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| **Intraoperative Consultation - Frozen Sections** |
| **Purpose** | This procedure provides instructions for giving the surgeon a rapid, preliminary diagnosis on tissue submitted intra-operatively, to assist with intra-operative decision-making and aid in triage of specimen material. |
| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | AlcoholOCT Media Xylene Mounting media | * Superfrost plus slides
* Cryostat specimen chucks
* Slide pens
* Disposable microtome blades
* Gloves, PPE
* Staining forceps
 | * + Cryostat, Leica 1850 (or 1100 - backup)
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| **Scope** | Histology staff; Pathology Assistant and Pathologist |
| **Sample** | Fresh, unfixed tissue. |
| **Forms needed** | Intra-Operative Pathology Consultation Request Form |
| Scheduling | Submission of tissue for frozen section should be communicated to the Pathology lab in advance of the surgical procedure whenever possible. The MPLS Pathology lab is staffed between 0530-1730 M-F. The St. Paul Pathology lab is staffed between 0730-1600 M-F. Evenings, weekends and holidays are covered by on- call Histology staff. The on-call Pathologist must be notified at least 30 minutes **before** the surgical procedure starts. |
| Special Safety Instructions | Frozen sections involve fresh, unfixed tissue; follow universal precautions and use appropriate PPE.Disposable microtome blades may be safely stored in the cryostat to facilitate sectioning. To prevent accidental injury, microtome blades should not be placed into the blade holder until just before use. Microtome blades should be carefully removed immediately after use and discarded in the Red Biohazard Sharps container. |
| Stock Solutions | HEMATOXYLIN 1 EOSIN Y BLUINGALCOHOL, 100%XYLENEAll Stock solutions are purchased premade from Cardinal Health. Storage: Flammable, Hematoxylin is general storage. |
| **Working Solutions** | ALCOHOL, 95%Alcohol, 100% ................................................. 95.0 ml  Distilled water ................................................... 5.0 ml  |
| **Quality Control** | * CoPath Intraoperative Log containing time received and time result reported
* Pathologist will discuss stain and tissue section quality directly with the Histology Staff or Pathology Assistant in ‘real time’ so corrections can be made to ensure accurate diagnosis.
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| **Procedure** | Preparations: * Have a chuck with OCT in the cryostat ready to receive a specimen.
* Have the reagents in the staining line at their proper levels.
* Have charged slides available, labeled with patient's last name, first initial and case number (see Step 5 below).
* Use only solvent resistant pens or pencils to label slides.
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|  | **Step** | **Action** | **Related Document** |
|  | 1 | An unfixed specimen is received in a container labeled with patient’s name, medical record number, date and time of surgery, description of specimen and initials of collection personnel. Occasionally the submitted material will be folded in a blue towel, but it must still be labeled with requested information. |  |
|  | 2 | Time stamp the request slip. The Pathologist should be notified immediately. An Intraoperative Pathology Consultation Request Form will accompany the specimen. |  |
|  | 3 | Contact another Histotech or the Pathologist Assistant for assistance in staining if necessary. |  |
|  | 4 | Accession tissue in Copath to obtain the next surgical number. DO NOT take the time at this point to completely accession the case. Details can be added after frozen is cut. |  |
|  | 5 | Labeled slides will have two identifiers, the part ("A", "B", etc), and designate the cryostat section (CS) numbers: CS1, CS2 and CS3.  |  |
|  | 6 | Prepare a chuck by placing enough OCT on the chuck to cover its surface. Place heat sink on top of OCT. Turn on peltier. |  |
|  | 7 | Give the Pathologist the prepared chuck. The Pathologist will orient the specimen on the chuck and give to the Histotech or PA to place in cryostat. |  |
|  | 8 | Cover the specimen with OCT in sufficient quantity to allow a clear zone of OCT between the specimen and the edge of the chuck. The heat extractor mechanism in the cryostat may be used, providing the tissue sample is firm. Do not use heat sink on very soft tissue, or tissue that must stand on edge; this may result in misorientation of tissue specimen. |  |
|  | 9 | Place the chuck in the chuck holder, fasten securely and face tissue until it is full face unless otherwise directed by Pathologist. This is the first section (CS1). Mount tissue on slide. Slide is given to the Histotech or PA for immediate fixation and staining. |  |
|  | 10 | Turn the handle 2-3 times and mount section on slide. This is the second section (CS2). Place into fixative and begin staining. |  |
|  | 11 | Turn the handle 2-3 more times and mount on slidefor the third section (CS3). Proceed with fixation and staining. The Pathologist may request more than the standard 3 slides. Follow standard labeling, sectioning and staining *only per request of pathologist.*  Allow that sufficient tissue remains for fixation and routine processing for permanent section. |  |
|  | 12 | Slides are stained in the following manner:* Fix in 95% Alcohol.... …….......….20 seconds
* Rinse until clear in water
* Stain in Hematoxylin…….............1 minute
* Rinse in water
* Bluing………................................5-10 dips
* Rinse in water
* 95% Alcohol ................................10 dips
* Eosin…….....................................5-10 dips
* 95% Alcohol.....................…….....10 dips
* 100% Alcohol……........................10 dips
* Xylene (2 changes)......................dip until clear
* Coverslip with synthetic mounting media
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|  | 13 | Routine cleaning will be done **after each case.** Carefully sweep up excess tissue/ OCT and discard in the Red Biohazard trash. The microtome blade is removed and placed in the Red Biohazard Sharps container. Interior surfaces are cleaned with 80% alcohol. Cleaning is documented on Histology Preventative Maintenence Form. |  |
|  | 14 | When the Pathologist has called the surgeon with the results, document both the time received and the time the result was called. Document in the intraoperative report. If the time difference is more than 20 minutes, document the reason for the delay in CoPath-QA. The Pathologist will also write the results on the request form in the designated area. | [SOP 1.67 Intraoperative Diagnosis.doc](file:///C%3A/temp/SOP%201.67%20Intraoperative%20Diagnosis.doc) |
|  | 15 | Finish accessioning the case in Copath and include all collections takenThe tissue in OCT used for frozen section is placed in a labeled yellow tissue cassette for processing/ permanent section and marked as "FS".  In rare cases where there is scant tissue for special collections, the Pathologist may choose to HOLD this tissue as Frozen Tissue Collected. The tissue/OCT button is carefully removed from the chuck, wrapped in foil, placed in an appropriately labeled bag and placed into designated -70 tissue storage. This must be documented in CoPath under Histology Data Entry/Edit and noted in the gross dictation. . If tissue is saved in the -70° C freezer, document in accession entry/edit using the “Frozen Tissue Saved” retrieval flag and in Histology Data Entry/Edit function. |  |
|  |  | **CUTTING TISSUES WITH SPECIAL INFECTIOUS RISK****(INCLUDES HIV/AIDS VIRUS or TB):** |  |
|  |  | **Protective Clothing:** Wear double gloves, eyeglasses or gog­gles, N 95 mask and gown. |  |
|  | 1 | Place the mounting medium onto cryostat freezing specimen holder, and at the appropriate time, add tissue. **DO NOT** allow dispenser to touch tissue. Do not use Cytocool on suspected infectious tissues. |  |
|  | 2 | Cut and stain tissue according to procedure.  |  |
|  | 3 | Remove outer layer of double gloves, withdraw slide fromfixative with clean forceps, and proceed with stainingprocedure (please refer to the Staining Manual and/or postedbench reference). |  |
|  | 4 | When technical procedure is completed, put on clean gloves and place residual tissue in 10% formalin to fix thoroughly before it is handled again. |  |
|  | 5 | When cutting is completed, remove disposable blade and discard in appropriate BIOHAZARD SHARPS container. With protective clothing in place, remove the knife holder from the microtome. Then wipe clean with two changes of Sanicloths, Cavicide and absolute Alcohol. |  |
|  | 6 | If complete defrosting/disinfecting is desired, the cryostat must be shut off long enough for the microtome area to reach ambient temperature (usually overnight).Wipe all exposed surfaces inside and outside the cryostat chamber with a gauze soaked with Cavicide disinfectant and/or a Sani-cloth. Then wipe all surfaces with a towel or sponge that has been soaked in 100% Ethanol. Let the surface area air-dry with the chamber cover open. |  |
|  | 7 | Disposable blades are stored in the cryostat; for safety concerns, the blades are not mounted in the blade holder on the microtome until actual use. They are removed immediately after each case. |  |
|  | 8 | Be sure cryostat temperature is set to -18°C to -25°C. The cryostat temperatures are documented daily on the Histology Preventative Maintenence Form. |  |
|  | When results are available, the Pathologist contacts the surgeon, verifies the patient’s identification, and communicates the results to the surgeon. The Pathologist completes and signs the Intraoperative Consultation Requisition Form, providing contemporaneous documentation of the intraoperative consultation diagnosis. This information may be dictated for transcription, or directly entered into Copath. The Pathologist dictates the results that comprise the final written Surgical Pathology report**. All frozen section slides are permanently stained, mounted, labeled, and filed with the rest of the slides from the case. The residual frozen tissue is routinely processed and a paraffin section** prepared for comparison with the intraoperative interpretation. In rare circumstances for small biopsies, the frozen section material may be required for specialized diagnostic studies. The Pathologist will make appropriate notation of this exception in the final report. |
| **Interpretation/****Results/Alert Values** | Nuclear material BlueCytoplasm and connective tissue Pink to Red |
| **Result Reporting** | 1. Preece, Ann, A Manual for Histologic Technicians, 3rd ed, 1972.
2. Childrens Hospitals & Clinics, Minnesota, Histology/ Pathology Department, Equipment Manual
3. Children's Hospitals & Clinics, Minnesota, Laboratory Safety, Hospital Safety and Procedure Manuals

Note: CAP ANP.12050 and ANP.12075 |
| **References** | Frozen section quality data is documented quarterly. |
| Training Plan/Competency Assessment |  |
| **Authorization** | **Medical Director** | Dennis Drehner, DO | 3/9/09 |
|  | **Medical Director** | Peter Helseth, MD | 5/1/12 |
| **Medical Director** | Megan K. Dishop MD | 6/27/15 |
|  | Megan K. Dishop, MD | 6/28/17 |
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|  | **Designee** | **Signature** | **Date** |
| **Annual Review** | **Technical Specialist** | Dave Slinger | 4/16/09 |
| Pathology Assistant | Melissa Turner, PA | 4/29/10 |
| **Technical Specialist** | Dave Slinger | 2/8/11 |
| **Pathology Assistant** | Melissa Turner | 5/30/12 |
| **Pathology Assistant** | Melissa Turner | 7/6/15 |
| **Histology Supervisor** | Prabha Chintapalli | 6/20/17 |
| **Histology Lead** |  | Angela Dubbelde |  | 5/14/2019 |  |
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