

MD PATHOLOGY

***Specimen
Collection Manual
and User's Guide***

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Introduction

MD Pathology is a general surgical pathology and cytology laboratory serving Texas Health Plano, Texas Health Allen, Texas Health Flower Mound, Texas Health Frisco, Texas Health Center for Diagnostics and Surgery, Baylor Medical Center at Frisco, and physician offices located in Plano, Frisco, Allen, Carrollton, Lewisville, Flower Mound, McKinney and North Dallas. MD Pathology also assists with Laboratory Medical Directorships at autonomous medical facilities.

The goal of the MD Pathology Laboratory is to provide excellent service to our clients and patients. The rules and guidelines set forth in this manual have been formulated to help achieve this end. We ask your cooperation in abiding by them. Should any difficulties arise, please direct your problem to a pathologist, pathologists' assistant or a histotechnologist. We welcome any suggestions that will enhance our contribution to the care of your patient.

The Laboratory at MD Pathology is staffed during daytime hours, five days a week. Most routine tests are performed during the day on Monday through Friday (excluding holidays). Pathologists are available on call 24 hours per day, seven days per week.

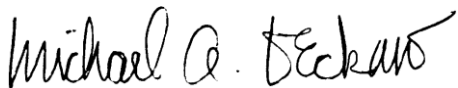
In addition to highly skilled and board certified surgical pathologists, the MD Pathology team includes pathologists with subspecialty certification and/or fellowship training in cytopathology, hematopathology, breast pathology and gastrointestinal pathology.

Although we perform much of the testing in our own laboratory, some specialized procedures are referred to outside reference laboratories and require a turnaround time that may vary from one to ten days. In special circumstances this turnaround time may be shortened if the pathologist is consulted beforehand.

The MD Pathology histology/cytology is accredited by the CAP Laboratory Accreditation Program (CAP # 48360-01), is registered with CLIA (45D0881398), and participates in a rigorous internal and external quality control program. Nevertheless, errors will rarely occur and should be brought to the immediate attention of a pathologist. If you encounter results that are inconsistent with the clinical status of the patient please advise and we will re-evaluate the specimen at no extra charge to the patient.

The laboratory welcomes and encourages you to tour our facility or review pathological material on your patients. The Laboratory is on the Texas Health Plano campus, on the ground floor (Suite G36) of Medical Office Building III on the same campus. Telephone inquiries should be directed to (972) 981-3107.

The contents of this guide reflect the general operating policies of the MD Pathology Laboratory.



Michael A. Deck, M.D.
Chief Pathologist and Laboratory Director

General Operating Guidelines

Contacting MD Pathology

The following telephone numbers may be used for reaching various staff at MD Pathology:

Reaching Pathologists; General Inquiries; Specimen Pick-up	(972) 981-3107 (If you get the voice mail system, please indicate your location and that you have a pick-up for MD Pathology).
Histology Laboratory	(972) 981-3108 (Staffed 2:00 a.m. to 5:00 p.m.)
Gross Room (Specimen Processing)	(972) 981-8065 (Staffed 9:00 a.m. to 6:00 p.m.)

Ordering Pathology Examinations

Pathology consultations are ordered using paper requisitions provided by MD Pathology. Requisitions may be ordered from MD Pathology by calling (972) 981-3107. Orders will only be accepted from physicians licensed to practice medicine.

All specimens sent to the laboratory must be accompanied by a written requisition. Requisitions must be legible and complete. Incomplete requisitions may be cause for specimen rejection. Care must be taken so that all sections of the requisition are completed including the patient identification, the billing instructions, the specimen(s) submitted (clearly identifying the site of origin and laterality if appropriate) with type of examination requested and with pertinent clinical information (e.g. the clinical impression, the duration of the problem, the differential diagnosis, "rule out" conditions, pertinent past history such as prior tumors or inflammatory disease, other biopsy or cytology findings, etc.). ***The importance of clinical information cannot be overemphasized and will have direct bearing on the quality and specificity of the pathological opinion rendered!*** It is the responsibility of the individual collecting the specimen to label the container(s) at the bedside (or immediately upon removal from surgical field) with the patient's name and second patient identifier such as date of birth or medical record number, date and time of collection, the anatomic site of origin, and initials of person collecting the specimen, or the person receiving specimen immediately from the surgical field. Double check and confirm that the name on the container and requisition are indeed the patient's. When pre-printed labels are used, it is easy to assume that the label has the correct patient name when it, in fact, does not. Identifiers may be in machine readable format, such as a barcode.

Please see that the specimen container is carefully sealed. Only non-leaking containers should be used.

For fine needle aspiration of palpable masses, we recommend that a pathologist skilled in this technique perform the aspiration. Please refer to the Pathologist Performed Fine Needle Aspirations (FNA) section below.

Specimen Processing

Specimens received in the MD Pathology laboratory by 16:30 (4:30 p.m.) will be processed that day, and the pathologist will read the slides on the following working day. Exceptions will be made when the patient would be better served by allowing large specimens to fix in formalin overnight. See the section below entitled "Report Turnaround Times" for information regarding the time to issuance of the final report.

Results that are needed within a short time frame should be brought to the attention of the pathologist, preferably by a telephone call at the time the specimen is obtained. Specimen processing and interpretation can be expedited if clinically required.

Specimens submitted for frozen section are processed immediately. Results are usually available within twenty minutes from the time the specimen is received in the laboratory. Cases requiring multiple frozen section blocks (e.g. evaluation of margins from a large skin excision) will, at times, require more time with most cases completed within 30 to 45 minutes. Paraffin ("permanent") sections are available as described below.

Packaging and Labeling the Specimen(s)

All specimens submitted to the Laboratory must be accompanied by a requisition. The specimen container(s) must be labeled with the patient's name, another unique identifier such as the medical record number or birthdate, date and time of collection, initials of person collecting the specimen, anatomic site of origin, and should be in an appropriate fixative (see below). The patient's identity should be verified at the time of collection. All specimens must be placed into a leakproof container, labeled appropriately while at the patient's bedside, and placed inside a plastic specimen bag for transport to the laboratory. For specimens with removable lids, the label should be affixed to the side of the container rather than the lid. It is not acceptable to label the transport bag and not label the specimens inside. The requisition and accompanying paperwork should be placed in the outside pocket of the specimen bag to avoid contamination. Specimens received without appropriate labeling will be rejected.

Surgical and cytology specimens may be delivered directly to the MD Pathology Laboratory in Medical Office Building III, on the Presbyterian Hospital of Plano Campus, Suite G36. Courier pick-up may be arranged by calling (972) 981-3107.

Rejection of Specimens

In the ultimate interest of patient wellbeing and quality results, the laboratory may reject specimens for the following reasons:

- 1) No laboratory requisition.
- 2) Requisition label and specimen label do not match.
- 3) Inappropriate container or preservative.
- 4) Insufficient, inappropriate or contaminated sample.
- 5) Excessive delay in transport of specimen to laboratory, compromising specimen integrity.
- 6) Additional reasons specific to the requested procedures listed in the sections below.
- 7) Unlabeled or incompletely labeled specimen. Specimens rejected due to discrepancies in the specimen labeling or ambiguities in specimen identification shall be addressed by first contacting the individual who filled out the requisition or the submitting physician, either in person or over the telephone. If the discrepancy is resolved to the satisfaction of the person performing the gross examination of the case, the discrepant information is noted on the requisition with a notation on the requisition as to the person who provided the corrected information and the pathology staff member who obtained the corrected information. The specimen shall then be accessioned as usual. If the individual who prepared the requisition or the submitting physician is not available, or if the discrepancy is not satisfactorily resolved, the specimen(s) are returned to the physician's office/facility for correction and completion of a Specimen Identity Confirmation form. Once discrepancies are resolved, the specimen(s) are returned to MD Pathology and processed accordingly.

The most common and potentially most serious problem is mislabeling of patient samples. Great care must be taken to avoid this error. The specimen container must be labeled as promptly as feasible while still at the patient's side.

Result Reporting

For most specimens, reports should be available before the close of business on the next working day following the submission of the specimen. Cases requiring special stains, decalcification, additional sections, or consultation with additional expert pathologists will require additional time (as much as a week or rarely longer). In such instance the pathologist working on the case will keep the ordering physician informed of preliminary impressions and updates as the workup progresses. Written reports will be delivered either by Courier or by U.S. Mail. Faxed reports are issued upon sign out unless the submitting physician has specifically requested otherwise. Electronic delivery of

reports (PDF) via an online report viewer is available upon request. Please contact MD Pathology for further details.

On occasion, amended reports will be issued. The amended version, which will contain the original report with the changes, will clearly be marked as such across the top of the first page. The pathologist of record will telephone amendments carrying significant clinical implications to the ordering physician.

Addendum reports will be issued when indicated, and shall contain all of the information in the original report as well as the additional findings (usually ancillary tests, results of consultations, additional study, etc.).

Surgical Pathology

Surgical pathology entails histologic examination (i.e. microscopic examination of very thin tissue slices) of organs and tissues, also known as histology or "gross and microscopic examinations". Order these examinations for tissue biopsies, organs removed at surgery, or portions thereof. For liquid specimens, body fluids, smears, scrapings, fine needle aspirations, etc., please refer to the "Cytopathology" section below.

Requisitions

All specimens submitted for surgical pathology examination must have an accompanying anatomic pathology requisition slip.

The source of the specimen (i.e. anatomic location including laterality if appropriate), the ordering physician, the day and time collected, the preoperative and postoperative diagnoses, and any special procedures desired (i.e. stain for AFB, Fungi, Pneumocystis) should be stated. In addition, the ***submitting physician should be consulted as to*** what relevant clinical information is to be included on the requisition.

If multiple specimens are obtained on the same patient, the source (i.e. anatomic site) of each specimen should be explicitly indicated on the requisition. Each requisition can accommodate up to six separate specimens.

Please specify the source of the specimen in the "specimen" section of the requisition and on the specimen container. Do not confuse diagnoses with specimen sources. Sources are anatomic sites. Diagnoses or "rule outs" are important information to provide to the laboratory but belong in the pertinent clinical information section.

<u><i>Appropriate Specimen Labeling</i></u>	<u><i>Incorrect labeling...</i></u>
Skin, right hand	Mole
Left inguinal tissue	Hernia sac
Mass, back	Lipoma
Small bowel	Rule out Whipple, Sprue

Specimen Packaging and Labeling

- 1) Each specimen must be placed in a sealed leak-proof specimen container and labeled at the bedside or promptly upon passage from the surgical field.
- 2) The label shall include the patient's name, another unique identifier such as the medical record number or birthdate, the date the specimen was collected, and the specific anatomic site of origin of the specimen.
- 3) The label should be placed on the container itself rather than the lid to insure unequivocal identification even while the lid is removed or should it become dislodged in transit. Specimens from separate sites should be placed in separate containers.

Specimen Handling/Preservation

Prompt and appropriate preservation of specimens is mandatory for optimal diagnostic yield. Detailed instructions for the various types of specimens routinely encountered follow. Do not hesitate to contact the pathologist if there are any questions.

1) *Routine Pathology:*

- a) Place in 10% neutral buffered Formalin. Be sure to choose a container that is large enough to accommodate the specimen and sufficient volume of Formalin. A 10:1 ratio of Formalin volume to specimen volume is optimal.
- b) For specimens which must be submitted fresh in one of the larger, non-prefilled containers, deliver to MD Pathology promptly or arrange urgent pick-up.
- c) Refrigerate if delays in delivery are anticipated.

2) *Frozen Sections:*

- a) Place the specimen in a closed-labeled container in the fresh state without fixative, preferably on saline-soaked gauze.
- b) For specimens composed of one or many small particles which are likely to lodge within the loose weave of the gauze (i.e. curettings, small mucosal biopsies), saline-moistened Telfa should be substituted.
- c) Notify MD Pathology immediately when the specimen becomes available.
- d) Leave the specimen unfixed. Addition of fixatives (i.e. Formalin) renders specimens unsatisfactory for frozen section.

3) *Cytology Specimens (e.g. fluid):* See "Cytopathology" Section for details.

4) *Tissues For Histopathology And Culture* (routine culture and sensitivity, AFB, fungal, viral, and anaerobic cultures):

- a) Separate specimens are required for histopathology and for culture (e.g. routine, anaerobic, acid fast/fungal).
- b) If there is a specimen that cannot be conveniently subdivided, place it in a closed sterile container and notify MD Pathology immediately upon obtaining the specimen.

5) *Lymph Nodes and Cases of Suspected Lymphoma:*

- a) Contact MD Pathology in advance.
- b) Submit the specimen fresh in a closed sterile container on sterile saline-moistened Telfa.
- c) Notify MD Pathology immediately upon availability of the specimen.
- d) See the following section on Flow Cytometry.

6) *Estrogen/Progesterone Assay, S-Phase Analysis (Ki-67), and HER2/neu Analysis:*

- a) In most cases, these studies may be obtained on routinely submitted (i.e. Formalin-fixed) specimens using immunohistochemistry and/or FISH. Submit as for routine pathology (#1 above).
- b) Please note the time the specimen was removed from the patient (cold ischemia time) and the time the specimen was placed in formalin on the requisition so that we may ensure that the specimen is formalin-fixed for the proper amount of time for optimal accuracy. The time between removal and placement in formalin should be minimized, as results may be compromised should the cold ischemia time exceed one hour.

7) *Muscle and Nerve Biopsies:* These specimens are referred to the Neuropathology Laboratory at U.T. Southwestern Medical School for definitive processing.

- a) These cases should be arranged with MD Pathology in advance.
- b) Special kits for both muscle and nerve biopsies are kept in the laboratory. These kits come with detailed instructions, which should be reviewed by the surgeon prior to the case and followed carefully.
- c) Once the specimen is obtained, MD Pathology should be notified immediately.
- d) A supplemental clinical information sheet accompanies the kit and must be completed by the surgeon or one of the attending physicians.

- 8) **Bone Marrow Biopsies and Aspirates:** Bone marrow evaluations should optimally include bone marrow aspiration (smear slides, tubes and clot), bone marrow biopsy core (and touch preps slides) and peripheral blood smear with CBC results. For your convenience, MD Pathology provides bone marrow specimen kits. Please call 972-981-3107 to be supplied with these kits as needed

Bone Marrow Specimen Collection Kit Contents:

- 1 Lavender (EDTA) tube 6 mL
- 2 Green (Sodium Heparin) tubes 6 mL
- 2 10% Neutral Buffered Formalin jars 40 mL
- 10 slides
- 2 slide transport containers
- Ice pack (please place in freezer and allow it to freeze before using)
- Biohazard transport bag

HOW TO SUBMIT A BONE MARROW SPECIMEN

1. Complete the MD Pathology Hematopathology requisition (See example requisition in Addendum B)
2. Label all specimen jars and slides with patient name, date of birth, date of service and specimen type as outlined below in bold.
- 3.

a. Bone Marrow Aspiration:

Bone marrow aspiration is used to make smears and to prepare a clot. A total of 2 pulls should be made.

Pull#1: BM Aspirate - No anticoagulant – withdraw up to 4cc into syringe

NOTE: Attempting to draw more aspirate will result in the specimen being diluted with peripheral blood.

BM Aspirate Slides

Make 4-6 aspirate smear slides – Label slides as **BM ASP**

Slides can be prepared in a similar method to the “Slides prepared at bedside” section of this manual, using a watch glass or extra slide to hold a portion of the aspirate to allow for making slides. Individual spicules (bone marrow particles) should be picked up via pipette and deposited in a drop onto the slide.

Allow slides to air dry completely before putting into slide holders.

BM Clot

Allow specimen to clot completely in syringe. Remove plunger and slide slot into 1 formalin jar. Label jar as **BM CLOT**.

Pull#2: BM Aspirate - After re-directing needle, use a heparin anticoagulated syringe and withdraw up to 6-8 cc of additional aspirate. Dispense this aspirate equally into:

1-2 Sodium Heparin tubes – Flow cytometry/Chromosome/FISH analysis

1 – EDTA tube – Molecular/FISH/Flow cytometry analysis

Recap tubes and mix by inversion 6-8 times to prevent clotting. Label these tubes as **BM ASP**

NOTE: The minimum volume per tube is 1cc, but this low volume may result in testing limitations, as will omission of one of the tube types. Optimally 2-4cc per tube (in at least 1 tube of each kind) should be obtained.

b. Bone Marrow Core Biopsy

Bone marrow core biopsy should be at least 1cm in length and should be from a different site than the aspiration was taken from. Slide the core from the needle onto a clean slide.

Touch Prep Slides:

Make at least 2 touch imprints of the core while it sits on the slide, and submit the slide the core was sitting on. Label the slides as **BM CORE TP**.
Allow slides to dry completely before putting into slide holders.

Core Biopsy

Roll the core into 1 formalin jar and label as **BM CORE BX**.

c. Peripheral Blood

Peripheral blood samples can be submitted as either of the below with a CBC report:

1. 1-2 smears labeled as **PB or CBC**. Allow smears to dry completely before putting into slide holders.
2. 1 EDTA tube labeled as **PB or CBC** (MD Path will then make smears from this tube)

- 9) **Immunofluorescence** (Immune complex studies, Goodpasture's syndrome, certain infections such as Legionella, etc.):
- a) Notify MD Pathology in advance of obtaining specimens for immunofluorescence.
 - b) Place the specimen in a closed container on a saline-moistened Telfa pad, or placed in Michele's or "Immunofluorescence Holding" solution. Notify MD Pathology immediately upon availability of the specimen.

- 10) **Electron Microscopy**: MD Pathology is not equipped with an electron microscope. Cases requiring EM will be referred to an institution with appropriate expertise on a case-by-case basis.

- a) These cases must be arranged with MD Pathology in advance, as a specific fixative (glutaraldehyde-based) is recommended and can be provided by MD Pathology if the laboratory is given proper notice.
- b) If this special fixative cannot be obtained in a timely manner, the specimen should be fixed immediately in generous quantities of 10% neutral buffered Formalin. Loss of resolution under EM may occur with Formalin fixation.

11) **Calculi**:

- a) Submit in dry container without addition of preservatives with a completed anatomic pathology requisition. A gross examination will be performed and the specimen forwarded for crystallographic analysis.
- b) If crystallographic analysis is the only service desired, please submit calculi directly to the clinical laboratory.

12) **Cases of suspected gout**:

- a) Place specimen in reagent grade alcohol (95%).
- b) If alcohol is not available, place the specimen on saline-moistened Telfa and arrange to have promptly delivered to the MD Pathology laboratory.

13) **Renal biopsies**:

- a) Needle biopsies of the kidney should be scheduled with MD Pathology in advance to ensure proper handling and prompt transportation.
- b) The tissue cores should be placed into a properly labeled specimen container on saline-moistened Telfa.
- c) Notify MD Pathology of the specimen's availability or bring the specimen and the appropriate paperwork to MD Pathology.
- d) The specimen will be sent by a stat courier (within two hours) to a local reference laboratory for evaluation and diagnosis.

14) **Testicular biopsies**:

- a) Testicular biopsies for infertility should be scheduled with MD Pathology in advance to ensure proper handling.
- b) The biopsy should be placed in a properly labeled container and 10% neutral buffered formalin should be added in a 10:1 ratio.

- c) If touch preparations are made, they should be properly labeled and air-dried.
- d) Please notify MD Pathology when the specimen is available.

Specimen Transport

Arrangements for transportation of specimens to MD Pathology will be made on an individual basis with each physician/facility.

Specimen Rejection

Specimens not submitted according to this protocol may be rejected as inappropriate. Specifically, specimens submitted without proper labeling, without a complete requisition, and without appropriate preservation will be subject to rejection. Rejected specimens will not be discarded without first contacting the operating physician. An opportunity to remedy the reason for rejection will be provided if the pathological interpretation and patient well-being is not compromised. In the event of insufficient or no fixative, fixatives will be added upon discovery by MD Pathology personnel.

Pathological Examination and Reporting

Gross examinations on specimens received Monday through Friday (excluding holidays) prior to 4:30 p.m. are performed the day the specimen is submitted. The pathologist may elect to allow overnight fixation for certain large and complex specimens prior to performing the gross examination.

Specimens received after 4:30 p.m., on weekends, or on holidays are examined the next working day. If there is a more urgent need for gross examination and tissue processing, call MD Pathology.

The vast majority of specimens are given both gross and microscopic examination. A specimen may be given a "gross examination only", but at the discretion of the pathologist. The pathologist reserves the right to perform histologic examination on all specimens submitted as "gross only" or "identification only".

Processing for histologic examination is performed the night of the gross examination (unless more prolonged fixation is required), and the histologic sections are available for the pathologist's study the following morning. For uncomplicated cases, final written reports are completed and sent the working day following gross examination. Cases requiring special stains, decalcification, additional sections, or consultation will be reported as soon as is feasible. In diagnostically challenging cases in which a delay is anticipated, the pathologist will maintain communication with the submitting physician and keep him or her abreast of the progress on the case prior to rendering the final report.

Final reports are automatically faxed and/or electronically transferred to each physician/facility on a daily basis (Monday through Friday), and formal copies are mailed to each physician via U. S. mail.

On weekends and holidays, the pathologist on call gladly performs histologic examinations upon special request. Clerical support for production of the written report, however, may not be available until the next working day. Physicians requiring interpretations over weekends or holidays should make advance arrangements with MD Pathology.

Cytopathology

Cytopathology involves deduction of the underlying disease process through the microscopic study of intact individual cells and multicellular fragments. Specimens amenable to cytologic examination include body fluids, fine needle aspirations, and touch and scrape preparations. For solid pieces of tissue or organs, please refer to the "Surgical Pathology" section above.

Requisitions

Specimens for cytologic examination should have an accompanying anatomic pathology requisition. The source of the specimen, the ordering physician, date and time collected, relevant clinical findings (especially history of prior therapy, biopsies or malignancy), and desired special procedures (if any) must be stated.

Specimen Labeling

If prepared slides are submitted, the patient's name and a second patient identifier (including patient ID number or date of birth), and the date collected must be written on the frosted end of the slide(s) in pencil (ink will be dissolved by the solvents used in processing, rendering the labeling illegible).

For liquid and semisolid specimens, each should be placed in a sealed container labeled with the patient's name and a second patient identifier (including patient ID number or date of birth), date and time collected, and site of origin of the specimen.

Specimen Collection and Handling

Specimen collection techniques play a major role in rendering an accurate cytologic diagnosis. A basic concept and goal in specimen preparation is to reduce artificial cell changes while producing an optimal diagnostic cell sample. In order to achieve this goal, certain collection techniques must be followed to ensure that an optimal cell sample is achieved. Failure to follow the collection techniques for each specimen type can result in delay in processing time, loss of cellular material, loss of cellular detail, and having to recollect a specimen, which is an unnecessary inconvenience for the patient. The collection techniques are outlined below for each specimen type.

RESPIRATORY TRACT

1) *Sputum and tracheal aspirations:*

- a) Collect specimen in a clean specimen container.
- b) Have the patient produce an early morning deep cough directly into the sputum container. The patient should be advised that oral and nasal secretions do not yield satisfactory material. If the patient cannot produce sputum spontaneously, he can usually be induced to do so by inhaling an aerosol containing an irritating or mucus-dissolving agent under the instruction and/or supervision of respiratory therapy. Submit the specimen fresh and refrigerate. **Do not add fixative.**
- c) Label the container properly and submit with the appropriate requisition.
- d) Three consecutive early morning specimens are recommended to maximize diagnostic yield when screening for malignancy.

2) *Pneumocystis carinii:*

- a) Patient must be HIV positive or immunosuppressed (i.e. transplant patient, chemotherapy, etc.).
- b) Collect a fresh sample in a sterile specimen container. Highest yield is obtained from transbronchial biopsies, but lung tap or bronchoalveolar lavage specimens are also useful. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) **NOTE:** Clearly mark on the requisition that "special stains for Pneumocystis desired". Tracheal and bronchial brush specimens, tracheal aspirates, bronchial washings and sputum are low-yield specimens and are discouraged.
- e) Deliver immediately to the Laboratory and place in the refrigerator.

3) *Bronchial washings:*

- a) Collect washings in an electrolyte solution (i.e. saline or Plasma-lyte).
- b) Submit washings as fresh, unfixed specimens. **Do not add formalin.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

4) *Bronchial brushings:*

- a) Preserve the specimen by cutting off the disposable brush and placing it in a sealable tube containing 5-10 ml of saline, and cap tightly.
- b) Submit as fresh, unfixed specimens. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

5) Sinus washings:

- a) Irrigate the sinuses with any balanced salt solution (Plasma-lyte).
- b) Collect the specimen in sterile specimen container. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form.
Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

6) Ciliary Motility:

- a) At start of case, call Pathology to notify the pathologist of impending ciliary motility assessment.
- b) **NOTE:** Cellular death begins as soon as the sample is removed from the body with progressive loss of ciliary motility. Maintaining the specimen at body temperature helps delay this inevitable ciliary motility loss.
- c) Biopsies or brushings must be taken from portions of the aerodigestive tract lined by ciliated columnar epithelium. This includes the nasopharynx, trachea and bronchi.
- d) Specimens should be placed promptly into normal saline which has been prewarmed to approximate body temperature.
- e) Attempt to maintain the specimen near body temperature during transport by warming the specimen against your skin during transport.
- f) Specimens should not be dropped off unattended, but rather handed directly to the pathologist as soon as possible after collection.

BODY CAVITY FLUIDS

The following collection procedures apply to urinary tract fluids, **pleural fluids, peritoneal fluids, pericardial fluids, synovial fluids, cerebrospinal fluids, breast cyst fluids, cul-de-sac fluids, and other cyst fluids.**

- 1) Submit as much fluid as is feasible (up to 100 ml of specimen) fresh and unfixed in a sterile specimen container. 5 ml is the minimum required. **Do not add fixative.** Volumes in excess of 100 ml may be discarded.
- 2) Label the specimen properly and submit to the Laboratory with the requisition form.
Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- 3) Deliver to the Laboratory immediately and place in the refrigerator.

NOTE:	Aspirate a suspected breast cyst by using a 23 or 25 gauge needle and a 20 ml syringe. Breast cyst fluid with any appearance other than clear and yellow (which has no significant cytologic value), should be examined cytologically, particularly when bloody. Collect fresh and unfixed in a sterile specimen container in the same fashion as body cavity fluids. Add an equal volume of saline if necessary.
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URINARY TRACT**1) Voided urine:**

- a) Hydrate the patient by having him/her drink as much water as possible for 1½-2 hours (i.e. one glass every 15 minutes).
- b) Have the patient void the urine and discard the urine at will during the period of hydration.
- c) Have the patient void the urine and discard it at the end of the hydration period.
- d) Collect the next voided urine (about ½ hour later) in a sterile specimen container. Collect the specimen fresh and unfixed. **If there is a delay in transport of specimen to MD Pathology, overnight/weekend, please add equal amount of reagent grade alcohol to specimen.**
- e) Collect 50-100 ml of urine. Volumes in excess of 100 ml may be discarded.
- f) Label the specimen properly and submit to the Laboratory with the requisition form.
Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- g) Deliver to the Laboratory immediately and place in the refrigerator.
- h) Repeat for three successive days if clinically indicated. **NOTE:** If patient cannot be hydrated, send random voided urine. Never send 24-hour urine or first morning urine.

2) Catheterized urine:

- a) Hydrate the patient with several glasses of water and exercise the patient if possible.
- b) Collect the specimen from the catheter directly into a sterile specimen container after a good urine flow is established. **NOTE:** Do not submit urine obtained at the time of initial catheterization since it has been in the bladder for some time and contains only degenerated cells.
- c) Collect 50-100 ml of fresh, unfixed specimen. Volumes in excess of 100 ml may be discarded. **If there is a delay in transport of specimen to MD Pathology, overnight/weekend, please add equal amount of reagent grade alcohol to specimen.**
- d) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- e) Deliver to the Laboratory immediately and place in the refrigerator.

3) Bladder washings, cytoscopic urine, renal pelvis washings, and ureteral washings:

- a) Have the patient void the urine.
- b) Inject electrolyte solution into the bladder and retrieve.
- c) Collect 50-100 ml of fresh, unfixed specimen in a sterile specimen container. **Do not add fixative.** Volumes in excess of 100 ml may be discarded. **If there is a delay in transport of specimen to MD Pathology, overnight/weekend, please add equal amount of reagent grade alcohol to specimen.**
- d) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- e) Deliver to the Laboratory immediately and place in the refrigerator.

4) Renal pelvis brushings and ureteral brushings:

- a) Preserve the specimen by cutting off the disposable brush and placing it in 5-10 ml of normal saline or Plasma-lyte in a sterile sealable tube and cap securely.
- b) Submit specimen fresh and unfixed. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

BREAST NIPPLE SECRETIONS

- 1) Write patient's name on a frosted-end glass microscope slide.
- 2) Gently squeeze subareolar area and nipple using thumb and forefinger. Allow only a drop the size of a pea to accumulate on the apex of the nipple if secretion occurs.
 - a) Support areola and nipple with one hand.
 - b) With the other hand, place slide upon the nipple. Touch the emerging drop of secretion onto the slide and allow it to spread slightly. Then draw the slide quickly across the nipple.
 - c) Allow the slides to air dry.
 - d) Repeat the procedure until all the secretion obtainable from the nipple is sampled.
 - e) Label the slides properly and place in a protective cardboard or plastic slide holder and submit to the Laboratory with the requisition form.
 - f) Deliver to the Laboratory immediately.

GASTROINTESTINAL TRACT**1) Esophageal washings:**

- a) Collect washings in an electrolyte solution (i.e. saline or Plasma-lyte).
- b) Collect washings as fresh and unfixed specimens. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form. Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

2) Esophageal, gastric and bile duct brushings:

- a) Preserve the brushings by cutting off the end of the disposable brush and dropping it in a culture or other clean, sterile tube containing 5-10 ml of saline.
- b) Collect as fresh and unfixed specimen. **Do not add fixative.**
- c) Label the specimen properly and submit to the Laboratory with the requisition form.
Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- d) Deliver to the Laboratory immediately and place in the refrigerator.

3) **Colon and rectal brushings:**

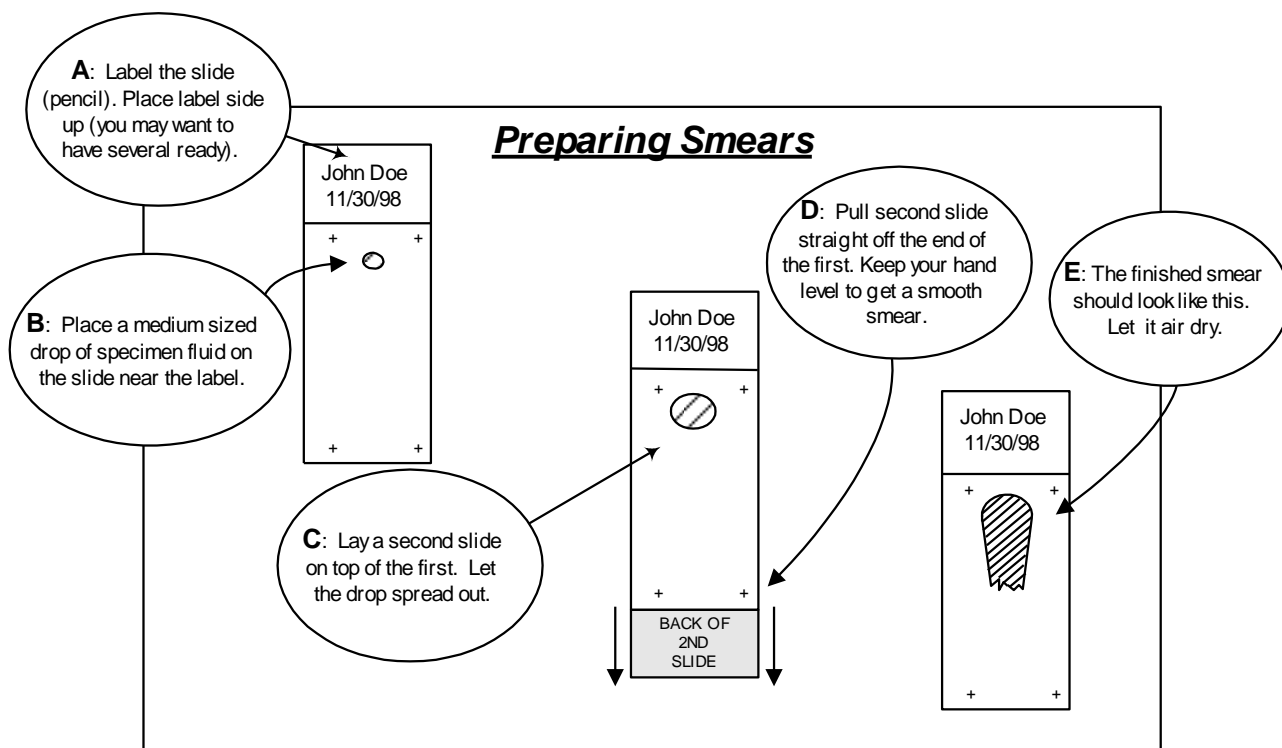
- a) Give the patient enemas (unless contraindicated) until return from the colon and rectum is clear.
- b) Preserve the brushings by cutting off the end of the disposable brush and dropping it in a culture or other clean, sterile tube containing 5-10 ml of saline.
- c) Submit as fresh and unfixed specimen. **Do not add fixative.**
- d) Label the specimen properly and submit to the Laboratory with the requisition form.
Refrigerate specimen if there is a delay in transporting it to the Laboratory.
- e) Deliver to the Laboratory immediately and place in the refrigerator.

4) **Oral cavity smears:**

- a) Write the patient's name on a frosted-end glass microscope slide.
- b) Scrape the lesion directly and firmly with the edge of a tongue blade.
- c) Smear the collected material on the glass slide evenly.
- d) Immediately fix the slides with copious amounts of spray fixative by spraying the slides from a distance of about six inches, or place the slides in a jar of 95% ethyl alcohol.
- e) Label the specimen properly and submit to the Laboratory with the requisition form.
- f) Deliver to the Laboratory immediately.

SLIDES PREPARED AT BEDSIDE (E.G. FINE NEEDLE ASPIRATES, SCRAPINGS OF SKIN LESIONS FOR TZANCK PREPARATION.)

For cytology smear preparation, refer to the illustration below.



Fine Needle Tissue/Fluid Aspirations (e.g. breast, thyroid, lymph node):

These guidelines are intended to help the clinician obtain the most optimal procurement of the specimen for cytologic interpretation. Additionally, we require that the clinician fill out a "Request for Fine Needle Aspiration" form which we will be happy to fax to the requesting clinician. This form will provide us with pertinent clinical history, the specifics of the lesion that was aspirated (size, shape, consistency), and the clinical impression of the clinician, etc., as previously discussed under the section entitled "Pathologist Performed Fine Needle Aspirations (FNA)" on Page 10 above. This clinical information, in conjunction with cytologic examination, will greatly aid the pathologist in arriving at the best diagnostic interpretation.

- a) Write the patient's name and date of birth on the frosted-end of the glass microscope slide.
- b) A drop of the specimen, no larger than 0.5 cm in diameter, is placed at the top of the slide.
- c) Immediately invert a second glass slide over the drop.
- d) As the drop spreads, pull the two slides apart horizontally in a single gentle motion.
- e) The resultant smear should be an even (homogeneous) distribution of the specimen with a feathered edge.
- f) Allow the slide to fully air dry before placing it in the protective slide carrier.
- g) Make as many air-dried smears as the specimen permits (up to six and, at the minimum, two). **NOTE:** We prefer dried (unfixed) slides in this laboratory.
- h) If aspiration fluid remains in the syringe, carefully cap the syringe, and label it with the patient's name, second identifier (e.g. date of birth), site of aspiration, date and time of collection, and your initials.
- i) Fluid aspirations should be refrigerated until transferred to our laboratory for processing.
- j) Submit the slides and any fluid aspiration specimens to the laboratory with the requisition form.

5) Tzanck preparations to assess for herpesviruses:

- a) Write patient's name and date of birth on frosted-end glass microscope slide.
- b) A fresh, clear vesicle should be selected. **Old, crusted lesions are unsatisfactory.**
- c) The vesicle should be unroofed, and the base scraped with a small scalpel.
- d) The scrapings from the blade should be immediately placed at the top of a clean, labeled slide.
- e) Immediately invert a second glass slide over the drop.
- f) As the drop spreads, pull the two slides apart horizontally in a single gentle motion.
- g) The resultant smear should be an even (homogeneous) distribution of the specimen with a feathered edge.
- h) Allow the slide to fully air dry before placing it in the protective slide carrier.
- i) Make as many air-dried smears as the specimen permits (up to six and, at the minimum, two). **NOTE:** We prefer dried (unfixed) slides in this laboratory.
- j) Submit specimen to the laboratory with the requisition form. No refrigeration is necessary.

If there are any questions about appropriate handling, do not hesitate to contact MD Pathology.

VIRAL STUDIES

Viral culture or antigen assays are the recommended studies for identifying viruses. While less sensitive and specific, cytologic examination is a useful and rapid adjunct in certain clinical situations. If cytologic examination is desired, specimens should be submitted as described in the preceding sections. A separate specimen must be submitted for viral culture or antigen testing and should be submitted directly to an alternate laboratory.

Specimen Rejection

- 1) Specimens not submitted according to this protocol may be rejected as inappropriate. Specifically, specimens submitted without proper labeling, without a complete requisition (including appropriate history), and without appropriate preservation will be subject to rejection. The laboratory will retain rejected specimens and the submitting physician will be contacted promptly. If the problem may be rectified, processing will commence upon resolving the cause for rejection.

- 2) Specimens with sparse cellular material, excess blood contamination, excessive clotting, or excessively thick smears may be unsatisfactory (or suboptimal) for examination or interpretation. In such instances, a written report stating that the specimen is unsatisfactory will be issued and sent to the submitting physician.

Specimen Processing

Liquid and semisolid specimens will be processed at the discretion of the pathologist to yield optimal results, and may include such techniques as cytocentrifuge preparations, cell blocking, direct smearing or monolayer techniques.

Pathological Examination and Reporting

- 1) Routine cytologic processing occurs Monday through Friday. Specimens received in the laboratory prior to 4:30 p.m. will be processed that evening and will be available for interpretation the following working day.
- 2) A pathologist will examine all specimens.
- 3) The same guidelines for reporting as stated for surgical pathology apply to cytology specimens.

Flow Cytometry

The MD Pathology Flow Cytometry Laboratory performs Leukemia/Lymphoma Immunophenotyping assays for clinical diagnostic testing. Clinical disorders or scenarios for which Flow Cytometry may be helpful include:

- Evaluating lymphocytosis of undetermined etiology.
- Identifying B- and T-cell lymphoproliferative disorders involving blood and bone marrow.
- Distinguishing acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML).
- Immunologic subtyping of ALL.
- Distinguishing reactive lymphocytes and lymphoid hyperplasia from malignant lymphoma.
- Phenotypic subclassification of B- and T-cell lymphoproliferative disorders.
- Recognizing monoclonal plasma cells.

Diagnostic hematopathology has become an increasingly complex subspecialty, particularly with neoplastic disorders of blood and bone marrow. While morphologic assessment of blood smears, bone marrow smears, and tissue sections remains the cornerstone of lymphoma and leukemia diagnosis and classification, immunophenotyping is a very valuable and important complementary tool.

Cellular immunophenotyping, characterizing cells by using antibodies directed against cell surface markers, is generally regarded as a fundamental element in establishing a diagnosis of tissue involvement by hematolymphoid malignancies, when used in conjunction with morphologic assessment. It is also an essential component in subclassification of hematolymphoid malignancies, when present.

This test is not appropriate for and cannot support diagnoses of sarcoidosis, hypersensitivity pneumonitis, interstitial lung diseases, or differentiating between pulmonary tuberculosis and sarcoidosis (requests for CD4/CD8 ratios). This test is not appropriate for quantitative lymphocyte subset enumerations or quantitative CD34 enumerations. Specimens sent for these purposes will be rejected.

Specimens submitted for evaluation for leukemia, lymphoma, plasma cell neoplasms, and myelodysplasia are appropriate to send for Flow Cytometry.

Requisitions

All specimens submitted for Flow Cytometry must have an accompanying requisition form, including either an MD Pathology Hematopathology requisition form for blood and bone marrow specimens, or an anatomic pathology requisition form for tissue and cytology specimens. If the specimen is a tissue or cytology specimen, special note should be made if leukemia or lymphoma is suspected, or if Flow Cytometry is specifically requested. As with other specimens, the following information should be included: patient name and date of birth, pertinent clinical history, clinical or morphologic suspicion, specimen source, i.e. Peripheral Blood, Bone Marrow Aspirate, etc, and date and time of collection.

Specimen Requirements

Flow cytometric analysis can be performed on anti-coagulated peripheral blood and bone marrow aspirates, as well as tissues or body fluids.

Submit one of the following specimens:

1) Blood

Container/Tube: Green top (Sodium Heparin) or Purple top (EDTA)

NOTE: Lithium Heparin Green Top tubes are NOT acceptable. The anti-coagulant must be Sodium Heparin. Lithium Heparin samples will be rejected.

Specimen Volume: 2-5 mL; minimum volume 0.5 mL

Collection Instructions:

- Do not transfer blood to other containers.
- Include 1 to 2 unstained EDTA blood smears, if possible. If unable to provide EDTA blood smears, then send EDTA tube to laboratory for smears to be made.
- Specimen should be labeled with patient name, date of birth, "Peripheral Blood" and date and time of collection.

Specimen Storage: Room temperature, do not refrigerate, do not allow specimen to freeze.

2) Bone Marrow

Container/Tube: Green top (Sodium Heparin) or Purple top (EDTA)

Specimen Volume: 1-5 mL; minimum volume 1 mL

Collection Instructions:

- Specimen should be labeled with patient name, date of birth, "Bone Marrow Aspirate" and date and time of collection.

Specimen Storage: Room temperature, do not refrigerate, do not allow specimen to freeze.

Additional Information:

- Label specimen as bone marrow, with the date and time of collection.
- If chromosome analysis, FISH or molecular tests are also desired, volume above the minimum for flow cytometry will be required. Samples requiring these tests should be handled in the same way as the flow cytometry samples. See section 8 of the Surgical Pathology section above for further information on specimen requirements for these tests.

3) Body Fluid (e.g. Serous Effusions)

Container/Tube: Body fluid container or body fluid transferred into Sodium Heparin (green-top) or EDTA (purple) blood collection containers.

Specimen Volume: 20 mL; minimum volume 5 mL

Collection Instructions:

- The volume of fluid necessary to phenotype the lymphocytes or blasts in serous effusions depends upon the cell count in the specimen. Usually 20 mL of pleural or peritoneal fluid is sufficient. Smaller volumes can be used if there is a high cell count. When a sample is very paucicellular, analysis may not be successful.
- Label specimen with patient name, date of birth, specimen source, date and time of collection

Specimen Storage: Refrigerate. Do not allow specimen to freeze.

4) Spinal Fluid

Container/Tube: Sterile vial

Specimen Volume: 1-5 mL; minimum volume 1 mL

Collection Instructions:

- Spinal fluid specimen should arrive within 48 hours of collection.
- The volume of fluid necessary to phenotype the lymphocytes or blasts in spinal fluid depends upon the cell count in the specimen. Usually 3-8 mL of spinal fluid is sufficient. Smaller volumes can be used if there is a high cell count. If cell count is <10 cells/uL, a larger volume of spinal fluid may be required. When cell counts drop below 5 cells/uL, the immunophenotypic analysis may not be successful.
- Label specimen with patient name, date of birth, "CSF" and date and time of collection.

Specimen Storage: Refrigerate. Do not allow specimen to freeze.

5) Tissue

Container/Tube: Sterile container with 15 mL of tissue culture medium (RPMI is preferred to maintain cell survival. Sterile Saline or Hank's Balanced Salt Solution can be used if sample can be sent immediately)

Specimen Volume: 5 mm³ or larger biopsy; minimum volume 1 mm³

Collection Instructions:

- Specimen is best when placed in RPMI solution, and received within 24 hours of surgery. If unable to be sent within that time frame, the sample should be minced and refrigerated until it can be delivered. **Do not fix in Formalin.**
- Samples should be no older than 48 hours old when received.
- If sample is unable to be placed in RPMI, then it should be delivered within 12 hours of surgery to maintain cell survival.

Additional Information:

- A pathology/diagnostic report and/or a brief history should be provided.
- If, in the opinion of the MD Pathology Hematopathologist, the tissue is not adequate for flow cytometric immunophenotyping, the specimen may be rejected.

Specimen Packaging and Labeling

Special MD Pathology collection kits are available for blood and bone marrow specimens. Please call 972-981-3107 for questions regarding the availability of such kits. Tissue and fluid specimens should be submitted **WITHOUT FIXATIVE** as specified under the Surgical Pathology and Cytopathology sections.

Specimen Rejection

Specimens not submitted according to this protocol may be rejected as inappropriate, as mentioned in prior sections. Additionally, specimens will be rejected for Flow Cytometry if the specimen is submitted in Formalin or B5 fixative, is frozen, shows gross hemolysis, or is a peripheral blood greater than 72 hours old. Mild hemolysis is acceptable. Lipemia or icterus is acceptable.

Specimen Stability Information

Blood and Bone Marrow samples should be stored at ambient temperatures. Tissues and Body Fluids should be refrigerated with RPMI or other approved solutions.

Avoid extreme temperatures (hot/cold). Specimens should be shipped with cold packs whenever possible to prevent dramatic changes of temperature for all samples, even if the recommended storage is at room temperature. Samples that are transported to MD Pathology from a location on the Texas Health Plano campus do not require cold packs. Specimens should be analyzed within 24 hours of collection, if possible.

Interpretation / Reference Values

This test will be processed as a laboratory consultation. An interpretation of the immunophenotypic findings will be provided by a hematopathologist for every case. The report will include an immunophenotypic description of cells present in the sample analyzed and an interpretive conclusion based upon the correlation available data including clinical history, morphologic features, and immunophenotypic results.

Cautions

Specimens will be initially triaged to determine which, if any, of the immunophenotyping panels should be performed.

Viability will be assessed on all tissue and fluid specimens. In addition, viability will be assessed on all blood and bone marrow aspirate specimens more than 24 hours old. Cases with viability of less than 80% are considered suboptimal. Cases in which the viability is <50% are prone to false-negative results and, therefore, must be interpreted with caution.

Cerebrospinal fluid (CSF), fine needle aspiration, and small biopsy specimens have a higher frequency of insufficient specimens due to low cell counts and/or poor viability.

For tissue specimens, it is well recognized that a negative flow cytometry result does not exclude tissue involvement by hematolymphoid malignancy. This may be attributable to sampling bias, although some malignancies, such as Hodgkin lymphoma, typically are not detected by this technique. The tissue used for flow cytometry cannot be subsequently submitted for histopathologic evaluation. For this reason, this technique should be avoided in small biopsy specimens.

Method Description

A request for leukemia immunophenotyping initiates a process in the laboratory to determine the most appropriate and cost-efficient immunophenotyping antibody panel. Panel selection is based upon clinical history and the 2006 Besthesda Guidelines to determine which lineages should be evaluated. Immunophenotyping is performed by multi-color flow cytometry using monoclonal antibodies directly conjugated with different fluorochromes. Based upon the findings in this initial panel, additional testing may be required and will be added to fully characterize disease states with a charge per unique antibody tested.

If no abnormalities are detected by the initial panel, no further flow cytometric assessment will be performed unless otherwise indicated by specific features of the clinical presentation or prior laboratory results. In some instances, additional cytogenetic or molecular genetic studies may be indicated by the immunophenotyping results. In these cases, the ordering physician will be notified verbally by the pathologist interpreting the case.

Specimens are processed and reported Monday through Friday, with a typical analytic time of 1 working day. Results are typically available 1 business day after the sample is received in the laboratory. Results from STAT samples may be available the same day that the sample is received in the testing laboratory.

Any remaining specimen is retained in the laboratory for 7 days.

Appendix A: Handling Formalin

INTRODUCTION

Formalin is a 10% solution of formaldehyde in a buffered aqueous solution. It is a hazardous substance and potent irritant. It is also a known carcinogen. Formalin must be used in a well-ventilated area. The rule of thumb is if you can smell it, the concentration in the air is too high and there is a problem. Notify a supervisor if you smell any fumes. It should be kept over a sink or with a drip pan under the spout to catch a spill. It is important to keep your face a safe distance away from the formalin when using it. Wear gloves and protective eye gear when handling or dispensing formalin.

FIRST AID IN CASE OF CONTACT

If you should come in contact with formalin, wash with soap and water immediately. If it gets in your eyes, flush with water immediately or use an eye wash.

DISPENSING AND OPENING CUBITAINERS

When opening the container, place the box on the counter with the lid towards the ceiling. Gently pull the cap off and remove the screw cap and screw on the spout, making sure the valve is in the "off" position. The "off" position on the valve is with the handle to the right side; rotating it to the left will open the flow. Place the box with the spout pointing toward the floor over the drip pan or sink where it will be stored.

If the container will be kept on the counter with the spout hanging over the counter, place a drip pan filled with either kitty litter or diatomaceous earth, or an absorbent pad directly below. Keep an approved spill kit on hand should a spill occur.


FORMALIN SPILLS

If a moderate amount of formalin should spill on the floor locate the formalin spill kit. Spread enough of the granules over the entire area and let it soak for 10 minutes. The spill kit material reacts chemically with the formaldehyde to neutralize it. If possible close the door. Make sure to let all necessary personnel know about the spill and notify a supervisor. Using a dustpan and broom, collect and place the absorbed spill kit into a biohazard bag and discard appropriately. **If the formaldehyde fumes are very strong or for large spills, close the door to contain the fumes and notify your supervisor. DO NOT ENTER THE ROOM – call Haz-mat IMMEDIATELY!**

DISPENSING FORMALIN ONTO SPECIMENS

Place the specimen in a container large enough to accommodate the specimen and add enough formalin to cover it. A 10:1 ratio of formalin volume to specimen volume is optimal. Make sure the lid is threaded on the container properly and is leak proof. Label the container in the usual way. Place the specimen container in the sealable compartment of the specimen bag and place the requisition and billing sheets in the outside pocket of the specimen bag.

Addendum B: MD Pathology Hematopathology Requisition

 <p>6124 West Parker Road, Suite G36 Plano, Texas 75093 Phone (972)981-3107 Fax(972)981-3236</p>		<h2 style="text-align: center;">Hematopathology Requisition</h2>																																																																										
<h3>Account Information</h3> <p>Ordering Physician _____</p>		<h3>Specimen Information</h3> <p>Collection Date: ____/____/____ Time: _____</p> <p>Body Site: _____ Fixative: _____</p> <p>Specimen ID # (optional): _____</p> <p>(check appropriate boxes and/or specify)</p> <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> Blood __green top__purple top__other <input type="checkbox"/> Bone Marrow Core __Bone Marrow Clot__ <input type="checkbox"/> Bone Marrow Aspirate __green top__purple top <input type="checkbox"/> Fluid __CSF_Pleural_Peritoneal_FNA <input type="checkbox"/> Fresh Tissue </div> <div> <input type="checkbox"/> Paraffin Block # _____ <input type="checkbox"/> Smears # __air dried # __fixed # __stained <input type="checkbox"/> Slides # __stained # __unstained <input type="checkbox"/> Other _____ </div> </div>																																																																										
<h3>Patient Information</h3> <p>Patient Name: _____ (Last) (First) (MI)</p> <p>Date of Birth: MM ____/DD ____/YY ____ Sex: M / F (circle)</p> <p>Medical Record #: _____</p> <p><i>*Please attach faxesheet and a copy of both front and back of insurance card, as well as Drivers License or State ID card.</i></p>		<h3>TESTING REQUESTED (Check all requested tests)</h3> <h4>Comprehensive Hematopathology Consultation</h4> <p><input type="checkbox"/> Comprehensive evaluation with Morphology, Flow, FISH, Cytogenetic and/or Molecular studies as determined by MD Pathology pathologist (Checking additional boxes below unnecessary)</p> <h4>Morphology</h4> <p><input type="checkbox"/> Morphology Evaluation</p> <h4>Flow Cytometry</h4> <p><input type="checkbox"/> Leukemia/Lymphoma Assessment <input type="checkbox"/> PNH Assessment</p> <h4>Cytogenetics</h4> <p><input type="checkbox"/> Bone Marrow or Peripheral Blood <input type="checkbox"/> If normal, reflex to FISH</p> <h4>Fluorescent In Situ Hybridization (FISH)</h4> <p><input type="checkbox"/> Pathologist to select FISH probes</p> <p>Discrete FISH tests as follows:</p> <table border="0"> <tr> <td><input type="checkbox"/> 11q-/17p-</td> <td><input type="checkbox"/> BCR/ABL1 t(9;22)</td> <td><input type="checkbox"/> PML/RARA t(15;17)</td> <td><input type="checkbox"/> STAT</td> </tr> <tr> <td><input type="checkbox"/> +12/13q-</td> <td><input type="checkbox"/> 1q+</td> <td><input type="checkbox"/> t(8;21)AML1/ETO(RUNX1)</td> <td></td> </tr> <tr> <td><input type="checkbox"/> IgH/CCND1 t(11;14)</td> <td><input type="checkbox"/> +3</td> <td><input type="checkbox"/> inv(16)</td> <td></td> </tr> <tr> <td><input type="checkbox"/> IgH/BCL-2</td> <td><input type="checkbox"/> +5</td> <td><input type="checkbox"/> MLL (11q23)</td> <td></td> </tr> <tr> <td><input type="checkbox"/> BCL-2 t(14;18)</td> <td><input type="checkbox"/> +9</td> <td><input type="checkbox"/> 5q-/5</td> <td></td> </tr> <tr> <td><input type="checkbox"/> ALK</td> <td><input type="checkbox"/> -13/13q-</td> <td><input type="checkbox"/> 7q-/7</td> <td></td> </tr> <tr> <td><input type="checkbox"/> IgH</td> <td><input type="checkbox"/> 17p- (p53)</td> <td><input type="checkbox"/> 8</td> <td></td> </tr> <tr> <td><input type="checkbox"/> MYC</td> <td><input type="checkbox"/> PDGFRa (4q12)</td> <td><input type="checkbox"/> 20q-</td> <td></td> </tr> <tr> <td><input type="checkbox"/> MYC/IgH/CEN8 t(8;14)</td> <td><input type="checkbox"/> PDGFRb (t(5;12)</td> <td><input type="checkbox"/> RB1/13q34</td> <td></td> </tr> <tr> <td><input type="checkbox"/> BCL-6</td> <td><input type="checkbox"/> API2/MALT1 t(11;18)</td> <td><input type="checkbox"/> P53/cep17</td> <td></td> </tr> <tr> <td><input type="checkbox"/> MALT1</td> <td><input type="checkbox"/> FGFR1 (8p11)</td> <td><input type="checkbox"/> CyclinD1-IgH</td> <td></td> </tr> <tr> <td><input type="checkbox"/> TEL-AML1</td> <td><input type="checkbox"/> ALK/Lymphoma (2p23)</td> <td><input type="checkbox"/> FGFR3-IgH</td> <td></td> </tr> <tr> <td></td> <td></td> <td><input type="checkbox"/> Other _____</td> <td></td> </tr> </table> <h4>Molecular Genetics</h4> <p><input type="checkbox"/> Molecular studies based on findings</p> <p>Discrete Molecular tests as follows:</p> <table border="0"> <tr> <td><input type="checkbox"/> B Cell Gene Rearrangement</td> <td><input type="checkbox"/> PML/RARA by RT-PCR</td> <td><input type="checkbox"/> IDH1</td> </tr> <tr> <td><input type="checkbox"/> T Cell Gene Rearrangement</td> <td><input type="checkbox"/> AML1/ETO(RUNX1)</td> <td><input type="checkbox"/> IDH2</td> </tr> <tr> <td><input type="checkbox"/> BCL-1 (CCND1) by PCR</td> <td><input type="checkbox"/> inv(16)</td> <td><input type="checkbox"/> DNMT3A</td> </tr> <tr> <td><input type="checkbox"/> BCL-2 by PCR</td> <td><input type="checkbox"/> FLT3</td> <td><input type="checkbox"/> WT-1</td> </tr> <tr> <td><input type="checkbox"/> BCR/ABL by RT-PCR</td> <td><input type="checkbox"/> NPM1</td> <td><input type="checkbox"/> JAK2-V617F Reflex to JAK2 Exon 12-14</td> </tr> <tr> <td><input type="checkbox"/> ABL1 (Gleevec resistance)</td> <td><input type="checkbox"/> CEBPA</td> <td><input type="checkbox"/> CALRETICULIN (CALR)</td> </tr> <tr> <td><input type="checkbox"/> ASXL 1</td> <td><input type="checkbox"/> TP53</td> <td><input type="checkbox"/> MPL</td> </tr> </table> <h4>Other</h4> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p>		<input type="checkbox"/> 11q-/17p-	<input type="checkbox"/> BCR/ABL1 t(9;22)	<input type="checkbox"/> PML/RARA t(15;17)	<input type="checkbox"/> STAT	<input type="checkbox"/> +12/13q-	<input type="checkbox"/> 1q+	<input type="checkbox"/> t(8;21)AML1/ETO(RUNX1)		<input type="checkbox"/> IgH/CCND1 t(11;14)	<input type="checkbox"/> +3	<input type="checkbox"/> inv(16)		<input type="checkbox"/> IgH/BCL-2	<input type="checkbox"/> +5	<input type="checkbox"/> MLL (11q23)		<input type="checkbox"/> BCL-2 t(14;18)	<input type="checkbox"/> +9	<input type="checkbox"/> 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AML1/ETO(RUNX1)	<input type="checkbox"/> IDH2	<input type="checkbox"/> BCL-1 (CCND1) by PCR	<input type="checkbox"/> inv(16)	<input type="checkbox"/> DNMT3A	<input type="checkbox"/> BCL-2 by PCR	<input type="checkbox"/> FLT3	<input type="checkbox"/> WT-1	<input type="checkbox"/> BCR/ABL by RT-PCR	<input type="checkbox"/> NPM1	<input type="checkbox"/> JAK2-V617F Reflex to JAK2 Exon 12-14	<input type="checkbox"/> ABL1 (Gleevec resistance)	<input type="checkbox"/> CEBPA	<input type="checkbox"/> CALRETICULIN (CALR)	<input type="checkbox"/> ASXL 1	<input type="checkbox"/> TP53	<input type="checkbox"/> MPL
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<h3>Billing Information</h3> <p>Bill to: <input type="checkbox"/> Client <input type="checkbox"/> Insurance* <input type="checkbox"/> Patient</p> <p><i>*Please attach faxesheet and a copy of both front and back of insurance card, as well as Drivers License or State ID card.</i></p> <h3>Insured Information:</h3> <p>Primary Insurance: _____</p> <p>Secondary Insurance: _____</p> <p><small>Certain tests may require Medicare patients to sign an appropriately completed ABN form prior to the specimen being obtained. Please attach to requisition if required.</small></p>																																																																												
<h3>Clinical Information</h3> <p>Diagnosis Under Consideration: _____</p> <p>_____</p> <p>Clinical History: _____</p> <p>_____</p> <p>(please attach CBC report & ALL relevant clinical history to aid in case workup)</p>																																																																												
<h3>ICD-9 Codes (REQUIRED)</h3> <p>_____</p> <p>_____</p>																																																																												
<h3>Laboratory Use Only:</h3> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p>																																																																												

Bone Marrow Collection Instructions – Card Version

Submit specimens with a MD Pathology Hematopathology requisition in a Flow/Cytogenetics/Molecular Collection Kit.

Label all specimen jars and slides with

1. Patient Name
2. Date of Birth
3. Date of Service
4. Specimen type (as outlined below in bold below.)

NOTE: Allow all slides to air dry completely before putting into slide holders.

a. Bone Marrow Aspiration:

Pull#1: No anticoagulant

BM Aspirate Slides - Make 4-6 aspirate smear slides – Label slides as **BM ASP**

BM Clot - Allow specimen to clot completely in syringe. Remove plunger and slide slot into 1 formalin jar. Label jar as **BM CLOT**.

Pull#2: Heparin Anticoagulated - Dispense this aspirate equally into:

1-2 Sodium Heparin tubes – Flow cytometry/Chromosome/FISH analysis

1 – EDTA tube – Molecular/FISH/Flow cytometry analysis

Recap tubes and mix by inversion 6-8 times to prevent clotting. Label these tubes as **BM ASP**

b. Bone Marrow Core Biopsy - Slide the core from the needle onto a clean slide.

Touch Prep Slides:

Make at least 2 touch imprints of the core, and submit the slide the core was sitting on. Label the slides as **BM CORE TP**.

Core Biopsy

Roll the core into 1 formalin jar and label as **BM CORE BX**.

c. Peripheral Blood

Peripheral blood samples can be submitted as either of the below with a CBC report:

1. 1-2 smears labeled as **PB or CBC**.
2. 1 EDTA tube labeled as **PB or CBC** (MD Path will then make smears from this tube)

MD Pathology Quick Reference

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Rocio P. Calle-Rodrigue, M.D.
David L. Ring, M.D.
Alexandra R. Nichols, M.D.
Benjamin J. Williams, M.D.
Imran Shahab, M.D.
Rachel Rucker-Schmidt, M.D.
Christopher M. Schuler, M.D.

MD Pathology is an Anatomic Pathology Laboratory providing surgical pathology and cytology services for physician offices and surgery centers in Plano, Frisco, Allen, North Dallas, Carrollton and surrounding communities. Pathologist performed fine needle aspirations of palpable masses are available by appointment. MD Pathology is fully accredited by the College of American Pathologists (CAP # 48360-01) and is registered with CLIA (45D0881398). Our goal is to provide the highest quality services in a friendly, cost effective and timely fashion.

Submitting Specimens

- 1) Following these instructions will help insure the most timely and accurate results for your patients. Routine tissue specimens should be fixed in Formalin solution as soon as collected. Cytology specimens (usually fluids) and specimens for Flow Cytometry should be submitted fresh with no fixation added. Allow cytology slides to air dry. Label each specimen container (on the side, not the lid) or slide (on the frosted end in pencil) with the patient's name, date of birth, date and time of collection, collector's initials, and anatomic site of the specimen.
- 2) Complete a requisition to include patient identifying information, age, sex, pertinent clinical information and list each specimen submitted.
- 3) Please be certain to include billing information or complete the billing section on the requisition. Please note that the only billing option available is directly to the patients or their insurance plan.
- 4) Place the specimen(s) into a biohazard bag, with the requisition in the outer compartment. Place the biohazard bag at the pick-up point.
- 5) If your site uses "on-call" pick-up services, notify the courier (see next section) that you have a specimen to be picked up.

Please refer to the "Specimen Collection Manual and User's Guide" for more detailed information.

Contacting MD Pathology

<u>Reason for Call</u>	<u>Number</u>	<u>Notes</u>
Routine Specimen Pick-Up 8:00 a.m. to 5:00 p.m.	(972) 981-3107	If you get voice mail, please indicate that a specimen is to be picked up for MD Pathology, the place of pick-up, and the latest time that someone will be available.
Fine Needle Aspiration Biopsy	(972) 981-3107	If you get voice mail, please leave a detailed message.
Frozen Sections, Fresh Specimens, Reports, Questions, or to contact a Pathologist	(972) 981-3107	If no answer, follow the instructions on the voice mail message for contacting the pathologist on-call.
Supplies	(972) 981-3107	If you get voice mail, indicate the supplies needed and the name of the account.
Histology/Cytology Lab	(972) 981-3108 (972) 981-3236 Fax	Histology/Cytology is staffed from 2:00 a.m. to 5:00 pm. After hours leave voice mail or call (972) 981-3107.
Other information	(972) 981-3107	If you have questions not covered in this manual, please contact the pathologist on call.