Created January 21st, 2021 by Jeremy Deisch, MD; Edited 7/29/2023 by JKD

- General Principle: For all specimens with send-out testing requests (as indicated on requisition by
 clinician or instructed by supervising pathologist), there are three primary tasks that must be completed
 to ensure completion of testing
 - **Tissue acquisition** if not already performed during the procedure, transfer a portion of the specimen (prior to formalin fixation) to an screw-cap vial (with or without supporting media)
 - Front office notification: A copy of the requisition clearly indicating the need for send-out testing (i.e. send-out to cytogenetics written and highlighted) needs to be placed in the "gross room send-outs" box in the front office of surgical pathology. Notify TR on duty that you placed a copy of requisition in the box. Without this step, the front office will not know to send out, and no testing will be performed.
 - Specimen storage: After tissue acquisition and placing the copy of the requisition in the "gross room send-outs" box, the tissue for testing needs to be placed in the appropriate location.
 - For cytogenetics requests, place tissue in RPMI in the refrigerator in surgical pathology accessioning
 - For copper mayo test, place the **metal free specimen vial** in the refrigerator in surgical pathology accessioning
 - For thyroglobulin/parathyroid needle washouts, disaccharidase assay place vial for testing in -70 degree freezer in the red send-out box labeled "Mayo" on the second shelf.
 - For muscle biopsy, place the vial for mayo testing in -70 degree freezer in the red send-out box labeled "mayo" on the first shelf. If an additional portion is frozen for possible additional studies, place the second vial in the send-out box labeled "Surgicals" on the second shelf.
 - ANY TISSUE PLACED IN -70 DEGREE FREEZER MUST BE DOCUMENTED ON THE LOG THAT IS HANGING ON THE SIDE OF FREEZER. Items to include on the log:
 - Date, surgical/autopsy number, quantity of vials, location of vials in freezer (include shelf number & name of box), tissue type, diagnosis/comment of type of send out (ie thyroglobulin washout).

Genitourinary Tract Stones for Chemical Analysis:

- DO NOT TOUCH THE STONES keep the specimen sterile.
- Take a gross photo of stones while still inside the container.
- State in gross description: "Specimen sent out for chemical analysis."
- Return the specimen inside a specimen bag with a copy of the requisition to the 2nd shelf of the refrigerator in accessioning.
- Attach sticky note stating "DONE" on the specimen bag to let MLA know that specimen has been grossed and is ready to be sent out via the clinical lab.

Lymphoma Protocol:

- o Excisional Lymph Node Biopsy Submitted Fresh:
 - Specimen is processed in with assistance with attending pathologist covering IOC
 - Stamp requisition and assign downtime number (same procedure for frozen sections)
 - Bivalve/serially section submitted lymph node
 - Perform touch prep x 1 slide, stain with H&E
 - Pathologist reviews touch prep slide to ensure that 1) specimen is lymph node and 2)
 there is no obvious non-hematolymphoid process such as metastatic carcinoma

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- On requisition, pathologist indicates "Lymphoma Workup Initiated" on frozen section diagnosis line, also include the date and time of the consultation on the requisition.
 There is no need to call in the surgeon with the findings if the tissue is appropriate for lymphoma workup
- Perform three additional touch prep air dried slides do not stain. Make sure that
 these slides are property labeled with the patient name and accession number. These
 may be used later by hematopathology for further staining as needed. Place these slides
 in the appropriate slide holder slot in the frozen room
- A small portion of fresh tissue (~ 0.4 cm cubed) is transferred to RPMI for flow cytometry (RPMI is located in the accessioning refrigerator). Fill out flow cytometry order form (located in the IOC room). During business hours, take RPMI tissue and filled-out order form to flow cytometry lab and place it in the specimen bowl on the front desk. After hours, notify the on-call flow cytometry tech—pager 6469 (schedule of tech on call is posted next to the accessioning room door.
- Thinly section remaining lymph node tissue and submit in formalin for routine processing. Unless lymph node is very large, lymph node should be entirely submitted for histology.

o Needle Core Biopsies of Lymph Node Biopsies:

- With pathologist supervision and guidance, tissue is apportioned into proper fixative to ensure that there is 1) adequate tissue for morphologic analysis by routine processing (FFPE) and 2) flow cytometric analysis.
- If necessary tissue can be transferred from RPMI to formalin to ensure adequate tissue volume for morphology.
- Preference is given to maximize tissue volume for morphology (formalin), with a much smaller volume (often a single biopsy core) for RPMI and flow cytometry.
- In situations with only a small volume (such as single core or two small length cores) submitted, the entire specimen should be submitted in formalin for morphology only.
- Tissue cannot be transferred from formalin to RPMI it is not possible to perform flow cytometry on fixed tissue.
- THE ATTENDING PATHOLOGIST MUST BE DIRECTLY INVOLVED IN THE DECISION TO TRANSFER TISSUE BETWEEN FIXATIVES; Document the attending name who approved tissue transfer.
- RPMI is a clear pink solution (not a fixative) that is stored in the accessioning refrigerator.
- Document a gross description on requisition of the tissue sent include tissue size/description, fixative received, and if tissue is sent for flow cytometry. Always include your initials and date for all laboratory documentation.
- Fill out flow cytometry order form (located in IOC room). During business hours, take RPMI tissue and filled-out order form to flow cytometry (down the hall) and place in specimens bowl on front desk. After hours, notify the on-call flow cytometry tech–pager 6469 (schedule of tech on call is posted next to the accessioning room door.

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Pediatric case protocol (without sendout)

- o Fresh tissue snap freeze tumor and normal into two (2) snap-cap containers, if necessary
 - Snap-cap containers are located in the top drawer on west wall of IOC room, to the right of the sink labeled "frozen vials" SNAP-CAP CONTAINERS SHOULD NEVER BE USED FOR SEND-OUTS - they are only for in-house procedures.
- o Label container with thin sharpie & <u>circumferentially place scotch tape over the label</u>. Labels will wipe off in -70 degree freezer if they are not taped. Items to include on the label:
 - Patient nameMRN, surgical case number, "tumor" or "normal", anatomic site, date collected
- o Place specimen in -70 degree freezer in the "surgicals box" located on the second shelf down from top.

Rectal biopsy for Hirschsprung's Disease

- o Hirschprung's disease handling is typically done on **suction rectal biopsy** in children, typically indicated on the requisition as "rule out Hirschprung's" or "aganglionosis". If a requisition is received requesting "ACE stain" or other special handling but has an atypical site, procedure type, or patient age, consult with pediatric pathologist prior to further handling/processing
- o Submit tissue in **BROWN** cap
- o Write "rectal bx" on side of cap
- o Add comment to task list on top block (A1)
 - "cut 6 slides for Hirschsprungs"

POC for cytogenetics:

- o Must be taken fresh utilizing sterile technique <u>always prioritize fresh tissue</u> over formalin-fixed paraffin embedded block.
 - Sterile forceps and scalpels are located in back of "sharps" drawer on southwest wall of HT gross room by station 5.
 - If you need a sterile surface to cut tissue, use the lid of a sterile container located in lower cabinet on the southwest wall of HT gross room by station 5.
 - Any samples received at other locations (TMC, SH) should be transferred into RPMI before sending to HT.
- o Label RPMI container with patients name, MRN, date of birth, date of collection, tissue type
- o Use sterile instruments to sample villous tissue and place into RPMI:
 - Prioritize fresh tissue in RPMI over FFPE, as this allows for a larger range of testing.
 - If you are unsure if villous tissue is present, do not submit any tissue in RPMI. Instead, place the most likely villous tissue into 1 (one) cassette, and up to 5 cassettes of remaining tissue (ie decidua or blood clot) to submit to histology. Then, place large post-it note on requisition to notify attending that no villous tissue was seen or placed in RPMI & to consider FFPE blocks for send-out. The attending that is signing out the case will notify the front office of this send-out.
 - If you see villous tissue, place some in RPMI, and follow the procedure below.
- o Place labeled RPMI container on 2nd shelf of accessioning room refrigerator, to await send out.
- o Write on CMAPC (Chromosomal microarray Products of Conception) on requisition.
- o Make a copy of the requisition and place it in "gross room send-outs" bin in the front office.
- o Dictate in your gross that a sample has been sent out for CMAPC, or Chromosomal Microarray.

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Thyroglobulin/Parathyroid Hormone Assay:

- Upon receipt of specimen WITHIN ONE HOUR OF COLLECTION MAXIMUM, the MLA immediately will remove the 15 mL plastic conical tube along with the requisition and handoff directly to the PA on-duty. (direct handoff only).
- o PA duties:
 - Include the following documentation on the requisition under the pertinent specimen part:
 - Write test code for the appropriate send-out assay:
 - PTHFN- Parathyroid hormone, Fine-Needle Aspiration Biopsy (FNAB)-Needle Wash
 - o TFNAB- Thyroglobulin, Tumor Marker, Fine-Needle Aspiration Biopsy (FNAB)- Needle Wash, Lymph node
 - On the requisition, write a gross description what was received (ie approximately 10 ccs of red-tan fluid), as well as type of container received (ie screw-cap conical container). Also document the received date, as well as the time placed in the freezer. You must also write "specimen placed in -70 degree freezer within one hour of collection and sent out to Mayo laboratories."
 - Place tubes in the red "Mayo" bin on the second shelf of the -70C freezer within one hour of collection, awaiting send out.
 - Make a copy of the requisition for the front office, and place it in the bin labeled "gross room send-outs"
 - Give the original requisition back to the MLA for accessioning.
- Cytology personnel will enter the information in Cerner after the case has been accessioned as an MN case.
- o TR will enter the request in Mayo site, enter note in Cerner. Prepare the specimen for send-out on dry ice for the Mayo carrier to pick-up before 3:00 PM

Disaccharidase Assay:

- Upon receipt of specimen WITHIN ONE HOUR OF COLLECTION, the MLA immediately will
 give the bag of ice with the specimen container directly to the PA on-duty (direct handoff only).
- PA Duties:
 - Specimens are received ON ICE and FRESH without gauze, filter paper, saline, or any other support/transfer media or fixative.
 - Carefully transfer fresh tissue into a small white screw-cap vial (provided from HLA lab and located on south wall at PA on-duty station).
 - Label container with sharpie with the following information Patient name, MRN, date of collection, type of tissue (ie duodenum), "disaccharidase assay". Be sure to place tape over the label so it will not wipe off in the freezer.
 - Place screw-cap vial in -70 degree C freezer in red "mayo" box on 2nd shelf within one hour of collection maximum, to await send out. Be sure to fill out the specimen log hanging on the side of the freezer.
 - On the requisition, you must document the following information within the pertinent specimen part:
 - Gross description (specimen dimensions) of what was received
 - Type of container received (ie screw-cap conical container)

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- How the specimen is received (fresh & on ice).
- Received date
- Time placed in -70C freezer.
- Write "**DSAC**"
- You must also write "specimen placed in -70C freezer within one hour of collection and sent out to Mayo laboratories for disaccharidase assay." This must also be explicitly stated in the gross dictation (see below).
- Make a copy of the requisition and place in "gross room send-outs" bin in the front office by 1330 hours (if possible).
- Return the original requisition with the original (now empty) specimen container to the MLA for accessioning.
- o When dictating the case, the grosser should read what is written on requisition of that part for the gross description.
- o IN PTOE:
 - Should be accessioned in as "Biopsy no task"
 - Do NOT add a block

Muscle Biopsy for send out to Mayo:

- NOTE: Muscle biopsies should be scheduled M-F between the hours of 8am-4pm. If page is received on weekend, refer to on call pathologist to contact clinician. Should be done during the week, not the weekend. No STAT muscle biopsies on weekend. If receive page for information, the form and instructions are located in the front office (bottom left drawer under mail boxes)
- o To be completed by <u>pathologists' assistants only</u>
- o When fresh tissue arrives:
 - Assign an undesignated number. Can be MS case if coming from SH, Murrieta, FMO and EC
 - Snap frozen tissue must be in green screw top containers labeled with case number, patient information, and site of biopsy
 - A portion is snap frozen and placed in -70 degree freezer to be sent to mayo clinic
 - For pediatric cases: if enough tissue snap freeze another portion for possible DNA studies
 - A portion is snap frozen for future possible studies (can be in clear plastic or screw top)
 - For known diagnosis omit snap frozen for possible studies
 - A portion is placed in glutaraldehyde for ultrastructural studies
 - A portion is placed in formalin for light microscopy
- o Write down measurements and what was done on the requisition.
- o Make a copy of 2 copies of muscle biopsy form.
 - Place a copy of muscle biopsy form in Dr. Raghavan and Dr. Deisch box (whomever is neuropathologist for the month)
 - Notify the TR on duty about specimen. Place original muscle biopsy form in "additional work bin" within the office
- o Make copy of requisition form
 - Place original with formalin container in accessioning to be accessioned
 - Place copy with glutaraldehyde container in the fridge for EM. Please notify EM personnel via phone or email that there is specimen for them in the fridge.

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- o Muscle Biopsy: Template "GMUSCLE" insert with F9.
 - Received without fixative on saline-moistened gauze are ___ fragments of red skeletal muscle ranging from _x_x_ to _x_x_ cm. A portion of the muscle is snap frozen and placed in -70 degree freezer. A small portion is transferred to glutaraldehyde fixative for possible ultrastructural examination. The remaining tissue is transferred to formalin for routine processing. (Te, 1 cap)
- Nerve Biopsy for Peripheral Neuropathy: Future entry:)

Bone Marrow Biopsies:

- 1. When receiving the bone marrow core, please <u>document the time the tissue was placed in formalin in the bone marrow log notebook</u> in the Hinshaw Towers IOC room next to the back sink (under the phone). Time in formalin should be previously written on container lid by clinical lab staff. If time is stated on lid, skip to step 2.
 - **If formalin time is NOT written on lid, processing depends on the location the tissue is received:
 - Received from clinical lab? Check the bone marrow cart in clinical lab for more information (ie time of procedure, time accessioned, etc...) to help.
 - Received from off site (ie Murrietta)? Check the time accessioned.
- 2. After fixation in formalin for 2.5 hours, immediately decal the core with immunocal for 1 hour.
- 3. After decal, processing depends on the day:
 - Received Saturday After decal, place in 70% alcohol for holding, until it is loaded to the processor on Sunday. The 70% alcohol is located by the bone marrow log notebook by the sink.
 - Received Last Day of Weekend (most likely Sunday, but holidays differ) After decal, load directly into VIP processor.
- 4. Fill in bone marrow log with all pertinent information (date, initials, case number, formalin time in/out, alcohol time in/out, decal time in/out).
- Temporal Artery: DO NOT CUT CROSS SECTIONS OF TISSUE AT TIME OF GROSSING. Instead, place the tissue UNCUT into a brown cassette. Write "temporal artery" on the side of the cassette. Histology will make cross sections of the tissue during embedding.
- **Endomyocardial Biopsies**: When grossing endomyocardial biopsies, you must include specific information, and it must be dictated into the correct areas of the report:

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- o Within the clinical history, dictate the number of pieces/samples that are documented on the requisition adjacent to that specimen part. For example, if the requisition lists "4 samples" next to part A, you would dictate in the clinical history: "Part A 4 samples."
- During your gross, you must:
 - Use a yellow cassette
 - Write on the side of the cassette: 1) write the number of pieces actually seen inside the container, 2) draw a heart symbol, and 3) write minus signs for each tissue that is submillimeter in size. For example, three submillimeter tissues would require three minus signs on the side of the cassette.
 - In PTOE, you must document the number of portions of tissue actually seen inside the container at the time of grossing in the column labeled "Pieces." Confirm orders.
 - If there are fewer tissue pieces that you can see in the specimen container than the number documented on the requisition, you MUST ask another grossing staff member (must be a more senior resident, PA or attending pathologist) to check the bottle. For specimens with fewer pieces in the bottle than are indicated on the requisition form, follow the "lost/missing specimen" protocols for notification and tracking. For specimens with more tissue pieces identified than what is listed, no further actions are needed.
 - Click "high priority" on winscribe before sending dictation (as done with other biopsies)