

***Hematopathology Specimen Requirements and Handling***  
***(Bone marrow, peripheral blood, lymph nodes, tissues/fluids with possible or suspected lymphoma/leukemia)***

Concurrent microscopic morphologic evaluation is required for subclassification of lymphoid/leukemic diseases. As such, the ancillary testing discussed in this section is in addition to specimens submitted for pathology/cytology evaluations. (I.e., additional specimens should be submitted in formalin or cytology fixative, and/or blood or bone marrow smeared slides, unless otherwise directed by a pathologist. An alternative includes submission of adequate *fresh tissue* that can be split by Vista Pathology staff for microscopy evaluation and ancillary testing.)

Specimens submitted for flow cytometric analysis, fluorescent in situ hybridization (FISH), DNA-RNA molecular evaluation (eg, PCR), and/or cytogenetics require special handling, storage and transport. Specimens should be received by Vista Pathology within 24 hours of collection for best results.

- Fresh tissue is required for these tests, meaning these specimens should never be exposed to any fixative including formalin.
- Fresh tissue submissions should occur in RPMI, saline or anticoagulant, the latter only if peripheral blood or bone marrow specimen. Keep specimens at room temperature, and avoid excessive heat; cold pack if necessary to avoid excessive heat.

***RPMI tissue culture media may be obtained from Vista Pathology Laboratory for tissue/fluid submissions.***

- Specimens will not be rejected if received in less than 72 hours from collection. When tissue cellularity allows, each specimen is tested for viability prior to analysis. Depending on the specimen type, cellularity and the atypical population of interest, specimens may show degeneration changes at different times after collection. Evaluation of specimens received and processed after 48 hours of collection will be limited as leukocyte and lymphocyte subset percentages and immunophenotypic expression profiles may be affected.
  - Vista Pathology processes flow cytometry samples Monday - Friday, except major holidays.
  - On Fridays, we recommend that you call Vista Pathology for specimens requiring flow cytometry. This gives us a chance to direct a stat courier to your clinic for immediate delivery to Vista Pathology and timely analysis of specimen. If we cannot accommodate analysis in timely manner, we can discuss analysis options with you to best preserve specimen integrity.
- The table below outlines specimen requirements for flow cytometry and ancillary testing in hematopathology.

Flow Cytometry Specimen Requirements	
Peripheral Blood	Minimum 2 - 10 mL ^ in GREEN (Heparin) or LAVENDER (EDTA) tubes, see below
Bone Marrow Aspirate	1 - 2 mL^ in GREEN (Heparin) or LAVENDER (EDTA) tubes, see below
Bone Marrow Core	<p>In RPMI -or- In SALINE (minimum of 2mm<sup>3</sup>)^ , see below</p> <p>Consider submitting a separate bone marrow core in RPMI or saline if bone marrow aspirate is unobtainable. This separate bone marrow core is in addition to a bone marrow core submitted in formalin for pathology evaluation. Formalin fixed tissue is unacceptable for flow cytometric analysis.</p>
Fresh Tissue Biopsy	<p>In RPMI -or- In SALINE (minimum of 2mm<sup>3</sup> or 100 mg, 3-5 needle core biopsies)^</p> <p>Formalin fixed tissue is unacceptable for flow cytometric analysis.</p>
Fine Needle Aspirate (FNA)	In RPMI -or-In SALINE 10-100 mL preferred^ (Minimum 3 mL)^
Cerebral Spinal Fluid (CSF)	Minimum 3.0 mL^ in sterile tube (mix with equal volume RPMI)

\*FISH, DNA-RNA based PCR and cytogenetics test requests received by Vista Pathology are sent to our selected reference laboratory of choice for indicated testing. Each of these tests have similar specimen amount requirements to flow cytometry. If multiple testing modalities are required, the amount of specimen required to perform testing increases accordingly.

^Minimum volume is dependent on cellularity

***Peripheral Blood and Bone Marrow Aspirate Specimens***

Peripheral blood and bone marrow aspirate specimens should be anticoagulated and transported at room temperature as quickly as possible to the Vista Pathology Laboratory. For flow cytometric testing, EDTA or heparin are acceptable. Our testing does not require preservative-free heparin.

- For peripheral blood, in most cases, 10 mL is adequate for all analyses; however, greater volumes may be required for peripheral blood samples with very low white blood counts. If your institution routinely performs peripheral blood smears, please submit 1 (one) peripheral blood smear for concurrent review with the flow cytometric analysis.
- For bone marrow, 1-2 mL of anticoagulated marrow is sufficient. Clotted specimens may yield limited results or be rejected for analysis. Larger volumes of bone marrow

contain significant amounts of peripheral blood dilution and should be avoided. *In the absence of a suitable marrow aspirate, a marrow biopsy may be submitted fresh in tissue culture media (RPMI) or saline.*

For molecular DNA-RNA based testing (such as PCR), EDTA anticoagulation should be used as heparin can interfere with the assays; therefore, when requesting both molecular testing and flow cytometry on a shared sample, use EDTA anticoagulation. However, cytogenetic analysis (conventional karyotype) requires non-EDTA anticoagulation (e.g., sodium heparin). If specimens for DNA-RNA based molecular testing will arrive in the laboratory greater than 24 hours after collection, please refrigerate the sample to preserve the integrity of the DNA/RNA.

Specimen requirements and handling may vary depending on a number of clinical factors; therefore if additional questions arise, please contact the laboratory for more specific instructions.

#### ***Fresh Tissue Specimens***

A representative section of lymph node or other tissue should be placed in tissue culture media (RPMI) in the operating room and transported to Vista Pathology Laboratory as soon as possible. A supply of tissue culture media may be obtained from Vista Pathology Laboratory. Specimens for immunophenotyping by flow cytometry should NEVER be placed in formalin or any other fixative. If the specimen will take greater than 12 hours to reach the laboratory, it should be transported in RPMI with cold packs.

If additional questions arise, please contact the laboratory for more specific instructions.

#### ***Fluid Specimens, such as CSF, pleural fluid, peritoneal fluid, vitreous fluid, etc***

Fluids should be submitted in a sterile container and mixed with an equal volume of tissue culture media (RPMI in 1 part specimen to 1 part RPMI) as soon as possible and prior to transport to Vista Pathology. A supply of tissue culture media may be obtained from Vista Pathology Laboratory. At minimum 3 ml of fluid (and ideally, more if possible) will be needed for analysis, see table above.