Frozen Sections: Tissue Preparation and Sectioning

Final Approval: May 2010  Effective: May 2010  Next Review Date: April  2015

List all stakeholder(s) and dates of approval:

<table>
<thead>
<tr>
<th>Stakeholder Name(s): Geoffrey Werner</th>
<th>Date: 5/3/10 Reviewed</th>
<th>Revised</th>
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<tr>
<td>Stakeholder Name(s): Mark Magilner, MD</td>
<td>Date: 5/2010 Reviewed</td>
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<td>Stakeholder Name(s): Geoffrey Werner</td>
<td>Date: 4/12/13 Reviewed</td>
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<td>Stakeholder Name(s): Geoffrey Werner</td>
<td>Date: 3/26/14 Reviewed</td>
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Describe briefly the most recent revision made to this policy, procedure or protocol & why:

New Procedure

Purpose/Policy Statement:

For diagnostic purposes, a rapid technique is required for embedding tissue and performing cryotomy.

Definitions:

• n/a

STEPS / KEY POINTS

PROCEDURE:

1. The tissue specimen, labeled with the patient name and medical record number, and accompanied by a Surgical Pathology requisition, will be delivered by Surgery Dept. staff (who are responsible for “time-stamping” the requisition form and verbally notifying a staff member that a fresh specimen has arrived)

   OR

   messenger (who may not be aware of “time-stamping” the requisition form and this task will then be preformed by the Lab Assistant).

   In any circumstance, the specimen will be handed directly to the Lab Assistant.

2. Immediately notify the designated pathologist of the day (indicated by the Anatomical Pathology Schedule posted in the Pathology Gross Room) of the arrival of the FRESH sample.

3. Place the specimen and requisition at the Grossing Board that is in closest proximity to the cryostat equipment.

4. The fresh tissue is examined and dissected by a pathologist and/or pathologist’s assistant

   The Lab Assistant will:
   a. prepare slides with the patient name and Medical Record Number (MRN) using a permanent marker,
   b. assist the pathologist by providing any necessary reagents requested for alternative fixation or additional testing (RPMI, sterile cup, glutaraldehyde, etc.),

4. When labeling slides: utilize a permanent marker, label two clean glass microslides with the patient identification information (name, MR#) and designated as frozen section (FS), touch prep (TP) or scrape cytology smears (SC or smear) slides may also be required. Document the number and type of slide preparations on the department Slide Preparation Documentation slip that will be attached to the Pathology Requisition for data entry into CoPath.
5. The pathologist/pathologist’s assistant will select the appropriate tissue for the frozen section procedure.

6. O.C.T. compound is applied to a frozen section platform, the selected tissue is placed in the O.C.T. compound and the frozen section platform is placed in the cryostat chamber on the cryo bar.

7. After the O.C.T compound is partially frozen (begins to turn white), a heat extractor is placed on top of the tissue.

8. Cryospray may be used at this time to further expedite the freezing process.

9. After the tissue is completely frozen, the heat extractor is removed and the platform is secured in the microtome block holder.

10. Using microtome controls, bring block face to knife edge.

11. Shave conservatively into the block surface, taking thin cuts of 10-25 micrometers (trim thickness), to the desired depth of the first representative section of tissue.

12. When a uniform cutting surface plane is achieved, the cutting thickness is reduced to 5 micrometers (section thickness).

13. Using microtome controls, begin frozen block sectioning. As sections are cut, use a fine-hair brush to grasp edge of frozen O.C.T.

14. Using a quick-touch action, mount the first representative section onto the corresponding pre labeled microslide.

15. Immediately place slide in Methyl-Formalin fixative for at least 30 sec.

16. Repeat Steps 14-17 to take one additional level from this block on the second slide.

17. Stain slides with Hematoxylin & Eosin (see H&E STAIN FOR FROZEN SECTION).

18. Present slides to pathologist for diagnostic review with the specimen requisition form.

19. If the specimen has not yet been accessioned in LIS, complete this task now.

20. Label an embedding cassette for the specimen; following routine departmental guidelines (see SPECIMEN ACCESSION AND PREPARATION).

21. When the pathologist returns the requisition form and slides to the Gross Room, complete the following:
   - Verify with the pathologist that the frozen section sample in the cryostat is ready for formalin fixation, and transfer the sample from the Specimen Block Holder into the labeled embedding cassette (see SPECIMEN ACCESSION AND PREPARATION).
   - DO NOT LEAVE THE FROZEN SECTION SAMPLE FLOATING FREELY IN THE SPECIMEN CONTAINER.

ADDITIONAL STUDIES:

If necessary, distribute alternatively collected and labeled portions of the specimen with copies of the pathology requisition to their designated department/send out locations. The pathologist will initial the requisition and verbally confirm the orders. The pathologist’s initials and time-stamp will be highlighted by the Lab Assistant prior to delivering to the appropriate area of the laboratory.

RPMI – For FLOW CYTOMETRY

- Note on the requisition form the designated instructions to “PREP AND HOLD OR RUN per (pathologist’s initials)”. Highlight the pathologist’s initials and time-stamp, and place a copy on the FC work bench to notify the Medical Technologists and place specimen in designated refrigerated storage area.

For CHROMOSOME ANALYSIS

- Highlight the pathologist’s initials and time-stamp on the requisition. Submit copy of requisition form and keep specimen at room temperature. (See PATHOLOGY SENDOUT MANUAL).

STERILE CUP

- Note on the requisition form the designated instructions for any or all of the tests “SURGICAL CULTURE, AFB CULTURE, FUNGAL CULTURE per (pathologist's initials)”, highlight the pathologist's initials and time-stamp, and deliver a copy with the specimen to the Microbiology Dept.
GLUTERALDEHYDE

- Specimen remains in the Histology Lab designated refrigerated storage area for possible Electron Microscopy send out testing following pathologist’s review of H&E slides.

24. Verify that the frozen section slides are labeled with a departmental accession number OR place the requisition form and specimen container on the designated specimen shelf to await possible additional subsequent specimens from the same patient procedure.

25. Decontaminate the work area (see SPECIMEN ACCESSION AND PREPARATION), perform FS H&E stain set maintenance (see H&E STAIN ON FROZEN SECTIONS) and restock supplies as needed.

CALCULATIONS: N/A
CALIBRATION: N/A
QUALITY CONTROL: Cold chamber equipment temperatures and routine maintenance are documented in the Continuing Quality Improvement record book.
RESULTS: N/A

PROCEDURE NOTES:

1. Breast localization specimens are submitted in a plastic specimen bag with an accompanying Accu-grid; DO NOT disturb the tissue orientation in any way. This positioning correlates with X-ray films of the tissue and is diagnostically critical.

2. Routinely, the tasks of accessioning, labeling, staining and mounting slides are performed by a Lab Assistant or technologist; the tasks of embedding, freezing, and cutting tissue sections are performed by a pathologist or pathologist’s assistant who may request assistance from a histotechnologist.

LIMITATIONS OF PROCEDURE: N/A

Equipment/Supplies (If Applicable):
Disposable gloves
Laboratory coat
Eye protection
Surgical mask or face shield if there is a possibility of specimen or chemical release into the air

SPECIMEN: Fresh (unfixed) tissue specimen that is examined and dissected by the pathologist.

MATERIALS, REAGENTS:
Embedding medium - Tissue-Tek II O.C.T. - #4583
Cryomold - Tissue-Tek II #4557 & 4565
Microslides
Permanent Marking Pen – Secureline Marker II/Superfrost #1451
Canned quick-freezing spray - Surgipath Frostbite #FR-01000

Methyl Formalin Fixative
Methyl Alcohol . . . . . . 720 ml
37% Formaldehyde . . 80 ml

INSTRUMENTATION OR EQUIPMENT:
Frozen section H&E stain set.
Light microscope.

CAUTION: SUB-ZERO TEMPERATURE.
Wear appropriate protective equipment.
Use extreme caution to avoid hazards of exposure to sub-zero temperatures.

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Form Name & Number or Attachment Name (If Applicable):
surgpath08

Author Position:
Histology Supervisor

Review/Revision Authority (Position Not Individual Name):
Medical Director, Operation Manager, Histology Supervisor, Physician Assistant

Expert Consultant Position/s (Not Individual Name/s):
Pathology Physician Assistant

References (Required for Clinical Documents):

Is there a Regulatory Requirement? Yes □ No □
If yes, insert requirement information here:

Review History (No Changes):
N/A

Revision History (Note changes in area under header):
N/A

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N/A

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N/A