LYMPHOMA WORKUP PROTOCOL

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GENERAL INSTRUCTIONS

When a fresh lymph node or tissue is received for lymphoma workup, the following steps must be taken.

- **Specimens should be promptly handed**! If the resident on service is overloaded or not available, selected physician assistant, hematopathology fellow, or hematopathology attending on service should be notified for assistance immediately.
- Hematopathology attending on Heme 2 is responsible for the instruction of lymphoma workup protocol at the beginning of rotation and any questions during the rotation.
- Check essential clinical history if not provided on the requisition.
- Immediately thaw the cytogenetic and flow cytometry transport media in a container with warm water (thawed transport media may be available in the refrigerator).
- Label four glass slides for touch imprints with patient name and accession number.
- Wear sterile gloves and cut the lymph node/tissue on a dental wax board using a sterile scalpel.
- Cut the lymph node/tissue with one gentle slide per section and avoid chopping or squeezing.
- Make thin sections of the lymph node or tissue, ≤ 2.0 mm or thickness of a quarter.
- Do not leave the fresh tissue outside (particularly in a venting hood) for an extended period of time. If not grossed immediately, the specimen should be placed in a sealed container and covered with saline soaked gauze. Fix the thin sections in formalin immediately if not needed for flow cytometry or cytogenetic assays.
- If metastatic carcinoma is highly suspected, a frozen section should be performed or the stained touch imprints should be reviewed with attending immediately.

TOUCH IMPRINTS

- Make four air-dried touch imprints, send two slides to cytology lab for Diff-Quick staining, and then review the stains with attending.
- To make a good touch imprint, make a fresh cut through the tissue, blot gross bloody fluid off the surface with tissue gauze, and then gently touch a glass slide to the tissue surface several times.
- For needle core biopsies, gently touch or roll over the core on the slide.
- Avoid compressing or sliding the tissue on the slide.

NEEDLE BIOPSY or SMALL BIOPSY

- Please contact attending on Heme 2 service for further instruction.
- Count and measure all cores.
- For multiple cores or biopsies with sufficient tissue
  - Touch imprints: gently touch or roll over the specimen on the slide.
  - Flow cytometry and cytogenetics: cut small pieces from each core (~2.0 mm), mix together and split into flow cytometry and cytogenetic transport media.
  - Formalin fixation/permanent: if ≤3 cores received, submit one core per each cassette; if >3 cores received, submit no more than two cores per each cassette.
- For small or insufficient specimens
  - Submit the specimens entirely in a biopsy bag for permanent.
  - No touch imprints, flow cytometry, or cytogenetics

LYMPH NODE EXCISION

- Measure lymph node, section the node gently, and examine the cut surface for necrosis, nodularity, and other focal lesions.
- For small lymph node <1.0 cm, bivalve or cut along long axis with < 2.0 mm thin sections, and submit into 2 or more cassettes.
- For large lymph node >1.0 cm, cut along the short axis with 1.0 – 2.0 mm thin sections, and submit all or good representative sections into multiple cassettes.
- Do not fill in >2/3 of each cassette.

- Make a few small cuts of the intact capsule of a large lymph node to avoid retraction and crush of the subcapsular tissue after fixation.
- Make 4 touch imprints and submit cytogenetic and flow cytometric assays as instructed.

FLOW CYTOMETRY

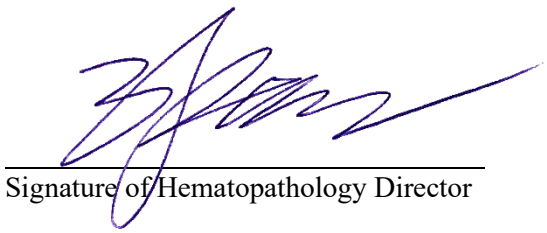
- For a large lymph node or tissue
  - Select a portion of tissue or multiple small pieces from different areas.
  - Total size ~3.0 × 3.0 × 3.0 mm, no need to submit more than 5.0 ×5.0 × 5.0 mm.
  - Gently chop the tissue into small fragments and place in thawed flow cytometry media.
- For needle core biopsies or small biopsies with sufficient tissue
  - Gently cut a small piece (~2.0 mm) from each core or small biopsy, or select the small fragments from the biopsies.
  - Mix the small pieces together and place in thawed flow cytometry media.
- For needle core biopsies or small biopsies without sufficient tissue, no need for flow cytometric analysis
- Fill in the flow cytometry requisition form. Flow cytometry panel selection:
  - For cases with no specific clinical history, select '*Low Cell Panel'*
  - In other circumstances, please contact attending on Heme 2 service
- The tissues on the same location when submitted in different containers can be mixed and submitted together.
- The tissues from different locations are submitted separately for flow cytometry assays.

CYTOGENETICS

- The tissue for cytogenetics must be handled in a sterile fashion.
- For a large lymph node or tissue
  - Select a portion of tissue or multiple small pieces from different areas.
  - Total size ~3.0 × 3.0 × 3.0 mm, no need to submit more than 5.0 ×5.0 × 5.0 mm.
  - Gently chop the tissue into small fragments and place in thawed cytogenetic transport media.
- For needle core biopsies or small biopsies with sufficient tissue
  - Gently cut a small piece (~2.0 mm) from each core or small biopsy, or select the small fragments from the biopsies.
  - Mix the small pieces together and place in thawed transport media
- For needle core biopsies or small biopsies without sufficient tissue, no need for cytogenetic analysis
- Fill in the cytogenetic requisition form and check the box of '*Chromosome analysis'*.

LYMPHOMA WORKUP GROSS DICTATION TEMPLATE

The specimen is received (**fresh vs. fixed**) in (**# containers**) for lymphoma workup, each labeled with the patient's name "#" and designated "#." The specimen consists of (**gross to include number of fragments, size, appearance, etc.**). The section surface of the lymph node reveals (**color, consistency, nodularity, necrosis, etc**). The specimen is submitted as per the Lymphoma Workup Protocol: (**XX**) touch imprints are made. Representative pieces are submitted for cytogenetic and flow cytometric analyses. The remaining specimen is submitted entirely into cassettes (##).



Signature of Hematopathology Director

02/06/2023
Date