**Background**

To establish a procedure on how to process core and open biopsies or resections for neuroblastic tumors.

**Materials**

* Between 3 and 5 small plastic vials to snap freeze tissue in for COG studies
* One (1) conical glass tube containing cytogenetics medium
* One (1) medium plastic vial containing cytogenetics medium for possible chromosome microarray analysis
* One (1) dewar containing liquid nitrogen to snap freeze tissue for COG studies
* One (1) extra-long forceps for removing small plastic vials containing tissue from liquid nitrogen
* Additional requisition and specimen labels
* Two (2) copies of the original surgical pathology requisition
* Two (2) cytogenetics requisitions
* Two (2) small biohazard specimen bags for transporting samples for cytogenetics

**Procedure**

Core Biopsies:

1. Carefully remove the folded Telfa pad containing the core biopsies from the specimen container. Unfold the pad and gently pour a small amount of RPMI medium over top of the cores. This will ensure the tissue remains moist and viable. Repeat this procedure as necessary to prevent drying and autolysis.
2. Photograph the cores.
3. Record the size and number of cores.
4. Surgeons will often request frozen sections on their cores to ensure they sampled from an area of viable tumor. Select one (1) of the better-looking cores to submit for frozen section analysis. Ink with eosin so the tissue is better visualized in the chuck.
5. Perform the standard frozen section procedure.
6. The attending pathologist will then evaluate the core for the presence of viable tumor. If the pathologist does not see viable tumor, he/she may instruct you to submit a different core for frozen section analysis or the surgeon to go back and take additional biopsies. If viable tumor was present in the core, the pathologist may instruct you to perform a pediatric tumor protocol on the remaining cores, depending on the number and quality.
7. If a pediatric tumor protocol is to be performed, consult with the attending pathologist to see which cores he/she would like you to submit for cytogenetics and COG studies.
8. Submit 2 samples of the core biopsies in cytogenetics medium, 1 for cytogenetics and 1 for chromosome microarray analysis.
9. Snap freeze some of the tissue (between 3 and 5 small plastic vials) for COG studies.
10. Thaw the core(s) from the frozen section analysis. Carefully place each core on a piece of cardboard, wrap it in lens paper, and put it in a biopsy bag. Put each core in its respective “FS#” cassette.
11. Fold the remaining cores back up in the Telfa pad and put them, along with the core(s) from the frozen section analysis, back in the specimen container. Fill the container with formalin and allow the cores to fix for at least 30 minutes (but preferably 2 hours) prior to submitting for routine histologic examination.
12. Put the sample for cytogenetics in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup”.
13. Consult with the attending pediatric pathologist before sending the sample for chromosome microarray analysis. If they want it sent, put the sample in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup.” If they are unsure, place in the bin on the door labeled “Pending Cytogenetics/Microarray” and await further instructions.
14. Put the samples for COG studies in the -80°F freezer in the most recently labeled “PTP” box (PTP-YY-##). In the pediatric tumor protocol logbook (located on the shelf above the accessioning computer) document the case number and part, the number of samples taken, and in which box the samples were placed.
15. Remove the fixed cores from the specimen container filled with formalin. Submit the “FS#” cassette(s) for routine histologic examination. Place the remaining cores on a piece of cardboard, ink with eosin, wrap them in lens paper, and put them in a biopsy bag. Place the cores in cassettes and submit for routine histologic examination. *Note: No more than 2 cores per cassette.*

Open Biopsies:

1. Take photographs of the external surfaces of the biopsy.
2. Weigh and measure the biopsy.
3. Ink the resection margin one color and any attached capsule a different color.
4. Bivalve the biopsy. Make sure to cut perpendicular to the capsule to show its relationship to the tumor. If the biopsy is large, make additional cuts parallel to the original cut on either side at intervals of 1.5 to 2 cm.
5. Photograph and describe the cut surfaces.
6. If the surgeon requests intraoperative histologic evaluation of the biopsy, take a sample from an area of viable-looking tumor and perform the standard frozen section procedure.
7. Submit 2 samples (at least the size of a pencil eraser) of the tumor in cytogenetics medium, 1 for cytogenetics and 1 for chromosome microarray analysis.
8. Snap freeze a minimum of 0.2 g (ideally > 1 g) of tumor for COG studies or at least 4-6 tubes.
9. Put the sample for cytogenetics in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup”.
10. Consult with the attending pediatric pathologist before sending the sample for chromosome microarray analysis. If they want it sent, put the sample in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup.” If they are unsure, place in the bin on the door labeled “Pending Cytogenetics/Microarray” and await further instructions.
11. Put the samples for COG studies in the -80°F freezer in the most recently labeled “PTP” box (PTP-YY-##). In the Pediatric Tumor Protocol logbook (located on the shelf above the accessioning computer) document the case number and part, the number of samples taken, and in which box the samples were placed.
12. If a frozen section was performed, thaw the frozen sample and place it in its respective “FS” cassette.
13. Put the remaining biopsy, along with the “FS” cassette, back in the specimen container and fill with formalin. Allow to fix for at least 2 hours before submitting sections for routine histologic examination.
14. After 2 hours have passed, submit the “FS” cassette for routine histologic examination. Put the remaining biopsy in cassettes and submit for routine histologic examination.

Resections:

1. Take photographs of the external surfaces of the tumor.
2. Weigh and measure the tumor.
3. Evaluate the integrity of the capsule. If the capsule is intact, ink it one color. If the capsule is disrupted, ink it one color where it is intact and a different color where it is disrupted.
4. Bivalve the tumor. Make additional cuts parallel to the original cut on either side at intervals of 1.5 to 2 cm.
5. Photograph and describe the cut surfaces. Document the presence of any residual adrenal gland. *Note: In ganglioneuroblastomas, distinct hemorrhagic nodules on an otherwise relatively uniform background may represent neuroblastoma clones arising from a more differentiated tumor and are an adverse prognostic indicator. It is, therefore, imperative to document and sample these areas for Shimada classification, N-Myc status, and cytogenetics. In treated tumors, it is important to estimate the percentage of necrotic tumor to viable tumor so as to evaluate the efficacy of the chemotherapy.*
6. Submit 2 samples (at least the size of a pencil eraser) of the tumor in cytogenetics medium, 1 for cytogenetics and 1 for chromosome microarray analysis.
7. Snap freeze a minimum of 0.2 g (ideally > 1 g) of tumor for COG studies or at least 4-6 tubes.
8. Put the sample for cytogenetics in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup”.
9. Consult with the attending pediatric pathologist before sending the sample for chromosome microarray analysis. If they want it sent, put the sample in a small biohazard specimen bag, along with a copy of the surgical pathology requisition and a completed cytogenetics requisition, and place in the refrigerator in the red bin labeled “Specimen Pickup.” If they are unsure, place in the bin on the door labeled “Pending Cytogenetics/Microarray” and await further instructions.
10. Put the samples for COG studies in the -80°F freezer in the most recently labeled “PTP” box (PTP-YY-##). In the Pediatric Tumor Protocol logbook (located on the shelf above the accessioning computer) document the case number and part, the number of samples taken, and in which box the samples were placed.
11. Place the specimen in an adequately-sized container filled with formalin and allow it to fix for at least 2 hours. Place paper towels in between slices to facilitate fixation.
12. Remove the specimen from formalin and submit representative sections for routine histologic examination. In small tumors, one full face of the tumor should be submitted. This should be accomplished with no more than 10 blocks. Larger tumors should be sampled at 1 block per cm of the largest tumor dimension. Sections should represent different areas of the tumor and show the relationship of the tumor to the capsule and adherent structures.
13. Map out your sections with annotations in the camera software (*see example image below*).



**Sample Dictation**

A. “left adrenal mass” Received fresh for pediatric tumor protocol in a large container is a 100-g, 12 x 10 x 7-cm well-encapsulated, soft, nodular tumor. The capsule (inked blue) is red-tan, smooth, and intact. The specimen is sectioned to reveal tan-pink, soft, lobulated and focally hemorrhagic cut surfaces. No residual adrenal gland is present. No capsular invasion is grossly appreciated. Tissue is submitted for cytogenetics and COG studies. Photographs are taken for reference.

Cassette summary:

A-1 through A8. Representative sections of tumor from periphery to include capsule (1 ss ea.)

A-9 through A-12. Representative sections of tumor from center (1 ss ea.)

Note: Please refer to the annotated photographs for the specific location of each section.