# Histology

Surgical Pathology Frozen Section Procedure for Breast Specimens with Liquid Nitrogen

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# Purpose

To establish policy relative to performing frozen sections in Surgical Pathology on breast tissue using liquid nitrogen.

# Materials

* OCT compound
* Frozen section fixative: flammable storage
  + Distilled water 495.0 ml
  + Glacial Acetic Acid 0.5 ml
  + Absolute alcohol 500.0 ml
* Liquid Nitrogen
* Solid stainless steel dewar
* Tongs or long forceps to hold specimen chuck
* Cryogenic PPE:
  + Cryogenic gloves
  + Buttoned lab coat
  + Face shield
  + Chemical splash goggles
* Freeze spray – must be *NON-flammable*
* 100% alcohol
* 95% alcohol
* 70% alcohol
* Xylene
* Gill’s III Hematoxylin
* Eosin
* Coverslip mounting media
* Coverslips
* 10% Bleach (sodium hypochlorite) for cleaning chucks
  + Bleach, household 10.0 ml
  + Distilled water 90.0 ml

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All reagents used in the Hematoxylin and Eosin staining circuit are gathered in the waste container at the staining area and manifested per established procedures. All flammable reagents are stored in flammable liquid storage cabinet or flame proof refrigerator.

# Procedure

Quality Control

1. All reagents, including stains are changed at the beginning of every day.
2. Tap water rinses are changed after every staining session.
3. Documented evidence of frozen section H&E quality is performed on the first frozen section of the day. Documentation is noted on the Surgical Pathology Frozen Section H&E Quality Control Record. Corrective action is documented.
4. The time of receipt and completion, notification to surgeon, of frozen sections are recorded on the Frozen Section Surgical Pathology Excel Sheet for that day, located on the S drive.
5. Turnaround time on a routine single frozen section should be no more than 20 minutes from receipt to completion and notification to surgeon.
6. Turnaround time on single frozen sections are monitored, with any delay noted, and included in the Monthly Technical Q.C. Report to Section Head.

Liquid Nitrogen Safety and Prep

1. Refer to MediaLab for additional liquid nitrogen safety and alarm protocols: 134845.977 “SAFETY – Liquid Nitrogen PPE”

134845.978 “SAFETY – Liquid Nitrogen, Silencing EAA and UH Alarms” 120353.64 “Liquid Nitrogen and Dry Ice”

1. When a breast margin frozen section case comes to the Frozen Section Lab, decanter liquid nitrogen into the dewar, filling only up to 50% full.

Specimen

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1. Fresh tissue is immediately delivered to the frozen section room, with no added fixative or fluid.
2. The specimen and request form is received in the frozen section lab.
3. The specimen is accessioned in SoftPathDx.
   1. Record the receipt of the frozen section on the Frozen Section Surgical Pathology Excel Sheet for that day.
4. The house officer or pathologist assistant examines the specimen and the gross description is written on the pathology request sheet.
5. After orientation and inking of the specimen, a portion, or possibly all of the tissue is placed on a brass chuck which has a thin layer of unfrozen OCT compound to anchor the sample to the chuck.
6. It is the responsibility of the house officer or pathologist assistant to communicate the identity of multiple frozen sections submitted at the same time. Communication may verbal or may be accomplished by setting a numbered cassette directly adjacent the chuck.
7. Place the brass chuck with the tissue sample in dewar filled 50% full of liquid nitrogen for 15 seconds using long handled forceps, cryogenic gloves, chemical resistant goggles, face shield & buttoned impermeable lab coat. Do not add additional OCT around the specimen.
8. After the specimen and OCT compound are completely frozen, place the chuck in the chuck holder in the cryostat. Tighten the chuck holder screw securely.
9. Prepare 2 slides with 1 section per slide.
10. Label each slide with the appropriate case number.
11. Cut each tissue section at 18-20 microns.
12. Stain slides with the following procedure.
    1. place slides with section in fixative
    2. rinse in 70% alcohol – 10 dips
    3. rinse in tap water 10 dips
    4. stain in Gill’s III Hematoxylin – 30 seconds each
    5. rinse in 2 changes of tap water 10 dips each
    6. Eosin - 30 seconds
    7. 95% alcohol – 10 dips
    8. 95% alcohol – 10 dps
    9. 100% alcohol – 10 dips
    10. 100% alcohol – 10 dips
    11. xylene – 10 dips
    12. xylene – 10 dips
    13. mount coverslip with mounting media
13. Present the slides to the Fellow, House Officer, or Pathologist.

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1. The Fellow, House Office, or Pathologist will notify the surgeon with diagnosis. With this notification, the patient identity and specimen source must be repeated to the surgeon for verification. The frozen section result must also be written on the request form.
2. Record the time of completion, notification of frozen section diagnosis to the surgeon, on the daily frozen section Excel sheet.
3. After completion of the frozen section, and verbal authorization from staff, the residual frozen section tissue is placed in an appropriately numbered cassette, melted, and placed in the container which it was received. The container is filled with 10% Neutral Buffered Formalin.
4. The container is then placed in the ‘to be grossed area’ and subsequently submitted for routine paraffin section after gross dictation.
5. The frozen section slides are saved and sent by courier at the end of the day to NCRC histology counter area so they can be included with the paraffin section slides of the frozen section, presented with the entire case, and filed with the case.
6. Following each frozen section the cryostat work and tools should be wiped down with 95% ETOH. Trimmings should be removed and placed in a biohazard plastic waste bag.

Decontamination

1. Cryostat should be decontaminated monthly and taken out of service immediately following a case known to be infectious.
2. Decontamination should be recorded on the Cryostat Temperature Q.C. chart.
3. Remove the cryostat blade and dispose in biohazard sharps container.
4. Remove the blade holder and soak it in, or wipe down and let it sit on the holder, with 10% aqueous bleach for 20 minutes. Rinse with copious amounts of water, rinse repeatedly in alcohol, and dry.
5. Soak all cryostat brushes and sectioning instruments as described above.
6. Lightly brush all tissue trimmings to the floor of the cryostat to form a pile. Remove the pile with an alcohol soaked swab, Swab out the cryostat chamber with absolute alcohol and wipe up excess alcohol with dry gauze. Dispose of tissue trimmings, gauze, and swabs in a plastic lined biohazard wastebasket.
7. Wipe out the chamber with 10% aqueous bleach solution. Let it sit for 20 minutes. Repeatedly wipe the chamber with water or 70% ETOH, followed by repeated wipes with absolute alcohol to remove any residual moisture.
8. Allow chamber to completely dry
9. Replace the cryostat blade holder, brushes and instruments. Lubricate where necessary.