LLUMC GROSS ROOM ORIENTATION MANUAL

An Introduction to **Safety** and **Quality** in the LLUMC Gross Room for Pathology Residents and Pathologists' Assistant Students

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LOMA LINDA UNIVERSITY
HEALTH

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PART 1: INTRODUCTION TO THE LLUMC GROSS ROOM

Welcome to the Loma Linda University Department of Pathology! This document is to assist you in orienting to the workflow, tools, and procedures that will help to ensure both quality and safety for everyone involved in the process of pre-analytic surgical pathology - everything that goes into producing both the gross descriptive report *and* high quality, well-sampled histologic slides that are requisite in producing an accurate and clinically useful pathology report. As PA students and pathology residents, you are the eyes and hands of the pathologists in the gross room. For questions relating to gross room workflow and procedures, utilize this document, the supervising gross room PAs, attending surgical pathologists, and the standard operating procedures (SOPs) for guidance.

STANDARD OPERATING PROCEDURES: SOP documents are located in the shared drive in the following pathway…

(SHARED → PATHOLOGY → GENERAL → MC AP POLICIES AND PROCEDURES → SURGICAL PATHOLOGY MANUAL MC → INDEX TO ANATOMIC PATHOLOGY PROCEDURE MANUAL MC)

EVALUATION CRITERIA: Over the course of your rotation, your evaluations will incorporate input from a variety of sources. Evaluation criteria vary based upon the type of rotation (PA student / Pathology resident) and are tailored to the experience level of the student/resident. Direct observation by supervising PAs and attending pathologists, quality of interpersonal interactions, openness and response to feedback, and quality of work produced, and post-analytic feedback from the attending surgical pathologists are the primary data points assessed. Rotating PA students are evaluated by the supervising PAs, and pathology residents are evaluated by the surgical pathologists *and* the supervising PAs.

SCHEDULING/WORKFLOW: Schedules for attending pathologists, PAs, pathology residents, PA students, and neuropathologists are posted in the gross room bulletin board. The schedule is useful for determining what pathologist will likely be reading a particular case and, if complex, should be consulted with questions as to the ideal approach to sampling the specimen. The current workflow from specimen receipt to signout is as follows:

DAY 1: GROSSING	DAY 2: PREVIEW	DAY 3: SIGN-OUT
• Specimen receipt • Accessioning • Gross examination	• Histology slides prepared • Preview cases • PM RFS interpretation • Accessioning	• Sign-out cases with “SR” attending pathologist • PM RFS technical coverage • Afternoon RFS interpretation

This workflow diagram demonstrates the typical cycle for pathology residents. For pathology residents, in addition to the above-listed duties, residents should address their QA forms arising from the previous day’s grossing immediately after morning conference. If you have a question about how to best fix a QA, seek guidance from the supervising PAs and/or the I/Q pathologist.

The PA student workflow is more uniform, as case preview and sign-out days are not applicable to their rotation. PA students are expected to proofread and edit their gross descriptions from the previous day and address any QA forms that may have arisen from the previous day's specimen grossing. Afterwards, PA students should take cases that are commensurate with their level of experience and the daily workload. It is the responsibility of the supervisory PAs to determine the appropriateness of case selection by PA students.

INSTRUMENT BOX SIGN-OUT: Color-coded gross room instruments (i.e. scalpel handles, forceps, scissors, etc) are assigned to students/residents at the beginning of the rotation. It is the responsibility of the student/resident to maintain the integrity and cleanliness of this set and return the set in good condition at the end of the rotation. The supervising PAs maintain a sign-out log of case sets.

PART 2: GROSS ROOM SAFETY

SAFETY DATA SHEET: The SDS is an online resource that contains information about chemicals and potential hazards in the gross room. In the histology laboratory, due to the need for a variety of histochemical and processing procedures, there are numerous potential hazards that may be encountered. In the gross room, however, the list of chemicals is much more limited. Aqueous formaldehyde solutions and a selection of biologic disinfectants are the primary agents encountered in the gross room. Mechanical ventilation systems and utilization of proper personal protective equipment (PPE) will minimize the risk from these chemicals. However, in the event of equipment failure (e.g. power failure disabling mechanical ventilation, liquid spills), information about the chemical in question can be found in the online SDS. The path to locate the SDS is as follows: 1) Open Internet Explorer; 2) From the home page, select “Resources”; 3) Select “General Interest”; 4) Select Safety Data Sheets – from Maxcom; 5) Click on “Common MSDS” link to open Maxcom site.

EMERGENCY CODES/EVACUATION PROTOCOL: In the event of an emergency, instructions to evacuate will be relayed by the emergency intercom system and/or supervising laboratory personnel. Evacuation protocols (horizontal and vertical) are maintained in the **Safety Manual**, located on the shelf in the histology front area. Below is the color code alert system utilized at LLUMC:

- **CODE RED – Fire**
- **CODE PINK – Infant Abduction**
- **CODE PURPLE – Child Abduction**
- **CODE YELLOW – Bomb Threat**
- **CODE GRAY – Combative Person**
- **CODE SILVER – Hostage/Active Shooter**
- **CODE ORANGE – HAZMAT Spill/Release**
- **CODE GREEN – Missing High Risk Adult**

In the event of an emergency, instructions will be relayed over the intercom, by supervisors, or emergency responders. Please follow their instructions for your personal safety and to assist those responding to the incident. It is the responsibility of all personnel to know the locations and use of the following:

- Emergency telephones: Grey telephone in the transcription office (on wall next to copier). Will work even if there is a power failure. There is a list of emergency contacts posted adjacent to the phone. Do not use this phone for non-emergency communication.

- Flashlights: Battery powered flashlights are attached to wall in the frozen section laboratory, histology, and the transcription office.

- Eyewash stations: Exposure of the eyes to dangerous chemicals is particularly worrisome; damaging the membranes covering the cornea can result in permanent blindness; prompt treatment can minimize risk of long term visual deficits.

  There is one eye wash station in the gross room, one in the histology laboratory.

  Turn on eye wash station, hold eyes open with your fingers, and **flush for at least fifteen minutes**. Remove contacts and seek medical attention.

- Emergency showers: The emergency shower is located in the histology laboratory. The concept is similar to that for the eyewash station. Remove contaminated items of clothing, jewelry, and **flush affected area for at least fifteen minutes**. Then seek medical attention.

- Fire extinguishers:
  - *PASS* acronym is a reminder of the appropriate use of a fire extinguisher
    - P: ***Pull*** pin
    - A: ***Aim*** the extinguisher
    - S: ***Squeeze*** the trigger
    - S: ***Sweep*** extinguisher from side to side

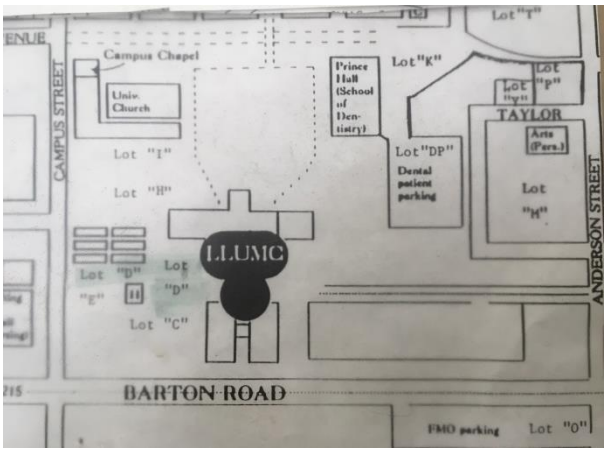
- Alarm pull stations: There are several fire alarm pull stations in the department. The closest to the gross room is in the hall across from the frozen section laboratory.

- Panic button: A small button is present in the upper left hand corner of the first resident grossing station (closest to the telephone). Pressing the button triggers an alarm in security. Security personnel will call and come to the gross room when summoned.

- First Aid kit: First aid kit is located in the histology laboratory.

- N95 masks: Located in the frozen section laboratory (on cart adjacent to wall to left of microscope table). Fit testing for N95 masks is performed at the time of PA student/resident orientation.

EVACUATION PROTOCOL: In the event of an evacuation, the Surgical Pathology designated assembly site is parking lot “DP” – Dental Patient parking. See the map to the right.



EARTHQUAKE RESPONSE: “Duck, cover, and hold”

PERSONAL PROTECTIVE EQUIPMENT (PPE): All personnel in the gross room (PAs, PA students, Pathology residents, observers (medical students, potential applicants) are required to wear proper PPE while in the gross room. PPE items are available on the cart immediately adjacent to the door to the gross room. For those directly handling specimens (or in very close proximity to the specimen), wearing the following items is required:

- Eye protection (either face shield, personal eyeglasses, or protective goggles)
- Waterproof apron
- Protective sleeves
- Gloves
- Shoe covers (to minimize tracking of biologic agents on shoes)

For those observing and not directly handling specimens (i.e. with a low risk of exposure), a protective waterproof apron is generally sufficient. Two additional points to remember when handling specimens is to remain as close as possible to the negative pressure ventilation at the grossing stations to reduce exposure to formaldehyde fumes. In addition, if there is a suspicion for an airborne infectious agent (e.g. tuberculosis) in a particular specimen, examination within a fume hood in the gross room is required.

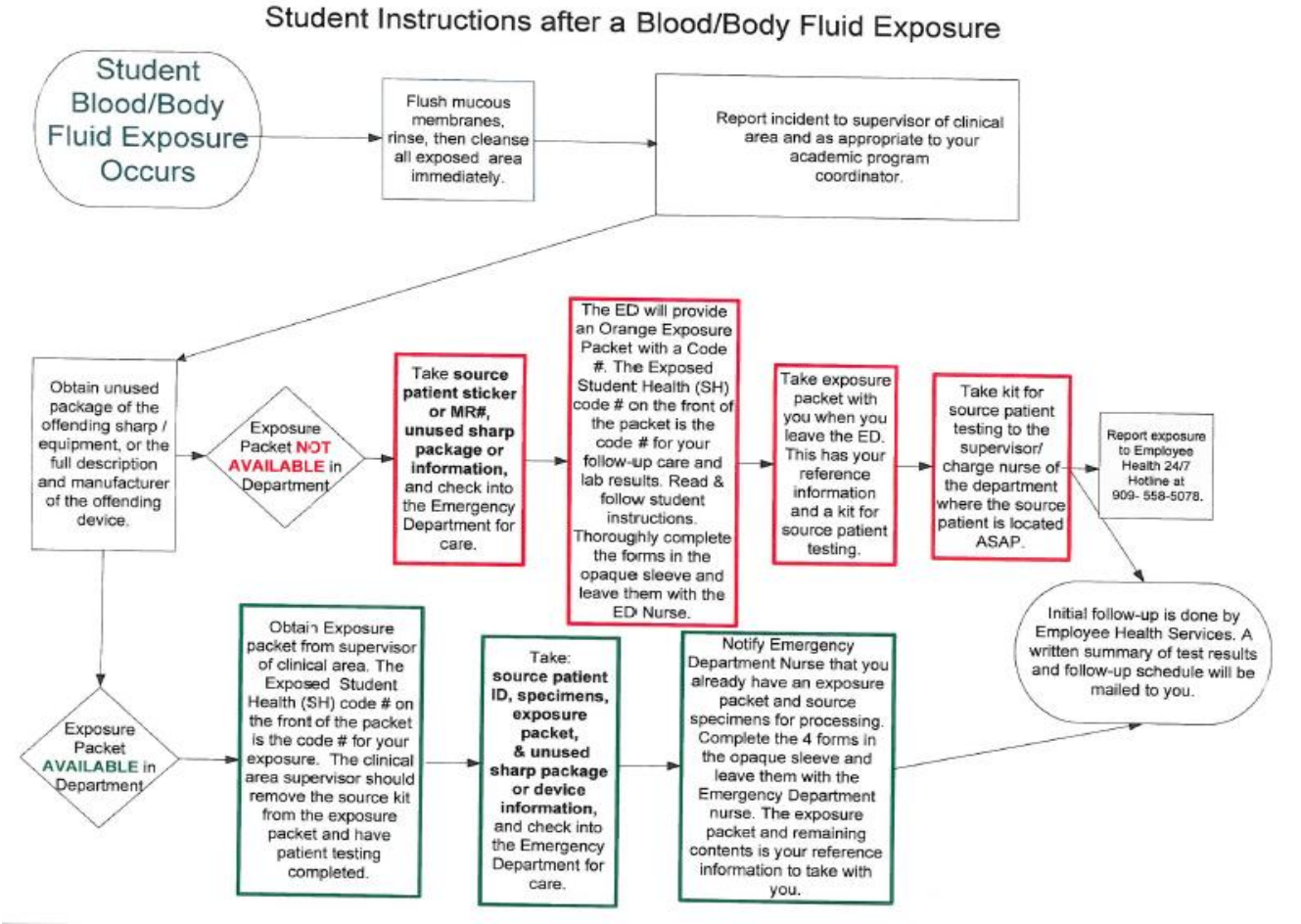
FORMALIN SAFETY: In anatomic pathology, 10% formalin solution is used (3.7% formaldehyde solution) for tissue fixation, appropriate for histologic processing, reduction in potential infectivity, and prevention of tissue breakdown. Formalin is clear and colorless, with a characteristic pungent odor. Possible routes of exposure (inhalation, direct contact, ingestion) are minimized by both use of PPE (see above) and environmental controls (ventilated grossing stations), as well as being mindful of specimen handling techniques to reduce exposure. There are procedures in place to handle formalin spills, depending on the volume of solution involved.

- < 10 mL = **minimal spill**. No special procedures required. Can be cleaned up quickly with paper towels.
- 10 mL – 1 L = **small spill**. Use formalin spill kit to clean up solution. Can be readily handled by gross room personnel.
- > 1 L = **large spill**. Call Safety Officer and/or Hazmat to handle spill cleanup.
- Both small and large spills must be reported to laboratory manager (Daniel Sorace)

Please see the LLUMC Pathology Formalin Safety Training Documentation for further information.

INJURY/EXPOSURE PROTOCOL: Utilizing proper sharps precautions (e.g. disposing of used and/or unnecessary sharps, keeping an organized and clean grossing station, knowing the locations of potential sharps at all times) helps to minimize the risk of injury. However, there are occasional injuries that occur in specimen handling. Although there are risks at all steps of specimen handling, the most common are of injury is in cuts to the hands/fingers in the frozen section laboratory. In addition, some specimens contain intrinsic sharps hazards, such as localization needles, metal staples, bone fragments, that can pose a risk to those handling specimens. Although all cases should be handled as potentially infectious, extra precautions (i.e. “double gloving”, prolonged formalin fixation prior to grossing) may be taken in cases with known high-risk infections such as hepatitis C and/or HIV.

In the event of an injury and/or exposure, all LLU students will follow the LLU Student Exposure Protocol. Important components of this protocol are initial response at the site of exposure (e.g. cleaning the affected sites), collection of relevant data (name and MRN of source patient), obtaining a Student Health code number, and reporting the exposure to the **Exposure Hotline (909-558-5078)**. See the diagram below for more detail.



LLU Pathology Residents will follow the Hospital Employee Injury/Exposure Protocol

RADIOACTIVE SPECIMEN HANDLING PROTOCOL: There is a slim possibility that a specimen containing a radioactive localization seed may come through the gross room during your rotation. Typically, there are seed localized partial mastectomy specimens. The pathologists and PAs have received appropriate training in the complex handling of these specimens. The general principles lie in minimizing the exposure to the small amount of radiation emitted by these seeds while localizing the seed, removing it from the tissue, documenting the number of seeds retrieved, and placing the seeds in a shielded container. Afterwards, retrieval of all seeds is confirmed by negative testing of the grossing area using the Geiger counter and a Neoprobe. Due to the complexity of handling these specimens and potential for radioactive contamination of the field if a seed is ruptured, handling of these cases should only be performed by staff PAs.

FOOD/DRINK IN THE PATHOLOGY LABORATORY: Given the exposure risk inherent to gross room personnel, food and drink are not allowed in the gross room, frozen section room, or histology laboratory. Food can be stored in the refrigerator in the pathology department break room or disposed of in trash cans prior to entering these areas. Do not dispose of food items (including wrappers, cans) in trash cans in these restricted areas. Doing so is presumptive evidence of violation of this restriction and may be cited by site inspectors if encountered.

PART 3: COMPUTER ACCESS IN THE GROSS ROOM

Computers play an integral role in the workflow of the LLUMC gross room. The benefits of the integration of the pathology laboratory information system (LIS) are myriad, with the greatest benefit being the rapid accessibility of case information by all pathology personnel, regardless of their proximity to the gross room. The detriment of the reliance on these systems is that when they are not working properly (such as in situations when the network connection is down), “down-time” procedures have to be followed, stress increases, and productivity and efficiency is greatly reduced. Fortunately, these instances are relatively uncommon.

The laboratory currently uses several key systems for specimen processing. **Cerner** is the anatomic pathology LIS, and is critical for all steps of specimen accessioning, processing task entry and fulfillment, photographic documentation and storage, and all steps of diagnostic report generation. In addition, billing processes and non-printable case notes are functions of the Cerner LIS. However, Cerner is not a dictation/transcription system. For these functions (dictating gross and microscopic reports), **Winscribe Internet Author** is the system utilized at LLUMC. The hospital-wide electronic medical record (EMR) system is an EPIC product, referred to at Loma Linda as **LLEAP**. LLEAP is the primary source for clinical documentation, clinical laboratory results, etc. In the gross room, LLEAP is useful for obtaining relevant clinical information pertinent to specimen sampling; surgery operative reports, radiology reports, and endoscopy notes are useful for complex specimens. This portion of the orientation manual is dedicated to initial setup and confirming proper function of the relevant subsystems.

COMPUTER LOGIN: At LLUMC, the Windows Password set by the individual user is used to access many of the patient care functions, in particular those that are not specific to pathology. Verify that your login is successful. If not, call the LLUMC Help Desk (X48611) for password reset. After successful login, verify that your personal home drive and the pathology shared drive are listed under the “my computer” function in the start menu.

CERNER APPBAR SETUP: The LLUMC Cerner Pathology Module (AppBar) requires a different login and password than the above described Windows password. This should be given to you during your orientation. At initial setup, the link to these systems will not be on your desktop and have to be accessed from the **LLUMC VIP** home page. Find the relevant links (Apps → Ancillary Desktop → Cerner AppBar; Apps → Clinical Desktop → LLEAP), right click each, and choose “create desktop shortcut” for each. Shortcuts to the required apps will be on your desktop from this point forward.

Initially, the Cerner AppBar loads without the buttons needed to launch the applications required by gross room personnel. These have to be added and/or organized by the user. Once this setup is complete, however, the users and personalized AppBar will load properly henceforth, regardless of the station that the user happens to be at. To set up the AppBar, click on the setup button and select **customize**. From this function, you will select the following “buttons” from the left, add them to the right side of the column. This is also the field that you can re-order the buttons as you so desire. Add the following buttons:

- **Online Review**: Used for viewing and editing reports that are not finalized. This is where you will edit your gross descriptions.
- **Case Findings**: This function is for viewing reports that have been finalized by the responsible pathologist. This will be useful for follow-up on cases of interest. This is also a good place to search for cases by several available parameters (patient name, responsible pathologist, date received/verified).
- **Pathology History Inquiry**: Useful for viewing all other surgical pathology reports available for a given case – what has this patient's previous pathology shown? This application is often configured to launch along with the "online review" function.
- **Processing Task Order Entry (PTOE)**: This function is essential in the gross room. This is where you will enter tasks that are required for appropriate specimen handling. These orders set into motion specific tasks that show up on work lists for the relevant department. For example, when you submit two blocks on an appendectomy specimen, you add the blocks in PTOE (select specimen "A", then "add task", type in "2", and hit enter). Up to 10 blocks can be entered at a time in this manner. You then place the appropriately labeled blocks in the formalin. The next morning, the two blocks that you entered into Cerner will show up on an embedding work list printed off in Histology. Histology will compare what is on their work list to what they received. If everything matches, all is well. However, if you either forget to enter the blocks in PTOE *or* only submit one block in formalin, the worklist and caps will not match. You (i.e. the person who made the error) will then get a Quality Assurance (QA) form and will have the distinct pleasure of fixing it.
- **Image Management**: Used for taking, storing, and viewing photographs. Images are linked to the surgical accession number, so all images (gross, microscopic, etc) are viewable from this function. Each user needs a personal folder to store images taken in the gross room.

One key point to remember when using Cerner applications is that just entering the changes/tasks does not save the changes to the system. Until you hit the "save" button (with an image representing an old-school 3.5'' floppy drive, typically in the upper left-hand side of the screen), your entries are only stored locally. Hitting the "save" button registers the changes with the server. This can be hard to get used to. If you enter data and then hit the close ("X") button or walk away and the application times out, **you will lose all of your work**. Always hit "save" before you walk away!

WINSCRIBE: The digital dictation system at LLUMC and related entities (FMO, SH, Murrieta). An individual user ID and password are assigned to each user and should be available at the time of orientation. The USB footpedal needs to be configured for **each user at each station**. This is because the settings are stored locally and are dependent on the hardware at that particular computer. Moving to an alternate PC will require setup for that station. Specific functions are assigned to each of the three "buttons" on the pedal; typical functions are rewind for the left button, record for the center, and forward for the right button. An additional item that needs to be configured is the microphone input volume. Find this setting and set microphone button to maximum. Otherwise, transcription will not be able to hear you.

GROSS ROOM PHOTOGRAPHY: Using the image management app from the Cerner AppBar, test to ensure that the TWAIN window opens appropriately. Similar to Windscribe, the camera will have to be configured at each station. Test to ensure that images import into Cerner appropriately for gross room photographs.

PART 4: GENERAL SPECIMEN HANDLING GUIDELINES

There are a wide variety of specimens that are analyzed in the LLU affiliated laboratories, ranging from small diagnostic biopsies to large, complex composite resection specimens. Each type of specimen can pose challenges, from diminutive biopsies that are at risk of being lost or cut through in the histology lab (an “exhausted block”), to the large complex specimens that are difficult to orient and determine the spatial relationship between the lesion and the sampled structures. These risks are mitigated by following the LLUMC handling guidelines as described below. One key point to remember that will help to prevent errors is to remember that each case submitted to pathology is from a person who, besides their own concerns, likely has a family that is greatly concerned about their well-being. Treat each case as though it came from your mother, father, brother, or friend. Don’t let workload pressures lead to skipping critical steps or cutting corners. Doing so can result in incorrectly staged tumors and resultant under/over-treatment, or switched biopsies resulting in unnecessary or delayed treatment. Your attention to detail and following specimen handling guidelines are key components of preventing these types of errors in the pathology gross room.

Rule 1: Only one case on the grossing board at a time. This rule is pretty much common sense. It’s easy to mix up specimens when your board is covered in them.

Rule 2: Only one specimen container open at a time. Another rule that is self-explanatory.

Rule 3: Always verify correct patient identifiers and specimen designations. This applies to the specimen container, printed and/or hand-written caps, and requisition. For example, when grossing a GI biopsy, you check that the surgical accession number, patient name, and MRN match on both the container and the requisition. You then inspect the cap(s) to verify that the accession number and alphabetical specimen designation match.

Rule 4: Always clean the board and instruments between cases. In most cases, cleaning the board only between entire cases is appropriate. However, when grossing specimens with friable tumors and/or those known to result in cross contamination, place a clean paper towel and wipe down all instruments between grossing each specimen. If this policy is not followed, all specimens grossed after examining a large, friable tumor will have small fragments of said tumor smeared amongst the tissues from the subsequent specimen. This is referred to as “buttering”, as the fragments are often stuck to the scalpel blade. This is a nuisance at best, and a diagnostic conundrum at the worst.

Rule 5: Do not reuse cotton-tip applicators, gauzes, or paper towels between cases. These items pose a great risk of cross contamination of tissue between cases. Failure to follow these procedures will result in contamination between cases.

Rule 6: Do not gross two large specimen of same type back to back. Following this recommendation will minimize tissue transfer between cases that is indistinguishable based of tissue type/histology findings.

GROSS ROOM WORKFLOW: The following workflow applies to all cases, regardless of complexity.

1) Place specimen container, labeled caps, and requisition on counter (adjacent to your cutting board)

2) Verify that the accession number, patient name, MRN, and specimen designations match

3) Dictate the case information from the requisition (see order of dictation below)

4) Dictate the patient name and identifiers from the specimen container label

5) After visual inspection and/or dissection of the specimen, dictate the gross description (e.g. what is in the bottle, how big is it, what lesions are present, etc)

6) Sample the specimen according to the level of complexity. Refer to white suggested sample size (hanging next to faucet in main gross room sink) for the ideal section size and thickness. An additional general guideline is to **aim for tissue sections that approximate the diameter and thickness of a nickel**. Some specimens can tolerate thicker sections (such as umbilical cords, brain tissue), some require as thin of sections as possible (fatty specimens, especially breast tissue, placental parenchyma). Some tissues cut best when they are cut a little thicker, in particular portions of bone for decalcification

7) Dictate the specimen sampling. For example, if you cut the specimen in half and totally submit it in one cap, say “(TE, 2 caps)”. Alternatively, you can say “(RS, 10 caps)”, if you put only a portion of the specimen in 10 caps, with some remaining in the container. (TE = totally embedded, RS = representative sections)

8) Dictate the cap key: This is where you indicate what is in each cap. This is one of the most important parts of the gross report, as without it, interpreting the glass slides is very difficult. What does each slide mean? Is it a margin and, if so, what margin is it and how was it taken (perpendicular, en-face). What tumor did you sample in the cap?

9) Close the lid securely to the caps, and place caps in formalin tray in the gross room

10) Enter caps and any relevant instructions (i.e. special embedding, pre-ordered stains) in PTOE.

11) Repeat for each specimen from the case

12) When complete, write your initials on the bottom of the specimen requisition. This step is necessary for ensuring that you receive credit and/or feedback for grossing the case

ORDER OF DICTATION: Below is the proper order of dictation for LLU pathology laboratories. This order is intentional. Deviating from this order will result in transcription having to jump from field to field out of order, greatly decreasing their efficiency.

1) From the Requisition
    a. **Pathology case number and patient name**
    b. **Clinical history**
    c. **RFS information**
        i. **Site of performance** – Was RFS done at LLUMC or SH?
        ii. **Specimen identifier** – What specimen was the RFS from?
        iii. **RFS diagnosis** – What did the pathologist say to the surgeon?
        iv. **Pathologist Name** – Who was the pathologist that did the intraoperative consultation?
        v. **Date of IOC**
        vi. **Time specimen received, time reported** – both of these times are required for tracking IOC turnaround time
        vii. **Routine vs. Complex** – Multiple concurrent specimens/cases, cases requiring subspecialty expert consultation should be dictated as complex. This again is for case time tracking
    d. **Specimens** – Read what specimens were submitted ("specimen A is duodenum, specimen B is stomach, specimen C is esophagus", etc)

2) From the Specimen Container: This part is in the gross description. Say "specimen A, gross description, labeled John Smith, duodenum". Then proceed with the gross description, specimen handling, cap key (as detailed above in workflow). Do this for each specimen.

CAP COLORS USED AT LLUMC: Different color caps indicate varying histology processing tasks. Below is a legend for cap colors. Follow this color code, and good things will come to you in the future…

- **GREEN**: Routine Processing. Nothing to see here.
- **TAN**: Special embedding. Used for muscle, nerve, temporal arteries, small tissue biopsies
- **YELLOW**: Immunohistochemical studies/special staining
- **GOLD/ORANGE**: Stat processing. These cases are cut first thing in the morning.
- **PINK:** Steps sections. Used primarily for GI biopsies.
- **WHITE**: Decalcified tissue

PART 5: HANDLING OF SMALL BIOPSIES

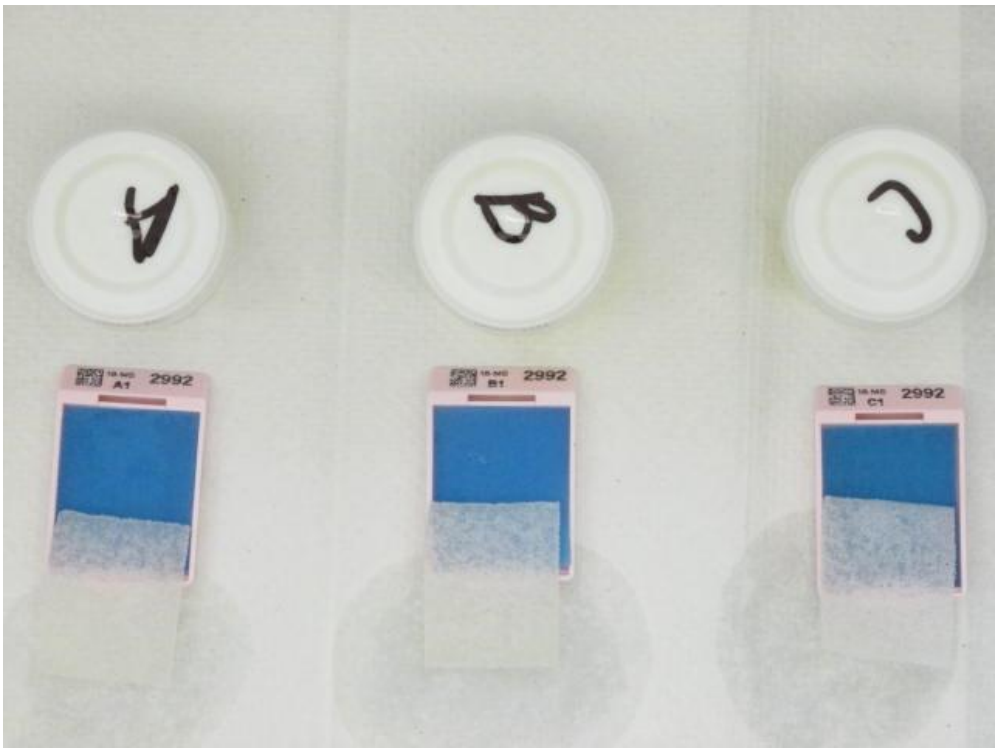
Great care must be taken when handling small biopsy specimens. Some, like gastrointestinal mucosal biopsies, are relatively routine and easily managed. However, some cases require significant attention when grossing the specimen to maximize diagnostic yield and reduce the chance of requiring a repeat biopsy. Here, I have laid out an illustrative example...

> *An interventional radiologist performs a biopsy on a mediastinal mass in a 70-year-old male with hemoptysis. In the gross room, you receive several very thin needle cores of tan tissue. At this point, even though you do not know what the final pathologic diagnosis is, there is a good chance that this will turn out to be a malignancy, possibly metastatic lung carcinoma. You think to yourself… "This small amount of tissue is going to have to be stretched very thin; in addition to the initial H&E sections, there will almost certainly be immunohistochemical studies performed to confirm the site of origin. Then, after the patient is presented at tumor board, Medical Oncology will request molecular studies looking for tumor mutations that can be targeted with directed chemotherapeutic agents. These tests all require tissue, and each round of additional tests is one step towards exhausting the block. Once exhausted, the clinicians will be forced to take the patient for another biopsy procedure, so that the necessary testing can be performed. This places the patient at further risk for complications, such as damage to vital structures, bleeding, infection, etc". Knowing that this biopsy specimen will be called upon for such exhaustive testing, you obviously do the right thing. You divide the cores into two separate blocks, wrap the specimen carefully, use an indicator dye (carbol fuschin) to assist in embedding and cutting, and order "PK CORE" so that unstained slides can be cut at the same time as the H&E section, minimizing tissue loss from trimming and facing the block. Recognizing your competence and mastery of grossing procedures, the pathologists, PAs, and treating clinicians all march single file into the gross room, give you a high-five, and proclaim the glory of your solid work ethic and knowledge of proper gross room procedure. Satisfied with yourself, you then proceed to gross the next case…*

The moral of this fable is that with proper attention to handling small biopsy specimens, diagnostic yield can be maximized, and repeat biopsy for treatment determination can be avoided in many cases. Below are specific guidelines for approaching these specimens.

GASTROINTESTINAL MUCOSAL BIOPSIES: Biopsies from anywhere along the GI tract (esophagus to anus) typically yield one to several small tan tissue fragments. When grossing these specimens, the following steps must be followed in order to ensure proper grossing. See image below for example of proper setup.

1) Organize all specimen bottles in alphabetical order with caps
2) Go down the row and confirm that the accession number/ID on bottle lid, bottle, and cap match, and that the numbers and site on the bottles match the requisition
3) Place cap on top of bottle
4) Place a **blue sponge** in each cap, and a **white sponge** adjacent to each cap – please ensure that sponges are wet, to avoid very small tissue fragments being absorbed by the sponge



5) For each individual specimen, “gross” the specimen, placing the tissue fragments on top of the blue sponge in the cap
6) Place a drop of marking dye (e.g. carbol fuchsin) on the tissue to aid in embedding and cutting
7) Cover the tissue and blue sponge with the white sponge that you previously set aside
8) Put lid on cap; ensure proper lid placement (it will “snap”)
9) Repeat for all specimens in the biopsy series
10) When done, **confirm that there are no leftover sponges** on the cutting board. If you setup the board as described in steps 1-4, and you have a leftover sponge on the board, you forgot to put a sponge in one of the caps. Stop and fix this now.

SMALL DIAGNOSTIC/INTERVENTIONAL RADIOLOGY CORE BIOPSIES: The guiding principle to handling these small yet critically-important specimens is to maximize diagnostic yield by conserving tissue at all steps. General grossing principles apply, with the following additional considerations.

- **Divide the specimen equally into two caps**; if there are two cores, place one core in each cap. If there are five cores, three go in one block, two in the second block. Don't cut the cores. This step allows both for diagnostic workup (i.e. immunohistochemical studies) *and* preserves precious tissue for molecular testing as required.
- Use tissue dye (e.g. carbol fuchsin) to stain tissue. This makes embedding and sectioning much easier and helps to decrease tissue loss in trimming. White tissue in white paraffin is difficult to see. Purple tissue is much easier to identify.
- Wrap specimen carefully in blue filter paper. Please fold the paper so that the tissue is both secure and the staff who is embedding the specimen can find easily find the edges of the paper (don't just wad the paper up). If you have questions, consult a supervising PA or surgical pathologist.
- If tissue is very scant, place tissue in tan cap, and enter a case note to "please conserve small amount of tissue".
- In PTOE, order package "**PK CORE**" on each block of diagnostic biopsy material. This package orders three tasks; 1) embed 1 block, 2) ribbon sections (not steps), and 3) 10 unstained slides. One point to keep in mind is that if there are multiple biopsies in the series, do not order unstained slides on every specimen. Consult with the supervising pathologist in cases such as these. Histology does not have time to cut ten extra slides on five to six specimens.

ENDOMYOCARDIAL BIOPSIES: Again, tissue conservation is key. An additional consideration for these biopsies is correlating the number of tissue fragments received and the number indicated on the requisition. The interventional cardiology laboratory will indicate the number of pieces that they submitted.

- Indicate the number of pieces as listed on the requisition form into the dictated clinical history.
- Write the number of pieces received on the side of the cap – This step alerts the embedder to look for any potential missing pieces
- Enter the number of pieces into the "Pieces" field for the appropriate block in PTOE.
- If any pieces are very small (and at risk for not surviving processing), say "one tissue fragment is very small, and may not survive processing", *and* write "(-)" on the cap, next to the number of fragments that you wrote from the first step.
- If the number of pieces in the container do not match with the number of pieces indicated on the requisition form, show the bottle to a supervising PA and add a case comment indicating a discrepency. Save the bottle on your bench until the case has been signed out.

PART 6: LARGE SPECIMEN CONSIDERATIONS

Specific guidelines for grossing the variety of large complex specimens is beyond the scope of this orientation manual. However, there are general considerations that can help in approaching these specimens.

1) For proper grossing protocols, utilize available reference materials
    a. **LLUMC grossing manual**– Please consult the LLUMC grossing manual for guidelines on specimen dictation and sampling. For a more complete overview of specimen approach (in particular for new trainees), consult published dissection manuals. Reviwe the relevant sections in these text for descriptions and images of specimen orientation, dissection techniques, and proper sampling of the specimen
    b. **CAP PROTOCOLS**– These checklists are available on the shared drive (see MASTER TUMOR SPREADSHEET). They are useful in that they provide a checklist of items that are required for pathologic staging (both in the gross description and specimen sampling). Use these to inform your approach to the specimen.
    c. **Gross Room Supervisory Staff:** The “IQ Pathologist” is the pathologist who is responsible for overseeing the gross room operations. In addition, the Pas are excellent resources for proper specimen approach. However, please do not consult staff until you have reviewed the grossing manual, CAP protocols for complex cases, and have reviewed and are familiar with the clinical information.
    d. **Pathologist who will be signing out the case** – In complex specimens (e.g. complex resection specimens, those that deviate from those described in reference manuals, specimens that are difficult to orient), the pathologist who will be reading the slides and signing out the case is the most important person to consult. If available on the day that you are grossing the specimen, this pathologist should be called to the gross room to “lay eyes and hands” on the specimen. If this is done, said pathologist will have a chance to give you input into their grossing preferences, decreasing the chance that you will be asked to pull the specimen days later. In addition, specimen orientation and interpretation of the gross description is much easier if the interpreting pathologist has seen the case themselves.

2) Pay close attention to section size and thickness. There is a sweet spot when it comes to diagnostic utility and section size. Too small, and context will be lost, and the relationship between the lesion and the adjacent tissues is lost. Too large, and histology quality suffers. Putting in too much tissue (“stuffing the cap”) results in poor fixation/infiltration, sometimes to the point that sectioning is impossible, and reprocessing is required. Tumor thickness is an important parameter for proper fixation, as the rate of infiltration of the tissue increases as the surface area/section volume increases. Sections that are too wide/tall can block the holes in the caps and impair the flow of reagents, leading to poor fixation. Again, as described above, aiming for sections the approximate width and thickness of a nickel is ideal. **Don’t be a cap stuffer!** Also, some tissues that are too thin may introduce similar challenges for histology. Dense tissues such as myometrium, bone tend to curve during processing, and sections that are ~ 1 mm thick are impossible to embed flat and cut a complete cross section. Also, very thin sections of dense tissues are more susceptible to “popping out” of the paraffin during

sectioning. Dense tissues such as bone and muscle should thus be cut into slightly thicker sections (2.5 to 3 mm) to ensure proper histology.

3) For specimens that are difficult to process, such as placental parenchyma, fatty soft tissues, lipomas/liposarcomas, breast tissue, colon, **get the tissue on the early run processor as close to the start time (1330 hours) as possible**. This ensures that the specimen spends maximal time in warmed and pressurized fixatives as possible, and helps to achieve optimal histology. For smaller specimens such as needle core biopsies, small skin punch biopsies, small GI mucosal biopsies, this is not as important, as duration of fixation is not as critical of a factor for quality histology.

4) **Large brain tissue specimens** (lobectomy, autopsy brain tissue) will never process appropriately using general surgical pathology protocols. This tissue is processed using a program that is specific for brain tissue, with much longer durations of fixation, dehydration, and infiltration. Placing these specimens in a formalin container on the fume hood in histology (to the right of the processors) is an alert to histology personnel that brain processing protocol is needed. Small brain tumor resection specimens do not need brain processing protocol, but will benefit from smaller, thinner sections and maximal processing duration (get on processor by 1330 hours).

5) **Specimens resulting in large volumes of "homogenous" tissue**: Occasionally, some procedures result in large volumes of tissue that is relatively grossly homogeneous. Examples would include endometrial biopsies from patients with hyperplasia, products of conception, some sinus content resections. In these cases, submit **up to 10 caps of tissue for initial sampling**, concentrating on areas that are grossly distinct in color, texture, etc. If the initial sampling is found to be inadequate the next day (e.g. no products of conception seen), additional sections can be submitted as needed. Submitting more than 10 caps in these cases should only rarely be indicated, and should be done so only at the guidance of a supervising pathologist (ideally the one who will be reading the case).

PART 7: FROZEN SECTION LAB CONSIDERATIONS

While operations in the frozen section laboratory follow many of the same principles of the gross room (such as maintenance of patient identification), there are obviously unique procedures, equipment, and time pressures that are not encountered in the gross room. Although a thorough review of the entirety of the LLUMC frozen section laboratory is beyond the scope of this orientation manual, the general workflow and important considerations will be covered here.

LLUMC FROZEN SECTION LABORATORY WORKFLOW: Below is the "twelve step procedure" of the routine frozen section laboratory workflow. Being familiar with this workflow helps to understand not only what the steps are, but why each step is important.

- **Step 1 – Frozen section notification:** The operating room staff will often notify gross room personnel by paging the anatomic pathology call pager.
- **Step 2 – Specimen delivery**: Surgery personnel bring the specimen to the gross room. Often, they will need to be reminded to place the specimen on the grossing counter and the requisition on the clean table.
- **Step 3 – Specimen receipt/requisition time stamp**: Write the patient name in the next available row on the reserved pathology case number list (prepared by accessioning staff, clipboard on clean table). Stamp the requisition with the IOC signature stamp (front of form if space is available, on the upper left side). Write the "time in" on the stamped area.
- **Step 4 – Initial gross examination**: Record a specimen weight and dimensions. Ink surgical margins as necessary. Open cystic structures, capturing the fluid in a container for pathologist/PA examination and determination of volume (remember 1 mL = 1 gram). For larger specimens prepped for IOC, use specimen handoff form to document specimen characteristics (copies on table in IOC lab), an important process for transmitting key details about specimen characteristics for final grossing.
- **Step 5 – Tissue selection**: This is one of the most critical steps, with great potential for loss of tissue or improper sampling. Please consult with a supervising PA or Pathologist prior to sampling**.**
- **Step 6 – Embed tissue**: Place the tissue selected (in the proper orientation – side down will be cut first) in a small amount of embedding medium on a ruler. Use this ruler and a pair of forceps to transfer the tissue to the tissue wells in the freezing temperature bar. Cover the tissue in OCT, place a labeled chuck over the tissue, and place a heat extractor on the chuck to aid in heat transfer. Allow specimen to freeze completely before attempting to remove chuck from bar.
- **Step 7 – Label slides**: While tissue is freezing, label two slides per specimen. Each (+) slide is labeled with the surgical accession number (MSXX-XXXX), determined from the reserved case list on the clean table (from step 3). The second line is the patient name (Last, First). The third line is the slide/specimen identifier, in the following format; Alphabetical specimen designation, number of chuck/block, a period, and slide number. For example, "A2.2" is the second slide from the second block from specimen "A".

- **Step 8 – Face the block:** Ensure that the blade angle is set at 24 degrees (ask for help in adjusting blade angle). Use the 40 micron trimming function on the cryostat (ask for guidance if you are not sure) to reduce the wear on the cryostat. Facing the OCT block requires trimming the block so a complete section that is representative of the volume of tissue in the block. While simple in statement, this process can be fraught with anxiety. For adequately-sized tissue fragments (i.e. not needle core or "fly speck" biopsies), the block should be faced deeply to ensure that the section is representative of the tissue. The purpose of deep facing is to reduce the number of false negative frozen sections, in which no tumor is seen on a very superficial section is seen at the time of intraoperative consultation, and the permanent sections that are faced deeper show tumor. If necessary, margin tissue can be exhausted during the IOC procedure, as the determination is most important during the procedure, not afterwards. Alternatively, on very small biopsy samplings (in particular if no additional tissue is expected – e.g. small brain biopsies), face the block very conservatively to preserve tissue.
- **Step 9 – Cut sections**: Standard procedure for IOCs performed at LLUMC are <u>two levels on two slides</u>: the superficial section goes adjacent to the label; the deeper section is furthest from the label. <u>For large samples/margins, cut and discard 10 sections between each level</u>. <u>For small "precious" specimens, do not intentionally discard sections between each level on the slide</u> (please discard sections that do not cut well or are not complete).
- **Step 10 – Staining**: Please utilize the automated stainer in the frozen section laboratory. Ensure that the water is turned on and flowing the in the water baths. Although slightly slower than hand staining, the automated stainer helps to ensure consistency in staining, and greatly assists in procedures where multiple concurrent specimens are being examined. Verifying proper reagent levels in the wells (H&E not higher than the alcohol level, and the highest concentration of alcohol is the highest level) will reduce reagent contamination (i.e. water being carried over to the coverslipping step).
- **Step 11 – Coverslip slides**: A poorly-coverslipped slide can impair examination; air bubbles introduce microscopy artifact, and globs of coverslipping medium on the back prevent alignment of the tissue with the microscopic plane of focus. Careful coverslipping can prevent these problems. With a wet slide (if dry, dip back in Clear-rite), add two drops of coverslipping medium (one over each tissue section). Slowly lower coverslip at an angle and allow medium to spread by surface tension. This prevents trapping of air bubbles under the coverslip. Using a paper towel, dry the bottom and sides of slide and deliver to appropriate tray next to microscope. Hopefully the pathologist is there and ready to read. If not, give them a call.
- **Step 12 – Transfer tissue to cap**: After pathologist has finished examination, ask them if the tissue is ready to be taken off the chuck (to make sure they don't want additional sections, etc – this may save you a headache). Properly label (accession number, block number) a cap of appropriate color. Hold chuck with frozen tissue under water (only use left sink over strainers) until OCT begins to slide off chuck. Place OCT with tissue on paper towel, trim excess with scalpel, then transfer tissue to cap in proper orientation (tissue facing up on chuck should face down in cap). <u>Do not try to melt all of OCT off over sink</u> – this may result in loss of entire remaining tissues! Always wrap small tissue fragments. Secure lid onto cap, and transfer to appropriately-labeled formalin container. Place in an empty plastic bin (to the left of the clean table) together with the specimen container (with specimen label).

CYTOLOGY/SMEAR PREPARATIONS: For some specimens, touch preps are very useful for providing a rapid assessment. In addition, the preservation of nuclear detail in these preparations is superior to frozen sections, as freeze artifact is avoided. The workflow for these preparations is simpler.

- **Step 1 – Prepare sample for touch prep/smear:** Section the lymph node, thyroid nodule, or whatever tissue of interest you are going to touch. Dab excess blood from the surface with a paper towel.
- **Step 2 – Prepare slide(s):** Write case number, name, and slide identifier ("A.1") to indicate first touch prep from specimen "A".
- **Step 3 – Ready Coplin jar with alcohol fixative**: Alcohol must be immediately available. Any delay in placing the slide in alcohol will introduce obscuring "air dry artifact". Be quick!
- **Step 4 – Touch slide to specimen**: You only get one touch. Be firm and decisive. Have forceps close by for when tissue section sticks to slide. Do not rotate or rub tissue (this distorts the cells). Transfer tissue immediately to alcohol jar.
- **Step 5 – Stain with automated H&E stainer**: No explanation needed. From this point forward, the protocol is identical to that for frozen sections (as described above).
- **Step 6 – Coverslip and deliver to tray**.

EXPOSURE RISK IN THE FROZEN SECTION LABORATORY: Dealing with unfixed fresh tissue in the frozen section laboratory poses a greater risk of exposure to personnel than the gross room workflow. As such, a greater degree of caution is required to minimize risk. Always read the requisition to see if high-risk infections are listed in the clinical history. In addition, notations may be listed on the surgery schedule if the patient is known to be infected with hepatitis C, tuberculosis, and/or HIV. A final notification method is by clinician communication. Following proper PPE protocols will minimize risk. All frozen section laboratory personnel will wear N95 masks if an airborne infectious agent is known or suspected. Prior to sectioning infectious tissue in the cryostat, please remove any uncessary supplies to the cryostat that will be contaminated and place in other "clean" cryostat. This will ensure that these supplies are available to use during the decontamination procedures required for the contaminated cryostat. Finally, cryostat/staining line contamination is to be avoided if possible. Tissue from known HIV/viral hepatitis positive patients is to be hand-stained in the back sink (on the left side of the cytology staining line), and decontamination procedures of the utilized cryostat will be performed following examination. Consult with supervising PAs when such cases are encountered.

Part 8: SPECIAL HANDLING REQUIREMENTS

LUNG WEDGE RESECTION: Always infuse wedge resection specimen with formalin using a small gauge (25G butterfly needle). Formalin "inflation" of the specimen helps to ensure adequate histologic analysis; specimens that are not inflated shows relative collapse of the airspaces, and makes assessment of the lung architecture more difficult. Attempt to minimize the number of times that you poke the specimen with the needle, as the formalin will slowly leak through the defects. Let specimen fix for several hours, then serially section and submit as usual.

STONE ANALYSIS: Dictate gross description and photograph specimen. State in dictation that "stones have been sent for chemical analysis". Send photocopy of requisition and stones to clinical lab for send-out.

TEMPORAL ARTERY BIOPSY: Do not cut specimen. Put in a brown cap and add comment in PTOE – "Temporal artery biopsy – please cut and embed demonstrating lumen". Add step sections and elastic stain.

ZEUSS FIXATIVE: Specimens that arrive in Zeuss are in that particular fixative for a very important reason. Do not transfer to formalin, or you will ruin the specimen! Zeuss fixative is required for immunofluorescence studies, and is routinely performed on renal biopsies, occasionally on skin biopsies. Always consult with a pathologist prior to removing from Zeuss.

FRESH SPECIMENS: When specimens are received fresh, it may be an oversight by the clinical staff. Or, it could be that special specimen handling is required (such as a skeletal muscle biopsy). Always read the requisition very carefully, and consult the patient's chart in EPIC prior to transferring to formalin. If in doubt, consult with PA/pathology staff.

PART 9: APPENDICES (NON-VERMIFORM)

Here are additional forms that you are either required to address (i.e. gross room training checklists, grossing capability check list) as part of your training. In addition, there are forms that are intended to assist in your experience in the LLUMC gross room.

CAP/ASCO Guidelines for Duration of Fixation for HER2 Analysis: > 6 hours, < 72 hours

LLUMC Early Run: Starts at 1330, out of formalin at 2030; late run starts at 1500, out of formalin at 2200

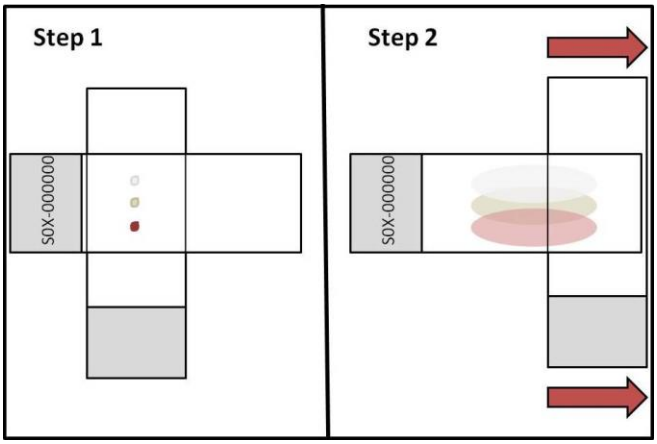
DAY 1 (PROCEDURE)	DAY 2 – 24 hours fixation (PROSECTION)	DAY 3 – 48 hours fixation (LOADING OF HELD CASES)	WEEKEND CONSIDERATIONS
ACCESSIONING Specimens are accessioned on the day of procedure; priority accessioning ensures that breast specimens can be grossed early in the morning the following day **SPECIMEN PREPARATION** Specimens are measured, differentially inked, sectioned in a manner to maintain orientation and integrity, and placed in sufficient volume of formalin to ensure thorough fixation	**PROSECTION** A priority is placed on prosection of breast mastectomy and lumpectomy specimens, including prophylactic mastectomies. The goal is to have sections of appropriate size and thickness ready when the 1330 early processor begins **SECTION VERIFICATION** Residents and PA students **must** review tissue section thickness and size with staff PA and/or pathologist prior to closing caps. Tissue that is too thick or large will impair proper processing, and will be trimmed prior to submission. **PROCESSING INITIATION** Blocks of breast tissue ready for processing will be placed in the Breast Early Run Basket (BERB) in the gross room. Gross room personnel will load this tissue when the daily early (1330) run is started.	**ACCESSIONING** Cases **grossed after 1330** (i.e. caps not ready for the early run) will be placed in the designated breast early run basket (BERB) in the gross room. These "held" cases will be transferred to the processor when the 1:00 run is started on the following day to ensure maximal fixation and processing duration on the processors. These cases will have an extra 24 hours fixation duration, and will undergo the full duration of tissue processing to ensure quality specimen processing and histology.	* Weekend processing run does not begin until Sunday at 1300, out of formalin at 2000 * Mastectomy specimens from Wednesday surgeries, if not grossed and ready for processing run on Thursday, will not come out of formalin until Sunday at 2000, and will be in formalin for **> 72 hours** * Mastectomy specimens from Thursday surgeries, grossed on Friday (+ 24 hours), will not come out of formalin until Sunday at 2000 **(> 72 hours**) * Mastectomy specimens from Friday surgeries, if not grossed until Monday, will in most cases be in formalin for **> 72 hours** * For the above scenarios in which formalin fixation duration is expected to be > 72 hours, a pilot section (if indicated) taken on the day of surgery to comply with the 6-72 hours fixation duration. Discuss these cases with supervising gross room personnel

GUIDELINES FOR GROSSING NEUROPATHOLOGY SPECIMENS

***** Remember to always place the specimen on a smooth, non-porous surface (e.g. specimen container lid), as nervous tissue is inherently "sticky", and will adhere to paper towels and gauze, never to be returned.

***** Keep specimen moistened. This is easily accomplished by placing a small amount of saline on the specimen. Nervous tissue will dry out quickly while the smear and frozen section are being prepared.

***** SMEAR PREPARATION: First, remove and inspect all fragments from the specimen container. Serially section any larger fragments. Select the tissue to be crushed. White matter is white, cortex is a light-tan, and neoplastic tissue is frequently dusky, gray to yellow, friable, or hemorrhagic. Up to three small (**< 1mm**) fragments from areas with varying gross appearance should be selected. Place specimen on labeled slide approximately 1 cm from the labeled end. With a second slide (held perpendicularly to labeled slide), apply **sufficient pressure** to crush the specimen. Move unlabeled slide across length of lower slide to smear the crushed tissue. Place labeled slide (with smeared specimen) immediately (**within 2 seconds**) in alcohol fixative. Any delay in fixation will introduce air-drying artifact. Stain with hematoxylin and eosin.



***** FROZEN SECTION: If adequate tissue is present, freeze approximately half of the tissue (unless the specimen is large). Remember to sample areas with varying gross appearance. **Note: Avoid freezing or smearing the entire specimen!!** If freezing an adequate amount of tissue requires freezing the entire specimen, wait until the pathology staff arrive to determine if the diagnosis can be made solely on the smear. This is because sections from specimens which have been frozen and subsequently formalin fixed and paraffin embedded show poor histology. One exception to the rule of not freezing the entire specimen is in the case of ACTH secreting microadenomas, in which no smear should be performed, and all tissue is frozen to look for the adenoma.

***** ROUTINE PROCESSING**:** In most cases, the entire specimen will be submitted for permanent sections. If entire submission takes > 10 blocks, consult with neuropathology staff to determine how much of the residual tissue to submit. Rare instances require submitting a small portion for electron microscopy or flow cytometry. In temporal lobe resections for epilepsy, the entire hippocampus (typically submitted separately) should be sectioned in the coronal plane and entirely submitted. If the remaining brain tissue is grossly unremarkable, submit one cassette per cm.

***** Wrap small specimens (e.g. stereotactic biopsies, spinal cord lesions) in formalin-moistened **lens paper** helps to avoid tissue loss. Larger specimens go in regular cassettes. Specimens are dictated as usual, keeping in mind that all issue is not just tan and pink.