AmeriPath®

A Quest Diagnostics Company

Indiana

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CYTOLOGY AND HISTOLOGY SPECIMEN COLLECTION MANUAL

AmeriPath Indiana
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PREFACE

The purpose of this manual is:

1. To provide instruction on the collection and submission of cytology and histology specimens.
2. To help ensure that the optimal specimen and information is obtained for accurate and timely diagnosis.
3. To save patients the unnecessary costs of repeated tests.

Detailed information is provided regarding specimen requirements, specimen collection and labeling, and data management. If you cannot find the answer to your questions in the manual, please call the laboratory.

Tests desired by clients that are not performed at AMERIPATH INDIANA are referred to qualified, licensed reference laboratories chosen for the quality of service provided including, but not limited to, accuracy, reliability, turnaround time, and range of services offered.

AMERIPATH INDIANA strives to maintain a reputation for quality, innovation, and dependability and we welcome comments and suggestions to further that effort.

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PHONE NUMBERS

After Hours call the Medical Society Exchange to have a pathologist paged: .....(317) 631-3466

Billing Customer Services ..... (317) 275-8112 or (866) 635-1917
Billing Questions

Client Services (7:30 am – 6:30 pm) ..... (317) 275-8111 or (866) 272-8799
(Report copies; supply requests; slide requests)

Courier Pick-Up (8:00 am – 5:00 pm)……..….. (317) 275-8111

Collection Information.....(317) 275-8111
Cytology (liquid specimens or slides)

Histology (tissue) Collection Information .....(317) 275-8111

Main Number: .....(317) 275-8000

Main Fax Number .....(317) 275-8018

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LABORATORY ACCREDITATION

College of American Pathologists; Laboratory # 7180519

U.S. Department of Health and Human Services; CLIA #15D1002565

Test Methodologies

Upon request, the laboratory will provide clients a list of current test methods, including performance specifications.

Results and Reports

Results of cytology and histology evaluations are entered into the AMERIPATH INDIANA computer system as the tests are completed. The reports are generated from the computer system and are sent through the mail, can be printed in the client's office, faxed or delivered by our courier.

The Client Services Department has been designed in order to give physicians, nurses, and office personnel, as well as hospital-based pathologists and laboratory personnel, a central location to call for report results and/or status.

Our Client Services Department is available to access specific reports and facilitate communications between our physician client and the pathologist.

Special Tests

Special tests are also available, and include:

- Cytogenetic Studies
- Flow Cytometry
- Fine Needle Aspiration biopsy and interpretation
- Immunohistochemistry (including Estrogen/Progesterone Receptor and Her2Neu studies)
- Molecular Pathology (including FISH and other molecular based tests)

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- Immunofluorescence
- Electron Microscopy
- Stone Analysis

Distribution of Manual

This manual is distributed to all physician offices and surgical centers utilizing AMERIPATH INDIANA services.

COMPLIANCE WITH FEDERAL AND REGULATORY AGENCIES

AMERIPATH INDIANA is committed to maintaining a culture which promotes prevention, detection, and resolution of instances of conduct that do not conform to:

- Federal and state laws
- Regulatory agency requirements
- Federal, state, and private payer health care program requirements
- AmeriPath Inc.'s ethical and business policies

As part of this commitment, physicians are notified of the following requirements. AMERIPATH INDIANA will update physicians as to changes in the medical necessity and billing compliance requirements relating to cytology and histology in order to protect both the physician and the laboratory.

Test Ordering and Specimen Submission

Laboratory tests may be ordered by physicians or other authorized personnel, such as physician assistants and nurse practitioners. All orders must be accompanied by a written requisition, including "add-on" tests. All specimens, including slides, must be appropriately labeled.

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Medical Necessity and ABNS

Laboratories can submit claims to federally funded health programs only for services considered medically necessary. An ICD code should be submitted with all orders substantiating the medical necessity of the testing. A notable exception to the medical necessity rule is that screening Pap tests are allowed once every three years. For Pap tests performed more often than every three years, or other tests which do not meet the federally defined criteria for medical necessity, an ABN must be completed. The ABN gives the patient advance notice that Medicare will not pay for the procedure.

OVERVIEW OF HISTOLOGY/CYTOLOGY SPECIMENS

General Information:

Once removed from the patient, specimens must be immediately preserved to avoid deterioration. See the following list for guidance in determining which section of this manual to refer to for the appropriate instructions.

CYTOLOGY	HISTOLOGY
Body Cavity Fluids	Breast Biopsy
Urine	Cervical/ECC Biopsies
Sputum	Endometrial Biopsies
CSF (Spinal Fluid)	Gastrointestinal Biopsies
Organ or cavity washings	Prostate Biopsies
Pap tests	Vas Deferens
Fine Needle Aspiration (FNA)	Skin Biopsies
Brushing Specimens	Other Tissue Specimens

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Submitting Specimens

A. LABELING: To avoid delays in processing and specimen rejection, please label accurately and completely. Specimens not properly identified will not be analyzed without further follow-up.

Always verify the identification of the patient by asking their name. Each specimen container (*not the lid*) **must** be labeled with the **specimen source** and at least **two unique patient identifiers.**

- the patient's first and last name
- specimen source/tissue type (laterality when applicable)
- a second unique patient identifier, such as:
  - date of birth
  - hospital / medical registration number
  - social security number
  - requisition number
  - accession number
  - other unique random number

  Note: Unique identifiers must also appear on the requisition for verification purposes. A location, such as a facility or room number, is not an acceptable identifier.

Multiple specimens should be placed in **separate** containers with each specimen source clearly identified. All slides that are submitted MUST be labeled with the patient's full name and site of sample.

B. REQUISITIONS: An AMERIPATH INDIANA requisition form must accompany each specimen submitted. We know that proper and timely billing of your patients is one of your concerns. In order for us to accomplish this task, we must have accurate patient information from you, which must be legibly written on the requisition accompanying the specimen. Please include the following:

- Complete patient name as it appears on the primary insurance card
- Complete address of the patient
- Gender of patient
- Patient's date of birth and social security number (SS# not required but is very useful for patient identification)
- Guarantor's name and date of birth if other than the patient

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- Complete insurance information. Attach a copy of the patient's insurance card(s), front and back, to the requisition.
- Appropriate ICD Code
- Patient's clinical history (This is a regulatory requirement because of the importance in aiding accurate diagnosis.)
- Requesting physician's first name, last name, and submitting facility (Circle appropriate choice if multiple physician names appear on the requisition.)
- Specimen type and source (mark checkboxes as appropriate). For specimen types or sources not preprinted on the requisition, write the information needed (ex. Endocervical brushing)
- Specimen collection date
    - For breast tissue specimens, include the following additional information:
        - Time specimen is removed from body (cold ischemic time)
        - Time specimen is placed in fixative
- The procedures or special test(s) desired

If the above information is not complete and legible, processing of the specimen may be delayed while we contact your office to obtain the required information.

The second copy of the requisition is for your office use and may be disposed of if not needed by your office.

Specimen Packaging

It is our policy at AMERIPATH INDIANA to meet all regulatory agency guidelines and to provide the best patient care possible. To do so, we must enforce the following procedures:

- All **requisitions** must have the patient's name, gender, tissue type, and all available pertinent clinical history.
- All **specimen containers** must be labeled with the patient's name, a second unique identifier, and specimen source / tissue type.
- All **slides, smears, and frozen section specimens** must be received with the patient's full name and specimen site, if more than one specimen is submitted on a single patient.
- Use only approved well-constructed containers and packaging materials.
- Secure container lids tightly to prevent leakage and seal the container into a biohazard bag. Fold the requisition and place the requisition in the outer pocket of the bag.

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The above procedures must be followed, otherwise the requisition and the specimen will be returned to you for appropriate identification via courier the following day, with a letter explaining the reason for rejection of the specimen, or otherwise held until full information / verification is obtained.

Additionally, specimens will be rejected when slides are broken beyond repair, a specimen is received in a syringe with an intact needle, or the names on the specimen containers, specimen slides and requisitions do not match.

Unacceptable Specimen Examples

- No or illegible patient identification on the specimen container, or the test requisition
- Mismatch between name of patient on specimen and name on test requisition
- Crushed slides
- No source indicated on test requisition for Non-Gyn specimens
- Expired liquid-based preservative/vial
- Non-Gyn specimens collected in a SurePath vial
- Specimens received in a syringe with needle attached
- Cerebrospinal Fluid (CSF) from a patient with suspected Creutzfeldt-Jakob Disease (CJD) Note*: Specimen is sent to a referral Prion laboratory*

Verbal Orders

Any additional testing which is verbally requested must be followed up with a written requisition within 2 working days in order to comply with regulatory requirements.

Courier Services

When specimens are ready to be picked up, place in the designated area for courier pick-up. If your office is not on a regular pick-up schedule, call AMERIPATH INDIANA at (317) 275-8111 to request a courier pick-up. Also, call if you have a specimen that requires immediate pick-up in order to maintain specimen integrity (such as fresh tissue). If you have prior knowledge of such needs, it is preferable to give AMERIPATH INDIANA 24-hour notice of such special needs.

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Ordering Supplies

All clients will be supplied with specimen containers, fixative (10% formalin), slides, cytology fixatives, media for collecting flow cytometry specimens, bags for submitting specimen containers, and requisition forms. We have a wide selection of supplies to fit each specimen requirement.

- Fill out the AMERIPATH INDIANA Supply Requisition form completely, including the clinic name, address, phone number, date of order, contact person and client account number.
    - If you are unsure what your account number is, please email dgxampindysupportsolutions@questdiagnostics.com or call 317-275-8111 to obtain your client account number.
- Indicate the amount of each supply you will need.
- Please fax completed form to 913-951-8221 or email to greatmidwestclientsupply@questdiagnostics.com

NOTE: Please allow up to 5 business days for your supplies to arrive from the time your request is received. If you require assistance, please call 317-275-8111 and speak with one of our Client Services Representatives.

SPECIMENS FOR CYTOLOGY EXAMINATION

Submitting Specimens to Laboratory:

All cytology specimens should be submitted to the Laboratory as soon as possible after collection. Delay results in deterioration of unfixed specimens. If delay (more than 1 hour) is unavoidable, please refrigerate unfixed specimens until they are picked up.

All Cytologic examinations are submitted on AMERIPATH INDIANA Requisition Forms.

Fluid cytology specimens are processed with the Thin Prep Technique. All fluid cytology specimens should be submitted fresh or with Cytolyt™ (*site'-o-lite*) preservative. Laboratory personnel will add preservative to fresh specimens after they are delivered to the laboratory.

DO NOT ADD CARBOWAX FIXATIVE (SACCAMONO FLUID) TO A CYTOLOGY SPECIMEN.

NEVER PLACE FORMALIN ON A CYTOLOGY SPECIMEN.

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If questions arise as to how a cytology specimen should be handled and you can't find the answer in this manual, call the Lab at (317) 275-8111.

Body Cavity Fluids (Pleural, Peritoneal and Pericardial)

- Equipment needed: clean containe, AMERIPATH INDIANA Requisition
- Perform tap.
- Place sample in a clean container (such as a sterile urine container).
- 50 ml is a sufficient sample for cytologic examination; other tests will require additional fluid.

Have specimen delivered immediately to the laboratory. If there is a delay, it should be refrigerated or a portion of CYTOLYT™ solution (30 ml) should be added to the specimen. Please note addition of fixative.

- Any body cavity fluid for flow cytometry needs to be in TTM (Tissue Transport Media)

The laboratory does not accept syringes with attached needles. Flush the barrel and needle into the specimen container, using the proper preservative, and dispose of the needle into a sharp's container.

Urine

A. A voided urine specimen should be obtained after the patient has been well- hydrated, and approximately three hours after the last void.

B. Do NOT submit the first morning void for cytology.

C. Clean catch samples are necessary. Female patients should be instructed to spread the labia during collection, then pass and discard a small amount of urine. Collect the remainder. Males should be instructed to pass and discard a small amount of urine. Collect the remainder.

D. Do not submit 24-hour collection for cytology. These are unsatisfactory because of cellular degeneration.

E. If the patient is unable to cooperate satisfactorily, a catheterized specimen should be obtained. If a specimen is obtained with catheterization, it must be clearly indicated on the requisition. Catheterization affects cellular morphology.

F. Have specimen delivered to the laboratory immediately. If a delay is anticipated, the specimen should be refrigerated or 30 ml of Cytolyt should be added.

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Sputum

A. Sputum specimens are of limited use in the morphologic identification of disease due to degeneration and frequent limited specimens.

B. Collect the sample in a wide mouth plastic container. The preferred specimen is that produced by a deep cough as soon as the patient awakens in the morning. The patient should be asked to rinse his mouth with water before coughing. If sputum production is scanty, several coughs over a period of 2 to 3 hours may be necessary to produce a sufficient sample.

C. Have specimen delivered to the laboratory as soon as collection is completed. If more than 2-3 hours delay is anticipated before processing, add 30 ml of Cytolyt and mix well. The specimen should be labeled with the patient's first and last name, another unique identifier and the specimen source. If three consecutive specimens are to be collected, all three specimens can be delivered on the third day of collection, clearly stating the collection date and time of each. A completed AMERIPATH INDIANA requisition form should accompany EACH specimen.

Brushings

Brushing can be performed on any surface lesion. Common sites for brushing cytology include bronchi, gastrointestinal sites, bile duct, anal canal, and pancreatic duct. Brushing specimens may be submitted as direct smears or the brush tip may be submitted in sterile saline or in Cytolyt™ media. NEVER SUBMIT A DRY BRUSH.

For direct smears:

1. Label the slides with the patient's full name and site of brushing using pencil.
2. Obtain brushing.
3. Smear brush in a rotary motion onto slide(s) and plunge immediately into a Coplin jar or other wide mouth receptacle filled with 95% alcohol.
4. Transfer the slides to an alcohol filled transport tube labeled with the patient's name, another unique identifier and site of brushing.

For brush tip submission:

1. Label a container with the patient's name, second unique identifier and site of brushing.
2. Fill container with 20 ml of Cytolyt™, sterile isotonic saline or transport media.
3. Plunge brush into fixative or saline.

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4. Vigorously shake the brush to dislodge cellular material into the liquid.
5. Clip the brush from the handle, retaining the head in the fluid media.
6. Submit the specimen along with a completed requisition, indicating clinical history, site of brushing and clinical impression as well as the necessary demographic information.

For skin "brushing":

1. Often time's skin vesicles are scraped or brushed to look for viropathic effects (Tzanck smears) or fungal organisms.
2. Direct smears can be made, or the brush tip may be submitted.
3. Break the surface of the vesicle (if present).
4. Scrape the bed of the vesicle or surface of the skin lesion with an endocervical style brush or a spatula. Do not use a cotton applicator as material will "stick" to the cotton.
5. Proceed as for other brushing.
6. When completing the requisition, make sure that the suspected organism is indicated in clinical findings.

Cerebrospinal Fluid

The specimen for Cytologic examination should be collected in a separate container, clearly labeled with the patient's full name, second unique identifier and specimen type. **As large a volume as possible should be submitted**. Have the specimen delivered to the laboratory without delay.

NEVER ADD CYTOLYT OR ANY FIXATIVE TO THIS SPECIMEN. Clinical history is essential in the interpretation of cerebrospinal fluid specimens and should be clearly stated on the accompanying requisition.

Nipple Discharge Specimens

NIPPLE SECRETIONS:

Equipment: Glass slides (available from the laboratory) OR Vial containing preservative CytoLyt.

Express material from nipple by gentle compression of the areolar area between thumb and finger. The initial secretions should be discarded as it contains degenerated cells. After discarding the first several drops, material is expressed directly onto the glass slide and gently spread over its surface.

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Cellularity tends to increase with each expression and 4-5 slides should be made. The slide is IMMEDIATELY dropped in 95% alcohol. Spray fixation is acceptable, but less desirable. DO NOT ALLOW TO AIR DRY. If using CytoLyt, express into vial.

- If secretion appears from a specific duct orifice or when pressure is applied to a particular area of the breast, it is advisable to note this on the request form and prepare a specifically identified slide from this material. This may be helpful in guiding a future biopsy.

Breast Cysts

Cytologic examination of most fine needle aspirates of breast CYSTS is often unnecessary. If the mass is clinically and radiologically cystic, most fluid can be discarded. Submit the breast cyst fluid if:

- The cyst is recurrent
- The fluid is bloody or brown
- The patient is postmenopausal and not on hormone replacement therapy

Note on the requisition how much fluid was extracted. If a delay in transport is anticipated (24 hours or greater), add an equal volume of Cytolyt and indicate on the requisition that Cytolyt has been added. If flow cytometry is requested, a portion of the sample should be collected in TTM.

Breast seroma fluids should be collected in the same manner as described above.

Anorectal Cytology

Standard colonic preparation is not required, but the rectum should be emptied prior to sample collection. Lubricants should not be used since it affects processing and interpretation of the sample.

1. Insert cytobrush 5-6 cm (2 inches) into the anal canal, past the internal sphincter, into the rectal vault.
2. Apply firm lateral pressure to the brush handle.
3. Withdraw in a firm downward spiral movement 10-12 rotations to ensure the device has made contact with the full surface area of the transformation zone.
4. Place the brush in ThinPrep preservative vial and agitate vigorously several times.
5. The sample must be fixed within 15 seconds to avoid drying artifact

NOTE: A swab or smear of the peri-anal skin is an unsatisfactory specimen site

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Cervical/Cytology (Pap test)

A. EQUIPMENT:

- Cervical spatula and endocervical brush or cervical "broom"
- Thin Prep vial

B. PROCEDURE:

1. Label the Thin Prep vial with the patient's name, date of birth and social security number (optional).
2. Open the vial.
3. Insert vaginal speculum WITHOUT ANY LUBRICANT. Two areas of cervix are to be sampled: the portio and the endocervical canal. If the patient has had a hysterectomy, the vaginal cuff should be sampled.
4. Visually inspect the cervix. Using the spatula, insert long arm into the external os and rotate in one direction at least one complete 360° turn. Immediately submerge into the vial, vigorously stirring in an attempt to remove the cellular material from the spatula into the liquid media.
5. Insert the cervix brush into the endocervical canal, rotate 180° and withdraw. IMMEDIATELY submerge brush into the vial, stirring the brush head against the side of the vial to dislodge the brush contents into the liquid media.
6. The cervical "broom" samples the ectocervix and endocervix at the same time. The device is inserted with the longer central portion into the Endocervical canal, rotated clockwise at least 360° and withdrawn. Immediately place the broom head into the liquid media, stirring and splaying the bristles in order to dislodge the cellular material.
7. Complete the AMERIPATH INDIANA requisition form as instructed previously, along with date of last menstrual period (LMP), and previous history (abnormal or not). It is important to correctly note the specimen source (vaginal vs. cervical/endocervical) and "hysterectomy" where appropriate.

NOTE:

- The assurance of good sampling from both the ectocervix and endocervix including the transformation zone is essential.
- If a suspicious area is visualized, a separate smear from that area may be obtained and submitted separately.

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Fine Needle Aspiration (FNA) Biopsy

Clinicians should have sufficient experience in order to perform FNA's in their offices. Lack of experience often results in inadequate or insufficient specimens.

Cells are removed from a mass using a fine needle, smeared on slides, stained and interpreted microscopically for a diagnosis. A "fine" or thin needle is defined as 22 or higher gauge. FNAB is a minimally invasive, cost-effective technique with high diagnostic accuracy.

The accuracy of FNAB depends upon the site and type of lesion aspirated the aspirator's experience, the quality of specimen preparation, and the pathologist's diagnostic skills. Fine needle aspiration biopsy may be performed on palpable (superficial) lesions or with radiological, ultrasound, endoscopic or bronchoscopic guidance (deep lesions).

SPECIMEN: Fine needle aspirates may be performed on any body site that can be reached with a fine needle.

REAGENTS:

A. Xylocaine or Lidocaine (1%) anesthesia without epinephrine (optional)

B. 95% ethanol

C. Tissue Transport Media or other tissue culture media (required for flow cytometry)

D. Cytolyt (optional)

- Use for CSF and body fluids

EQUIPMENT:

A. Glass slides with frosted end

B. Container filled with 95% alcohol (Surgipath Red Topped Slide Transport Containers)

- Slide transport tubes filled with 95% ethanol alcohol for transport of ethanol fixed slides

C. 22 to 25 gauge 1 1/2-inch needles (as preferred by the aspirator)

D. 10 ml syringes

E. Alcohol preparation pads

F. TB syringe for administering local anesthesia (optional)

G. Sterile gauze squares

H. Bandage

I. Pencil

J. Requisition

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K. Informed consent form

L. Slide transport containers for dried slides

PROCEDURE:

A. Obtain informed consent from the patient.

1. Complications include bruising and oozing from the biopsy site.

2. Surgical biopsy is the most common alternative to FNAB.

3. Site-specific complications for deep needle aspiration biopsy should be described to the patient if image guided FNAB is performed.

4. Informed consent must be obtained from the patient, guardian or legal representative.

5. Written consent should be obtained if the institution requires it where the procedure is performed.

B. Taking a "Time Out" – an effort to reliably identify the individual for whom the procedure is intended and to match the requested service to that patient.

1. Two unique patient identifiers are obtained relevant to the patient; acceptable identifiers would include:

a. the patient's name,

b. a medical record number,

c. telephone number,

d. photo ID,

e. date of birth,

f. or other person specific identifier.

2. Next, one must confirm the correct procedure, anatomic site, and availability of appropriate documentation.

Note: Marking of the anatomic site is required UNLESS the practitioner (often the pathologist) is in continuous contact with the identified patient from time of consent to initiation of the procedure and as long as the patient is an active conscious participant in the process.

3. The "time out" must occur in the location where the procedure is done.

4. The "time out" should include the entire technical team.

5. There should be an active affirmation either orally or by gesture by all participants (pathologist, assistants, and patient) that the patient, procedure and site are correct.

6. The "time out" procedure must be documented.

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C. Label 4 to 6 pairs of slides, using pencil on the frosted end, with the patient's name, and the number of the pass. Label any ancillary containers if used (i.e., TTM, Cytolyt).

D. The operator should be prepared to obtain material for ancillary tests, such as cell block preparations, molecular studies, flow cytometric studies or microbiologic studies if indicated.

E. The operator should wear examination gloves (standard precautions.)

F. Clean the skin over the mass with alcohol.

G. 1% Xylocaine anesthesia may be used to infiltrate the skin, being careful not to obscure the mass with too much anesthesia.

H. Two methods of aspiration may be used:

1. The "no aspiration" technique

a. A 25-gauge needle is placed into the mass.

b. Being careful not to occlude the needle hub, the mass is punctured.

c. Staying within the lesion, the needle is moved vigorously in a cutting motion withdrawing cells into the needle hub.

d. Remove the needle from the skin.

2. The aspiration technique

a. A 10-cc syringe is attached to the needle.

b. 1-2 cc of air are introduced into the syringe.

c. The needle is introduced into the lesion.

d. Using a vigorous cutting motion, simultaneously introducing negative pressure into the syringe by pulling up the plunger, cells are drawn into the hub of the needle. DO NOT DRAW CELLS INTO THE SYRINGE.

e. The plunger is released **before** the needle is withdrawn from the skin.

I. Prepare a paired smear

1. Express one drop of material on a labeled slide using a syringe.

2. Invert a second labeled slide on top of the drop.

3. Gently pull the two slides across each other, making a thin smear.

4. IMEDIATELY immerse one of the smears in the ethanol filled container jar.

5. Keep one smear drying in the air.

J. Rinse the needle by drawing tissue transport media solution (TTM) into the syringe and expressing the fluid back into the container until most of the hub is clear of material. Alternately this step can be done using CytoLyt solution (although no immunophenotyping can be performed on the CytoLyt specimen).

K. Repeat the aspiration 4 to 6 times as indicated by the amount of material expressed onto the slides.

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L. Ancillary tests (cultures, cytogenetics) can be obtained as indicated ~ additional material (or the needle rinses) may be designated for ancillary testing.

M. Apply local pressure between aspirations with a sterile gauze square.

N. After completing the series of punctures, apply a bandage to prevent any drops of blood from staining clothing.

O. Package the air-dried smears in a slide transport container.

P. Place the ethanol fixed slides in a slide transport tube that is filled with ethanol.

Q. Place the slides and the Needle rinse solution into a biohazard bag for transportation.

R. Fill out the requisition entirely, including, name, date of birth, ordering physician, site of specimen, clinical impression, number of slides, pertinent radiographic findings, and clinical history.

S. Transport to the laboratory.

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SPECIMENS FOR HISTOLOGY (TISSUE) EXAMINATION

Tissue specimens submitted to Pathology must meet certain requirements before they will be accepted for examination. If you have questions about the collection of histology specimens that are not answered in this manual, please call (317) 275-8111.

Specimen Containers

All specimens shall be placed in containers with lids tightly secured to prevent leaking. **Each specimen container (not the lid) must be labeled with the patient' first and last name, second unique identifier and specimen source.** Multiple specimens should be placed in separate containers with each source clearly identified (in addition to the patient's name and second unique identifier). ***See Submitting Specimens, Section A Labeling for specific labeling instructions.***

Most tissue specimens are submitted in formalin, which contains formaldehyde. Use caution when handling, as formaldehyde is toxic if inhaled or swallowed, and can be irritating to the eyes, respiratory system, and skin. There is a risk of serious damage to eyes. Repeated or prolonged exposure may increase the risk of cancer.

A formalin warning label must be attached to the container. NOTE: Most containers are pre-labeled by the factory before use. If a pre-labeled container is not used, please identify the solution containing the specimen on the specimen container (i.e., formalin, glutaraldehyde, saline, etc.).

Routine Specimen Requisitions

An AMERIPATH INDIANA Requisition Form must accompany the specimens. The requisition must be completed as outlined in ***Submitting Specimens, Section B - Requisitions***, including the following information:

- Date collected
  - If the specimen is **breast tissue**, also note the time tissue is removed from patient and the time specimen is placed in formalin (cold ischemic time).
- To the degree known, any preoperative and postoperative diagnoses.
- Pertinent clinical information. Lack of clinical information may delay or cause errors in diagnoses.
- The specimen source listed numerically if multiple specimens are submitted for a single patient.

Routine Tissue Fixation and Transport to the Lab

Place routine tissue specimens in a properly labeled container that contains 10% formalin solution. The size of the container should be proportionate to the size of the specimen so that the formalin: tissue ratio is approximately 20:1.

Place the specimen in a biohazard bag and place the requisition in the side pouch of the bag (if available).

Notify the laboratory of the need for a pick-up if your clinic is not on a regular pick-up schedule.

Intraoperative Tissue Consultations – FROZEN SECTIONS

To notify the laboratory of an intraoperative tissue consult call (317) 275-8111 and notify the lab when services are needed.

Label the specimen containers as described above.

Complete the AMERIPATH INDIANA requisition as previously instructed, including the following information:

- Clinical history/preoperative diagnosis
- Tissue removed; listed in numerical order if multiple specimens
- Any special instructions/information

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BONE MARROW

SEE ALSO: BONE MARROW ASPIRATION AND BIOPSY FOR CLINICIANS' MANUAL.

Dilution with peripheral blood, the most common problem with bone marrow aspirate smears, occurs when too much bone marrow is aspirated into the syringe. It is important, therefore, not to aspirate more than 1.0 ml of bone marrow into the first syringe, which is to be used for bone marrow smears and clot section. If additional bone marrow is required for special studies, such as flow cytometry, cytogenetic, and culture, then employ a second syringe to obtain the larger amounts of bone marrow.

Bone marrow smears can be obtained without the use of any anticoagulant, and it is usually not difficult to make them before clotting begins if you prepare the smears right at the bedside and work rapidly. Push a small amount of bone marrow (0.5 – 1.0 ml) form the syringe. Using a Pasteur pipette, corner of a glass slide, or wooden applicator stick, transfer a single drop of bone marrow to the end of another slide. Make a smear using the same "push" technique that is standard for making a peripheral blood smear. Submit 10 slides air-dried for routine histology. Alternatively, submit the entire aspirate in a purple top (EDTA) tube and smears will be prepared at our lab. Be sure to clearly label the tube as "aspirate" so it is not confused with a tube of peripheral blood.

An EDTA (purple top) tube of peripheral blood should always be simultaneously submitted; or alternatively, 2 unstained peripheral smears and the results of a recent CBC may be submitted. Be sure to clearly label the tube as "peripheral blood".

Bone Marrow Aspirate for Cytogenetic Studies

Preferred Method of Collection:

- Obtain cytogenetic sample immediately after sample obtained for morphology studies.
- Rinse barrel of syringe with sodium heparin and discharge excess, leaving <0.5 ml for a 3-5 ml sample, and less for smaller quantities.
- Minimum sample is 1 ml, with 3 ml optimum.
- Remove the needle, cap the syringe, secure with parafilm, and tape the plunger.

Alternate Methods of Collection:

- Collect sample in heparinized syringe and gently discharge into a red top tube.
- Collect sample in un-heparinized syringe and quickly but gently discharge into a sodium heparin green top tube.

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- If sample will be more than 4 hours in transit, collect sample in heparinized syringe and place in sterile transport medium*.

If aspirate is sub optimal (clotted, small sample, marrow is packed), also submit:

- Bone marrow core biopsy: One or more trephine needle samples (10 mm) collected in sterile transport medium*. Include "for Cytogenetics" on specimen label along with patient name and source.
- Peripheral blood: 3-6 cc in sodium heparin green top vacuum tube. Include "for Cytogenetics" and "Peripheral Blood" on specimen label along with patient name and source.

**Sterile transport medium may be obtained from AMERIPATH INDIANA.*

Maintain specimen at room temperature and call AMERIPATH INDIANA for immediate pick-up of specimen. Complete AMERIPATH INDIANA requisition, including clinical data and diagnostic information. AMERIPATH INDIANA will forward specimen to AMERIPATH ESOTERIC INSTITUTE (AEI).

Bone Marrow Aspirate for Flow Cytometry

Bone marrow aspirate is collected in a sodium heparin tube (dark green top tube) and immediately mixed well. Do NOT use lithium sodium tubes. Send CBC results and a peripheral smear with the bone marrow if available.

An EDTA tube (purple top) may also be submitted but is less preferable.

Store bone marrow sample at 20º to 30º C (Room temperature). DO NOT REFRIGERATE!

Specimens must be received in the laboratory as quickly as possible after collection.

SEE BONE MARROW ASPIRATION AND BIOPSY FOR CLINICIANS' MANUAL.

Kidney Stones

Stone (Calculus) Analysis:

Air-dry the stone and place in a clean, dry container (such as a sterile urine container). Stones transported in liquid or bound up in tape interfere with and delay analysis, compromising results. Label as instructed in the Submitting Specimens section of this manual and submit with completed requisition.

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Specific Specimen Requirements

The following specimens should be received fresh and require immediate processing. Contact the laboratory at (317) 275-8111 for instructions.

Diagnostic Lymph Node Biopsies /Lymphoma Protocol – Please contact AmeriPath prior to submitting a diagnostic lymphoma protocol specimen. Ideally, a portion of lymph node should be immediately fixed in 10% buffered formalin; a smaller portion of lymph node (at least 1 cm^3) should be submitted in tissue transport media (TTM); and a portion submitted for cultures (if clinically indicated) in the fresh state. It is imperative that a portion of a lymph node is received in TTM (*NOT* saline).

Breast Biopsies - must be fresh for frozen section study; otherwise place in formalin. Include on the requisition the time removed from patient and the time placed in formalin (cold ischemic time).

Products of Conception – submit a portion in TTM/RPMI (*NOT* saline or Hank's media) if cytogenetics testing is needed; otherwise, place in formalin.

Tissue biopsies for Immunofluorescence – must be received fresh in saline or immediately placed in Michel's transport solution.

Tissue for Electron Microscopy - Must be received fresh in saline or immediately placed in 2.5% glutaraldehyde (tissue fragments must be 1 mm or smaller).

Flow Cytometry Immunophenotypic Analysis:

- Tissue - at least 1 cm^3 piece of tissue should be sent in tissue transport media (TTM)
- Bone marrow – submit in a sodium heparin (green top) tube
- Body cavity fluids – submit at least 50 ml of fresh body cavity fluids
- Cerebrospinal fluid – as much fresh cerebrospinal fluid as can be safely extracted (minimum volume 1 ml)

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SPECIMEN COLLECTION GRID

AmeriPath Indiana Specimen Collection Grid

	Cytology	Cell Block Cytology	Histo-pathology	Immunohisto-chemistry	Special Stains (AFB / GMS)	Flow Cytometry	Bone Marrow	Microbiology
Ethanol (95%)	Smear fixation only	no	no	no	no	no	no	no
Tissue Transport Media (TTM)	yes, FNA rinses only	yes, FNA rinses only	no	yes, but MUST have tissue or cell block to stain	yes	yes	no	no
CytoLyt (contains fixative / methanol)	Yes. NOT to be used as smear fixative	yes	no	Not recommended (Cannot be used for reliable ER/PR)	yes	no	no	no
Formalin	no	no	yes, preferred standard tissue fixative	yes, but MUST have routine histology prior to stain	yes, but MUST have routine histology prior to stain	no	no, use Zenker's for core and clot	no
Zenker's	no	no	bone marrow only	yes, bone marrow only	yes	no	yes	no
Green Top Tube (Na Heparin)	no	no	no	no	no	yes	bone marrow aspirate only	no