

TECHNIQUES OF THE FROZEN

Good frozen section technique is learned gradually and only through experience. The resident should question the staff freely and attempt to observe all steps closely at first. He/she then should do as many things as possible and practice as much as possible. The steps of frozen section pathology are as follows:

1. Gross Tissue Examination

This step is probably the most important step and unfortunately is one that many pathologists have not yet learned. With practice one can become adept at predicting what a biopsy will show on the basis of its gross appearance, i.e., fibroadenomas of the breast have a characteristic whorled appearance; scirrhous breast cancer is retracted, stellate, and gritty.

The pathologist obtains gross clues not from just looking at the tissue, but also from feeling it and cutting it, i.e., soft or gritty. The pathologist records all gross expressions, i.e., size, adhesions, weight, similar to the recording of microscopic features.

2. Proper Communication With The Surgeons

This step is equally important as #1 and should occur either simultaneously with #2, before, or slightly after, but *before* any tissue is frozen.

The intercom is located in the frozen section suite; the room where the surgeon is operating will be obtained from the Pathology Department secretary transmitting the request for frozen section.

A list of operating room procedures appear the day before the planned surgery, and it is the responsibility of the resident and staff to be familiar with each case in *advance*. This means knowing what tissues have been removed previously, reviewing any previous diagnoses that our department has on file, and reviewing all previous slides on the patient. This is important because the present procedure may be related to previous ones. This "research" is the pathologist's equivalent of the medical history.

Example: A patient with a history of osteogenic sarcoma of the leg develops pulmonary nodules. Obviously, a comparison of the pulmonary material with the original material is essential in determining whether the pulmonary lesions are metastases or separate lesions.

After each case is thoroughly researched in advance, often there are additional questions and step #2 is for this purpose. Consider the possibility of obtaining microbiological cultures before contaminating the specimen. (Special research studies may also require sterile handling of tissues.) Consider preparing touch preps. When relevant, ink margins before cutting the specimens.

Based on steps #1 and #2 two critical decisions are made: Is a frozen section indicated and if so, what areas of the tissue should be frozen? The answer to this second part is often difficult, but is based on the question being asked and the pathologist's gross impression. Obviously, in a breast biopsy the pathologist should freeze the most suspicious areas.

3. Embedding The Tissue

The selected piece of tissue is then placed on a metallic holder and must be oriented a certain way so that the future section will reveal proper spatial relationships, this orientation depends on the question being asked. Sometimes orientation is not important; at other times it is of paramount importance.

The tissue is embedded in OCT mounting medium and is then placed either in cooled 2-methyl butane or the cryostat machine where it is properly frozen.

4. Cryostat

The machine, which cuts the tissue, is the cryostat. Certain things should be routinely checked in the operation of this machine:

a) Temperature

The temperature should be at -20°F for most tissues. For tissues with a large fat component, -40°F is optimal. This temperature is critical for optimal sectioning

- Too high, i.e., -10°F and the tissue will not stay frozen and firm and will not cut crisp.
- Too cold, i.e., -50°F and the tissue will crumble and become powder. The Ideal tissue should cut like butter, smooth and in one piece.

b) Blade sharpness and angle

The blade should be sharp and should be changed approximately once every 2 weeks. A dull blade cuts dull. Equally important is the blade angle. There is an optimal angle between blade and tissue:

- Too steep an angle and the tissue will crumble like it was too cold.
- Too shallow, then two things will happen. The section will alternately skip and not cut and then it will cut, but too thick.

This brings us to thickness and thinness of sections. In the rear of the machine is a device for adjusting thickness of sections. Ideal sections should be between 3 and 6 microns. But if the angle is too shallow even with the machine set at 3 microns, sections greater than 12 microns will be produced. If the blade angle is optimal, clean, thin intact sections should be obtained serially, i.e., with every cut.

When the tissue is cut the pathologist has the option of using a plastic tissue plate for ensuring that the tissue does not get folded, or manually knocking the tissue off the knife with a small brush. One's exact technique will vary with experience and individual preference. The tissue is picked up onto a slide by direct contact, taking care to avoid folds.

5. Staining

Once the tissue is on the slide it can be either air-dried or fixed in methanol. This depends on which staining procedure will be used. There are several stains available in the frozen section room and each has certain advantages.

	Advantage	Disadvantage
<i>Hematoxylin and Eosin</i>	<ul style="list-style-type: none"> • Looks similar to permanent section staining 	<ul style="list-style-type: none"> • Takes approximately 3 minutes
<i>Toluidine Blue</i>	<ul style="list-style-type: none"> • Takes 10-20 seconds 	<ul style="list-style-type: none"> • Different appearance than permanents
<i>Giemsa</i>	<ul style="list-style-type: none"> • Takes 10-20 seconds 	<ul style="list-style-type: none"> • Different appearance than permanents
	<ul style="list-style-type: none"> • Stains most cells with mucin-containing cells 	<ul style="list-style-type: none"> •
<i>Pneumocystis Stain</i>	<ul style="list-style-type: none"> • Rapid Toluidine Blue /"rapid" approx. 1 hour 	<ul style="list-style-type: none"> •

The choice of stain depends on what the pathologist is trying to demonstrate. The resident should practice all the stains and gain experience with their use.

The step-by-step procedures for each stain are listed in the operating room and in the histology room and are again summarized here.

6. Interpreting The Frozen Section

The results of one's labor now come to a climax when the resident and staff sit at the double-headed microscope and discuss the slide and render a diagnosis.

Since rapid diagnosis takes precedence over everything else in the operating room, oftentimes additional discussion and questions occur after the diagnosis has been rendered. The resident, however, should arrive at his/her own diagnosis and question the staff person without any hesitation.

7. Controls

In all science, controls are necessary. Since pathology is not an exact science, controls cannot be exact, but an attempt is made to check our frozen section accuracy. The tissue, which is frozen, is submitted for permanents and labeled "frozen section control." This should be kept separate from the other additional tissue submitted for permanents. In this way, the pathologist has a limited check on his frozen. If anything shows up on the permanents that is substantially different than the frozen, the surgeon or doctor taking care of the patient should be notified immediately.

GENERAL PATHOLOGY AND SUMMARY

The pathologist should always be conservative with frozen section, but accurate. A diagnosis of invasive breast carcinoma should be, in fact, invasive breast carcinoma with no hesitation if that is what the slide shows. Too many surgeons and too many pathologists imply an uncertainty in all frozen section and take the philosophy of waiting for permanents. If the frozen section is definite, and many are, a definite diagnosis should be made.

In the cases that are uncertain, it is here that a conservative approach should be employed. It is always better to call a malignant disease "benign" than it is to call a benign disease "malignant." This is because a diagnosis of malignancy carries with it a definite and irreversible action, i.e., mastectomy, whereas a benign diagnosis can always be revised after more thorough review.

As in so many things, experience is no substitute for knowledge, but knowledge alone is no substitute for experience. It takes years of frozen section experience to become a good diagnostic frozen section pathologist, and the resident should not be discouraged at first, but instead be encouraged to ask the advice of the staff for help and assistance in the frozen section room.