SPECIMEN COLLECTION

This directory serves as an aid in the collection and handling of specimens sent to the laboratory.

If you have any questions not resolved by this manual please call Doctors' Anatomic Pathology Services DAPS) at 870/930-3518. Questions resolved by use of this manual or by direct conversation with us, PRIOR TO COLLECTION OF THE SPECIMEN, will avoid problems that may compromise the specimen.

Hours of Operation

Hours of operation are Monday through Friday, 8:00 a.m. to 5:00 p.m. Pathologists do observe a call schedule for emergency situations.

SPECIMEN LABELING AND SUBMISSION

Prudent medical-legal practice and our laboratory accrediting agencies have strict guidelines for specimen labeling and submission. They also mandate rejection of improperly completed requisitions or incorrectly identified slides or specimens. Please note any possible expiration dates on supplies provided to your facility. For example, some specimens cannot be analyzed because of improper collection, preservation or degradation in transit. Other specimens may have prolonged turn-around-times because of lack of necessary patient information. Still other specimens will, by necessity, be rejected because of inaccurate or absent specimen and/or requisition labeling. Clients will be notified concerning rejection of problem specimen(s) upon receipt. To avoid delayed diagnoses and potential specimen rejection, please follow the following requirements:

Surgical Specimens:

Requisition:

The requisition is a three-part form with the pink copy provided for your records.

- Patient name
- Date of birth
- Physician name
- Insurance and/or billing information including name of insured, subscriber number, group number, name and address of insurance company.
- Site and type of biopsy

- Brief clinical history
- Collection date
- Please make special requests for stains, studies, or testing

Specimen container:

- Patient's name
- Specimen type and/or location (i.e. skin lesion, left shoulder etc.)

SURGICAL PATHOLOGY

Tissues for Pathologic Diagnosis:

General Requirements

Requisition:

- Patient name
- Date of birth
- Physician and clinic name and address
- Insurance and/or billing information
- Type of Specimen/Location

1. Once the tissue sample is removed from the patient, the specimen should be placed in 10% formalin unless a different or additional fixative is specified for a particular study. Tissues should always be placed in fixative without delay. The volume of formalin should exceed the volume of the specimen by up to 10x.

2. Label the tissue containers (not the lid) with the patient's name, tissue type and site of biopsy.

3. Secure the container lid tightly to avoid loss of formalin or possible leakage.

4. Complete requisition form including the patient's name, date of biopsy, tissue site and type, physician and clinic name and address, billing information, and special requests for the pathologist along with relevant clinical history.

5. Listing of age, last menstrual period and all exogenous hormones the patient has recently received, including birth control pills, should be provided with endometrial biopsies or curetting.

6. Dates and specimen numbers of abnormal Pap smears should be provided with cervical and endocervical biopsies.

7. Identify special reporting requirements (i.e. FAX numbers).

8. Please indicate any recent change of last name for patient.

9. If re-biopsy of a lesion, please provide date and the specimen accession

number of previous biopsy. Please include a copy of the Pathology / Cytology report, if the specimen was not performed by our laboratory.

10. Tissue samples from different sites or lesions must be submitted in separate containers.

11. Secure tissue container and requisition together in a specimen bag, in separate compartments.

12. Arrange for courier pickup or mailing. Be sure that containers are leak resistant and mailed in conformance with federal mail policies for transportation of diagnostic specimens.

Biopsies: Routine

Materials Required:

1. Collection container with 10% formalin
2. Requisition form.

Procedure:

1. Label the collection container with the specimen labels provided (not the lid) with the patient's name and tissue identification.
2. Complete requisition form including patient name, birth date, date of service, specimen site and information, and billing data.
3. Include clinical history on the requisition.
4. Place the specimen immediately into fixative container, tightly close the container lid, and forward to laboratory with the requisition.

Comments: Please provide as much clinical history as possible, including history of prior malignancies. This information is important to the interpretation of the lesion and may help in selecting the appropriate special stains and tumor markers needed to make an accurate diagnosis.

For all other specimen collection procedures not categorized above, which require special attention or assistance, DAPS should be contacted at (870)930-3518. A histotechnologist will give you special guidelines and instructions for the collection, preservation, and handling.

Biopsy: Bone Marrow

Materials Required:

1. Requisition forms, labels for patient name, specimen designation, time specimen was collected with biohazard bags.
2. B-Plus Fix container.
3. Two purple top tubes – one plastic capped tube for aspirate, one rubber stopper tube for peripheral blood.
4. Two Sodium Heparin green top tubes, one with 3-6 ml of aspirate for

cytogenetics and the other for 3-5 ml of blood or 1 ml of marrow for flow cytometry.

5. Stained or Unstained Peripheral Blood Smears (prefer submission of two slides). ***Please See Section on Peripheral Smear.***

Procedure:

1. Label the collection containers with the patient's name and specimen type.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Request studies on the requisition.
4. After preparing 5 touch imprints, the bone marrow core biopsy should be immediately placed in B-Plus Fix and note the time on the requisition form.
5. Express bone marrow aspirate material into a purple (EDTA) top tube. The peripheral blood specimen should be placed in a purple top tube.
6. If chromosomal or flow cytometry studies required, aspirate material must be submitted in separate green top tubes. If marrow aspirate is not obtained, a 10 mm bone core sample may be submitted in sterile RPMI fixative.
7. Place all the specimen tubes and the requisition form in a specimen bag, in separate compartments.

Comment:

Bone marrow biopsy specimens are performed for a variety of reasons ranging from evaluation of anemia to the diagnosis of metastatic carcinoma and leukemia. Complete patient history, as well as documentation of any supporting details are vital to the complete and accurate interpretation of the material.

Biopsy: Routine Cervical, Endocervical, and Endometrial

Materials Required:

1. Collection container with 10% formalin.
2. Requisition form.

Procedure:

1. Label the body of the collection container (not the lid) with the patient's name and tissue identification.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Provide clinical history, i.e. last menstrual period, prior biopsies or Pap smear information, and any history of hormone use including birth control pills on the requisition form.
4. Place specimen directly into formalin, close lid tightly and forward to

laboratory in a specimen bag, in separate compartments.

Comment:

Use of gauze pads to hold or place endometrial and endocervical samplings is discouraged because portions of the specimen are absorbed into the coarse weave and lost. Telfa or biopsy sponge is acceptable; placing the specimen directly into formalin is preferred.

Biopsy: Cone and Leep Conization of Cervix

Materials Required:

1. Collection container with 10% formalin.
2. Requisition form.

Procedure:

1. Label the body of the collection container (not the lid) with the patient's name and tissue identification.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Include on the requisition a history of prior Pap smear or biopsy results.
4. If necessary, orient cone specimen with surgical suture material.
5. Endocervical portions should be separated from ectocervical portions.
6. Immediately place the specimens in the fixative container, tightly close container lid and forward to laboratory with requisition, in a specimen bag, is separate compartments.

Biopsy: Endoscopic

Materials Required:

1. Collection container with 10% formalin.
2. Requisition form.

Procedure:

1. Label the body of the collection container (not the lid) with patient's name and tissue identification.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Place specimen directly into formalin, close lid tightly and forward to laboratory, in a specimen bag, in separate compartments.

Comment:

Use of gauze pads to hold or place endoscopically obtained specimens is discouraged because portions of the specimen are absorbed into the coarse weave and lost. Telfa or biopsy sponge is acceptable; placing the specimen directly into formalin is preferred.

Biopsy: Skin Excisions

Materials Required:

1. Collection container with 10% formalin.
2. Requisition form.

Procedure:

1. Label the collection container with the patient's name and tissue identification.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Include on the requisition form any clinical history, a gross description of lesion, and history of prior biopsies, if available.
4. Orient specimen as necessary using description or surgical suture material. Immediately place the specimen in the fixative container.
5. Tightly close the container and forward to laboratory with requisition, in a specimen bag, in separate compartments

Comment:

Each biopsy must be submitted in a separate container labeled with the site of biopsy. The consequences of submitting skin biopsies of several sites in a single container can be disastrous. Many dermatologic disease processes look similar microscopically. Please describe the lesion in detail, and provide as much clinical history as possible. Providing this information will help our pathologist to be very specific with his/her diagnosis.

CYTOPATHOLOGY

Cytology: General Requirements

Cytology Specimens:

Requisition:

1. Patient name
2. Date of birth
3. Physician and clinic name and address
4. Insurance and/or billing information
5. Type of Specimen
6. Clinical history including **LMP** or menstrual history as necessary using fields on the bottom of the requisition

All Pap smears and submitted Non-gynecological cytology specimens **must be** legibly labeled with the patient's name matching that on the requisition.

Fixation:

1. For gynecologic (Pap smears) specimens and other non-gynecologic cytology specimens, immediate fixation is essential for cytological examination. Air-drying begins as soon as the smear is made and proceeds rapidly although no grossly visible changes in the appearance of the smear or samples are present. Ethanol and Methanol are acceptable alcohol fixatives; however, isopropanol is not acceptable. Stage your exam so that fixative can be applied immediately to the specimen using one of the following methods:

a. Fixative should be sprayed on smears immediately. Spray fixatives should be applied to smears with the spray can held 6-8 inches away from the slide.

b. Thin Prep collected samples should be rinsed immediately into the Thin Prep vial using the supplied collection devices. The utensils used should be rinsed vigorously into the container, while swirling the device against the walls of the vial. It is imperative to remove any 'excess' mucous from the utensil used in the collection. The 'excess' mucous should be discarded into a biohazard container. The excess mucous will plug the filter during the processing of the Thin Prep vial.

c. Non-gyn specimens should be collected using a 1:1 ratio of CytoLyt™ fixative for adequate specimen preservation. This will be supplied in a pre-filled container or can be supplied in bulk quantity.

2. The lab has the ability to process all Non-GYN specimens that have been allowed to completely air dry using a Wright-Giemsa type stain. Air-drying of fine needle aspirates of the breast and thyroid are especially appropriate for this type of processing.

3. It is vitally important that you communicate to the laboratory the type of fixation used so the appropriate staining technique may be utilized.

4. Label slides with the patient's name prior to the procedure so that labeling will not delay fixation.

General Requirements

1. Complete requisition form including patient name, birth date, date of service, and billing data.

2. Samples from different sites (i.e. several aspirations of the same breast: lateral upper quadrant, below nipple, etc.) should be submitted in separate containers, or on separate slides.

3. Identify on the requisition the specimen site, and method of sampling of cytological specimens (i.e. FNA, lump, lateral upper quadrant etc).

4. Identify special interest or requirements for pathologists, and provide any relevant clinical history.

5. Last menstrual period (LMP) and all exogenous hormones the patient receives, or has recently received, including birth control pills, should be provided with cervical and endocervical smears.

6. Previous abnormal Pap smear and/or tissue specimen history should be provided when relevant to current examination.

7. Identify special reporting requirements (i.e. FAX report).

Gynecological Cytology Specimens

Once the sample is collected from the patient and smeared onto a slide, the Conventional Pap smear should be immediately spray fixed or immersed in a 95% methanol or ethanol.

Cytology: Cervical and Endocervical Smears (Pap Smears)

Principle:

The Pap smear is the most effective cancer-screening test in medical history, and is the only screening test, which has been associated with a 70-80% decrease in the death rate due to a prevalent cancer. The test is based on microscopic examination of exfoliated cells derived from the surface of the cervix.

Materials Required:

1. Frosted end microscopic slide
2. Cytology spray fixative or Pap fixative
3. Cervical spatula and/or other collecting device (cytobrush or cytobroom)
4. Slide holder
5. Requisition form

Procedure:

1. Schedule patient for sample collection mid cycle (obscuring blood from menstrual smears is a major source of less than optimal smears).
2. Instruct patient in advance not to douche for at least 24 hours prior to examination or engage in sexual intercourse for a t least 48 hours prior to examination.
3. Label frosted end of slide with the patient's name.
4. Complete requisition form including patient name, birth date, date of service, and billing data. Include on the form all clinical information (LMP, hormone use, prior Pap smear and biopsy results).
5. Prior to specimen collection, clean away any visible blood, mucous and or discharge from the cervix.
6. Obtain a vaginal smear from secretions in the posterior fornix. This material, along with the ectocervical scrape should be smeared evenly on

the slide adjacent to the frosted end. Fix immediately with fixative from a Pap pack or spray fixative. Spray should be held 6-8 inches from the slide while spraying. Abundant fixative should be applied to avoid cell degeneration and air-drying artifact. Do not allow cells to dry before fixing.

7. Sample the endocervix and smear the cells evenly on the remainder of the slide. Fix immediately with fixative from a Pap pack or spray fixative.
8. The Pap pack and cytobrush are recommended for use. The patient's name must be written on the frosted end of the slide.
9. A vaginal smear from the lateral vaginal wall is required for a hormonal evaluation (maturation index). Smeared material should be kept separate from the cervical/endocervical smeared areas.
10. Place slides in the slide holder, secure together with completed requisition and send to the laboratory is a specimen bag, in separate compartments.

Comment:

As good as the Pap smear test is, it is imperfect and must be interpreted in conjunction with the clinical history. Also the Pap smear is a screening tool, not a diagnostic procedure. Like any screening test, it has false negative and false positive rates. An annual Pap smear is the single best means of reducing the incidence of cervical dysplasia and cancer.

Cytology: Thin Prep® Pap Test

Principle:

Automation and new methodologies are dramatically changing gynecological cytology, particularly the Pap test. The FDA has approved the Cytyc Thin Prep Pap test as a replacement for the conventional Pap Smear. Studies indicate that it is more accurate than the conventional Pap smear in detecting premalignant and malignant lesions. All utensils used for the Thin Prep Pap tests should be immediately 'rinsed' rigorously in a Thin Prep vial and securely closed and stored at room temperature. Once a sample is collected in a Thin Prep vial, the life of the test in the vial is **3 WEEKS** from collection date and can be stored at room temperature.

The advantages of a monolayer slide include:

1. Elimination of obscuring inflammation and blood.
2. Elimination of air drying artifact, provided the specimen is placed in the fixative immediately after sampling.
3. A more uniform and representative sampling of the collected specimen
4. Residual sample available to perform additional testing on as needed.
5. Lowering of the ASCUS rate since air drying and obscuring inflammation are not present.
6. Increased sensitivity in detecting LOW GRADE and HIGH GRADE

DYSPLASIAS due to the greater ease in recognizing dysplastic cells on the Thin Prep monolayer slide.

Materials Needed:

1. Collection kit including cervix brush and plastic spatula or broom, and collection vial of CytoLyt
2. Completed requisition form

Procedure:

1. Schedule patient for sample collection mid cycle (obscuring blood from menstrual smears is a major source of less than optimal smears).
2. Instruct patient in advance not to douche for at least 24 hours prior to examination or engage in sexual intercourse for at least 48 hours prior to examination.
3. Label the Thin Prep vial with the patient's name.
4. Complete requisition form including patient name, birth date, date of service, and billing data. Include on the form all clinical information (LMP, hormone use, prior Pap smear and biopsy results).
5. Prior to specimen collection, clean away any visible blood, mucous and or discharge from the cervix.
6. Obtain a vaginal smear from secretions in the posterior fornix. This material, along with the ectocervical scrape should be placed immediately in the CytoLyt vial and agitated to remove the collected cells.
7. Sample from the endocervix with a cytobrush or broom. If the brush is used, the ectocervix is sampled with the spatula.
8. The brush/spatula or broom is placed into the vial of CytoLyt and agitated up and down against the bottom and sides of the container to collect as much material as possible. This should be done 10-12 times rotating the brush through your fingertips while swirling the brush in the vial.
9. Close the vial tightly, and place it with a requisition form into a specimen bag, in separate compartments, and ship it to the laboratory.

Comment:

Due to the significantly higher cost of the Thin Prep Pap test kit, ***we are asking you to use the kits we provide only on patients whose specimens will be sent to our lab for processing.***

Non-Gynecological Cytology

Please note: When CytoLyt solution is not available, a Thin Prep vial contains acceptable fixative

Cytology: Induced Sputum and Bronchial

Washings for Pneumonocycstis carinii Pneumonia

Materials Required:

1. Leak proof specimen container with CytoLyt
2. Requisition form.

Procedure:

1. Label specimen containers.
2. Complete requisition form including patient name, birth date, date of service, and billing data. Include requests for special stains or studies (i.e. Pneumonocystis carinii or fungus).
3. Enter washings into specimen container with precise identification of site (i.e. right upper lobe of lung, etc.).
4. If brushings are performed concurrently, rotate brush gently over slide to apply material.
5. Submit to laboratory with completed requisition form, in specimen bag, in separate compartments.

Comment:

Fresh specimens are not accepted. Only specimens with CytoLyt fixative are accepted for PCP (Pneumocystis carinii pneumonia) due to courier delay.

Spinal Fluid for Cytology

Materials Required:

1. Leak proof specimen container.
2. Preservative fluid (CytoLyt)
3. Requisition form.

Procedure:

1. Label specimen container with patient name and specimen type.
2. Complete requisition form including patient name, birth date, date of service, and billing data. Include requests for special stains or studies.
3. Place spinal fluid into specimen container with CytoLyt
4. Submit to laboratory with completed requisition form, in specimen bag, in separate compartments.

Comment:

Fresh specimens are not accepted. Only specimens with CytoLyt fixative are accepted due to courier delay.

Cytology: Bronchial, Colonic, Esophageal and Gastric Washings

Materials Required:

1. Leak proof specimen container

2. Preservative fluid (CytoLyt)
3. Slides with frosted ends (if brushings are obtained concurrently)
4. Spray fixative (if brushings are obtained concurrently)
5. Requisition form

Procedure:

1. Label specimen containers with patient's name, specimen type and identification of site. Label slides with patient's name.
2. Complete requisition form including patient name, birth date, date of service, and billing data. Include requests for special stains or studies (i.e. Pneumonocystis carinii or fungus).
3. Enter washings into specimen container with precise identification of site (i.e. right upper lobe of lung, etc.).
4. Add equal volumes of preservative fluid to each specimen. Close specimen container tightly.
5. If brushings are performed concurrently, rotate brush gently over slide to apply material and fix immediately with spray fixative**.**
6. Submit to laboratory with completed requisition form, in specimen bag, in separate compartments.

Cytology: Sputum

Materials Required:

1. Leak-proof plastic container with CytoLyt fixative
2. Requisition form

Procedure:

1. Label collection container with patient's name and specimen type.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. If the specimen is one of a series of samples taken, indicate position in series (i.e. 1/3 if first of three samples).
4. If all samples are collected into a single container, indicate this on the requisition form.
5. Have patient rinse mouth prior to collection.
6. Give collection container to patient.
7. Instruct patient to breathe deeply for 3 minutes.
8. Instruct patient to cough deeply from the diaphragm, with effort to expectorate material into collection container.
9. At short intervals, repeat the coughing attempts three more times with collection of all coughed up material.

10. Add equal volumes of fixative fluid and shake the container vigorously each time a new specimen is added to the cup (make sure that the container is tightly sealed before shaking).
11. Tightly close the collection container and forward to the laboratory with the completed requisition form, in a specimen bag, in separate compartments.

Comment:

1. Deep (cough from the diaphragm) specimens are necessary to provide information regarding the lower respiratory tract. The laboratory will determine adequacy of specimen by the presence of alveolar macrophages within the specimen.
2. A series of three sputums should be collected on three consecutive days, preferably first thing in the morning with hard, productive coughing is recommended. Morning collections take advantage of the accumulation of secretions during the night. Avoid collecting right after meals.
3. If there is difficulty in producing a specimen, collections may be facilitated by the moisture and steam of a preceding, long hot shower. For patients unable to produce sputum with repeated attempts, consider aerosol induced coughing and specimen collection.
4. Post bronchoscopy sputums may be productive with diagnostic material even with negative bronchial washings, brushings and biopsies.
5. Specimen consisting of saliva or nasal pharyngeal drainage will be reported as lacking alveolar macrophages, metaplastic cells or bronchial columnar cells. These specimens are inadequate for lesions of the lower respiratory tract and will not be considered as true negative studies.
6. With sputum samples positive for malignant cells, a primary of the head and neck region should be considered as well as malignancies of lung. Up to 10% of positive sputums may reflect malignancies of the head and neck.
7. Specimens requiring culture must be separately submitted in sterile containers without fixative. The culture specimens must be submitted to a laboratory specializing in micro biologic culturing (not DAPS)..
8. Specify the need for identification of pneumocystis carinii. Specimens will include examinations of Papanicolaou, Diff Quick and GMS as needed.

Cytology: Body Fluids

Materials Required:

1. Collection containers, leak proof.
2. Completed requisition form.
3. Preservative fluid (CytoLyt)

Procedure:

1. Label the collection container with the patient's name and specimen source.
2. Complete requisition form. Include patient name, birth date, date of service, and billing data.
3. Close container tightly, then forward specimen to laboratory with requisition form, in a specimen bag, in separate compartments.

Comments:

1. Body fluids to be processed in this manner include pleural fluid, ascitic fluid, cul-de-sac fluid and pericardial fluid.
2. The entire collected specimen should be submitted for cytologic processing after allocating-off specimens for other studies (i.e. culturing, cell count and protein analysis). It is our opinion that the yield and diagnostic sensitivity is increased when the entire specimen is received for cytologic preparation and interpretation.

Cytology: Urine

Materials Required:

1. Leak proof collection containers.
2. Appropriate fixative (CytoLyt).
3. Completed requisition form.

Procedure:

1. Label collection container with patient's name.
2. Complete requisition form, including patient name, birth date, date of service, and billing data. Patient's history of prior kidney or bladder abnormalities should also be noted.
3. Instruct the patient to void and discard the first morning specimen. The patient should drink as much as possible an hour or so prior to collecting urine specimens.
4. Mix an equal amount of fixative with the urine specimen. Forward specimen to laboratory with completed requisition form, in a specimen bag, in separate compartments.

Comments:

1. Urine should be identified as to type of sample (i.e. voided, catheterized, right or left ureteral or bladder irrigation fluid).
2. For detection of cancer, ureters and kidneys, a serial collection of specimens spaced over three days has proven to increase diagnostic sensitivity and yield.
3. Sensitivity and yield of urine specimen has also been shown to increase with optimal collection and preservation of specimen.

4. Unpreserved urine results in the rapid degeneration of exfoliated cells.

Cytology: Nipple Secretions, Smears

Materials Required:

1. Slides with frosted ends
2. Spray fixative
3. Physiologic saline
4. Cotton swab
5. Requisition form

Procedure:

1. Label slide with patient's name and site from which sample is obtained.
2. Complete requisition form, including patient name, birth date, date of service, and billing data. Also include any pertinent history on the requisition.
3. If there is no nipple erosion or ulceration, gently "strip" the area of the breast below the nipple and areola with a motion from beneath the areola towards the nipple surface. Do not massage the entire breast. The stripping motion will propel accumulated secretions within the ampulla of the larger excretory ducts.
4. With appearance of fluid on the nipple surface, touch a slide to the drop of fluid and draw the slide quickly across the nipple.
5. Repeat this process for the opposite breast.
6. If there is nipple erosion or ulceration, touch a slide to this area three times. With a different part of the slide in contact each time. This is conveniently done, starting with a contact position close to the hand holding the slide and then moving the application area of the slide further with your hand for the next two samplings.
7. Following touch preparation (#6) of the ulcerated area, try to express fluid (#3) and prepare slides if fluid is obtained.
8. If no fluid can be expressed, a swab may be dipped in saline and gently rolled and rotated on the ulcerated surface, and applied to a glass slide.
9. Immediately spray fix slides, allow to dry, place in slide container and forward to the laboratory with the completed requisition, in specimen bag, in separate compartments.

Comment:

The obtained smears can either be completely air dried or spray fixed. If spray fixation is elected, it is vitally important that smears be sprayed as soon as obtained. Partial fixation of slides develop air drying artifact that will only complicate interpretation and may even result in an unsatisfactory specimen. It is important to indicate the type of fixation (air dry vs. fixed smears) so that

the proper staining procedure may be selected at the laboratory. We recommend completely air drying smears to avoid fixation artifact.

Cytology: Tzanck Preps

Materials Required:

1. Thin Prep vial
2. Spatula
3. Completed requisition form

Procedure:

1. Scrape the vesicle
2. Place the sample into a Thin Prep vial

Comment:

The collection should come from the vesicle, which is suspicious. Please note on the requisition that the sample is submitted for a Tzanck Prep. Please contact DAPS at 870/930-3518 for any additional information.

Fine Needle Aspiration

Principle:

Fine needle aspiration provides a prompt, cost effective, safe, simple and useful evaluation of a mass through cytologic diagnosis, that is usually well tolerated by patients. It is often used as an alternative to surgery and may provide a definitive diagnosis that will determine therapy and/or assist in a planned surgical approach with effective utilization of operating room time. In general, any palpable mass can be evaluated by aspiration techniques. With ultrasound guidance, fluoroscopy and CT, most deep seated lesions may also be sampled. Lesions that are commonly sampled include: thyroid, breast, salivary glands and lymph nodes. Although the technique seems simple, it does require some practice and understanding of principles of aspiration.

Materials Required:

1. Hand grip syringe holder of preference.
2. Syringe, screw lock, disposable, 10 ml or 20 ml plastic with tight fitting barrel.
3. Needles of size preference. Most aspirates may be obtained with a needle no larger than 21 gauge. Most highly vascular structures (i.e. thyroid) are best sampled with a 23 or 25 gauge needle.
4. Frosted end labeled slides.
5. Specimen containers with appropriate fixative as needed (CytoLyt).
6. Spray fixative.
7. Alcohol or iodine solutions for sterilization of skin.

8. Cotton swabs for sterilization of skin.
9. Sterile gauze.
10. Adhesive tape.
11. Bandaids
12. Requisition form.

Procedure:

1. Label slides and specimen containers with the patient's name and site of aspiration.
2. Place labeled slides in three rows of four slides, each row for use with a "pass". This set-up supports three passes. Open container with fixative for needle washings.
3. Set-up a sterile field including preparation of syringe and syringe holders for initial and repeat passes.
4. Select needles and syringes to be used for the procedure. Place patient in a comfortable sitting or reclining position that allows easy access and aspiration of the lesion.
5. Palpate the mass to identify the depth of the target and its relationship to surrounding structures.
6. Assemble the syringe equipment.
7. Clean the skin over the aspiration site with an alcohol swab.
8. Immobilize the mass with the thumb and index finger of one hand or between two fingers of one hand.
9. Take the syringe equipment in the opposite hand and use a one handed withdrawal and release manipulation of the syringe plunger.
10. Place the needle against the skin at a determined puncture site and insert it into the mass area with a single quick motion without negative pressure on the syringe.
11. Once the needle is in the desired area, retract the plunger of the syringe to create negative pressure in the syringe and needle lumen. Minimal negative pressure is needed for highly vascular organs like the thyroid while more negative pressure is needed for dense fibrous organs like the breast.
12. Move the needle back and forth several times directing it in the same plane. *Avoid unnecessary redirection of the needle as this tends to produce unnecessary hemorrhage in the lesion.*
13. When material appears in the hub of the needle, the aspiration has been completed. Excess blood in the material will dilute the specimen rendering it unsuitable for microscopic diagnosis. One drop of material can usually produce 4 -6 smears.

14. Release the pressure in the syringe by releasing the syringe plunger.
15. Gently withdraw the needle from the lesion and apply pressure to the puncture site with sterile gauze.

Smear Preparation:

1. After the needle has been removed from the mass, detach the needle from the syringe using a surgical clamp or other appropriate instrument, fill the syringe with air, and reattach the needle to the syringe.
2. Place the bevel of the needle against a glass slide and express a small drop of aspirated material onto the slide.
    a. If too much material is expressed onto the slide, either re-aspirate a portion of the material by withdrawing the syringe plunger slightly, or spread the material out among several slides.
    b. If the cellular material is semi-solid, place a second slide on top of the material and pull the slides gently and quickly apart as the material spreads from the weight of the slide.
    c. If the aspirated material is diluted by fluid or by blood, use the same smear technique as for blood smears:
        i. Back the edge of a slide or cover slip into the drop and as the material spreads along the edge, move the slide/cover slip forward pulling the cellular material away from the fluid or blood.
        ii. Immediately fix the smear for Papanicolaou stain with spray fixative or 95% ethyl or methyl alcohol. Or alternatively:
        iii. Allow smears to air dry for Diff Quick staining. Write "air dried" on the end of the smears.
        iv. If staining is available, have the patient remain while adequacy of the aspiration pass is determined and repeat the procedure until the operator is satisfied with the adequacy of the material.
        v. Label all slides with the patient's name and site of aspiration.
    d. Complete requisition form, including patient name, birth date, date of service, and billing data.

Comments:

1. When the lesion is composed of solid tissue, the needle tip functions as a cutting instrument: as it is moved back and forth through the tissue, tiny tissue fragments become dislodged and collect inside the needle. When suction is added to this procedure the previously dislodged fragments are sucked into the needle and the tip of the attached syringe.
2. The needle should never be removed while any negative pressure is in the syringe. Such pressure may force aspirated material from the needle into

the syringe. This may make preparation of smears difficult and may start air drying of the material.

3. It is important not to dilute the cellular material with fluid or blood:
    a. If a cyst is encountered during the aspiration, evacuate all fluid from the cyst and perform a second aspiration on any residual mass. Express the fluid from the cyst into CytoLyt.
    b. If blood is aspirated into the syringe, stop the procedure and prepare slides. Express residual bloody fluid into CytoLyt.
    c. If puss is encountered, withdraw as much of the material as possible and perform a repeat aspiration in an adjacent area. (When an infectious process is included in the differential diagnosis, a culture of the aspirated material is often desirable).
4. Necrosis generally occurs in the center of large lesions because of an inadequate blood supply. If the first sample yields only necrotic debris, another sample obtained tangentially to the edge of the mass should be secured.
5. Local anesthesia is rarely required, as the discomfort caused by its application is about the same as the aspiration. Its use is dependent on the lesion location, the discomfort of the patient and the judgment of the operator.
6. Passing through layers of muscle while inserting the needle adds significantly to the discomfort of the patient, while making needle placement more difficult and uncertain. Also, small fragments of muscle may plug the needle, jeopardizing subsequent sampling of the target. The muscle and lesion may sometimes be manipulated so that aspiration technique does not involve the muscle.
7. The nipple and the areola of the breast are the areas most sensitive to pain from a needle stick. These areas should be avoided whenever possible. Masses in these areas can sometimes be pushed away from the nipple, immobilized and sampled through adjacent skin.
8. When a mass is located close to the chest wall, there is a possibility that the needle may penetrate and cause a pneumothorax. This can be avoided by moving the mass sideways so it rests on a rib. This not only prevents penetration of the chest wall but also provides good support for immobilization of the target.
9. Complications can vary as to the site of aspiration and cannot all be listed. The aspirator should be aware of these prior to aspiration and communicate them to the patient.

Other Cytology Related Specialty Testing

Human Papillomavirus (HPV) DNA Testing

Principle:
Testing is done directly from Cytyc's Thin Prep vial, using the Inform (TM)HPV (Ventana Medical System, Tucson, AZ). DAPS tests for the 'High Risk' Strains and 'Low Risk'' Strains of HPV. The National Institute of Health has concluded 'High Risk' Strains are present in at least 93 percent of cervical cancer. This test was developed and its performance characteristics determined by WCP Laboratories, Inc. in St. Louis, MO. This test has not been approved by the U.S. Food and Drug Administration. USFDA approval is not required per 21CFR809.30e.

Once a sample is collected in a Thin Prep vial, the life of the test in the vial is **3 WEEKS** from collection date and can be stored at room temperature. This test is approved for female collection only.

Materials Required:

1. Thin Prep Vial
2. Plastic Spatula, Brush, or Broom
3. Requisition

Procedure:

1. Collect sample identically as you would for a Thin Prep Pap Test.
2. Once collected mark 'HPV' if you want HPV Testing if the Pap is abnormal for all results ASCUS or above.
3. If you desire an HPV to be run by itself or in an adjunct to Thin Prep, *regardless of the result of the Pap,* mark 'HPV Only'.
4. A 'Standing Order' can be given to DAPS specifically stating when to perform testing for High Risk Strains of HPV. These requests will need to be submitted in writing detailing the clinic and physicians ordering the tests. These will be updated annually.

Comment:

Please note that this test can be used to triage patients with abnormal Pap results, specifically those of ASCUS or AGUS or as an individual test specifically looking for High Risk Strains of the Human Papillomavirus. The HPV test specifically looks for the strain or strains that lead to cervical cancer.

Chlamydia Trachomatis (CT) / Neisseria Gonorrhoeae (NG)

Materials Required:

1. Thin Prep Vial or M4 Collection Device *(Male or Female)*
2. Requisition

Procedure:

1. Collect sample identically as you would for a Thin Prep Pap Test or collect

the sample in an M4 collection device (M4 device is for CT / NG only). Please remove and discard any excess mucous from the sample as you would for a Thin Prep if you are using the M4 media. This device can be used for males from a urethral sampling by gently rotating the swab for proper collection.
2. Once the sample is collected in an M4 collection device, room temperature is acceptable.
3. Once collected mark 'Chlamydia' or 'Gonorrhoeae' for which test or tests you are requesting.

Comment:

PCR (Polymerase Chain Reaction) technology increases both the sensitivity and specificity of the sample in comparison to the other CT / NG testing available. These tests can be collected directly from a Thin Prep Sample or in their own collection device called M4. Once a sample is collected in a Thin Prep vial, the life of the test in the vial is **3 WEEKS** from collection date and can be stored at room temperature. The M4 collection device has a special utensil designed for male specimens. If a sample is collected in an M4 device, the sample must be refrigerated upon collection.

IMMUNOHISTOLOGY: MOLECULAR PATHOLOGY AND CYTOCHEMISTRY

Principle:

Immunohistology employs highly specific monoclonal antibodies to detect protein antigens in pathologic material to demonstrate specific types of cellular differentiation. *Molecular pathology* uses specific DNA probes to identify genetic material, to associate a disease process with an infectious agent or rearrangement of genetic material. *Cytochemistry* employs specific chemical reactions to demonstrate sub-cellular organelles or enzymes, which might be associated with certain types of cellular differentiation. These special studies are useful in establishing the correct diagnosis for a wide variety of pathologic conditions. These studies are also useful in determining a patient's prognosis for a given tumor, such as status of estrogen and progesterone receptors for breast carcinoma. Finally, these studies are also useful in helping the pathologist to determine if a given histologic pattern represents a malignancy, such as demonstration of basement membrane epithelium in prostate needle biopsies.

Materials Required:

1. Collection container filled with 10% buffered formalin for specimens not suspected of being lymphoma or leukemia (see next line). The pathologist will determine if these special studies are needed.
2. Sterile collection container lined with saline soaked gauze for cases suspected of lymphoma.
3. Glass Slides with frosted end for cytology specimens such as Fine Needle Aspirations or bone marrow aspirates.
4. A completed requisition form with proper patient identification, history and source of biopsy.

Procedure:

1. Label the collection container with the patient's name and tissue identification.
2. Submit routine biopsy material in 10% buffered formalin unless lymphoma or leukemia is suspected. The pathologist will determine if special studies are required after studying the routine preparation.
3. For suspected lymphomas, place the fresh tissue in a clean container, which does not contain any fixative material. The tissue should be immersed in saline.
4. Complete requisition form including patient name, birth date, date of service, and billing data. The pathologist will determine if additional studies are needed after initial examination of the specimen.
5. Please note that we can accept specimens Monday through Friday only, from 8:00 A.M. to 5:00 P.M. Advanced warning about specimens being sent fresh for lymphoma work-ups ensures the specimen will be properly processed upon receipt at the laboratory. If unsure of proper submission please call DAPS at 870/930-3518 prior to biopsy excision.

Other Specialty Testing Available

For the following specimens, please try to avoid a collection on Fridays in the afternoon, due to special handling. Please contact the Lab for special instructions.

ChromaVision

DAPS is currently using The Automated Cellular Imaging System, ACIS®, from Clarient to assist in the rapid, specific and sensitive detection of positive stained cells in immuno stained tissue sections. The pathologist can use 'Hot Spots', micrometer measurements, montage markers and visual confirmation

to assist in analysis.

Comment:
This procedure is done off existing tissue blocks processed in our laboratory. There are no special handling or procedure requirements to the clinician.

Chromosomal Studies

Currently, the DAPS does not perform chromosomal studies on tissue or fluid specimens. If requests for chromosome studies are received, we will forward them to the appropriate reference laboratory. Currently we use WCP Labs in St. Louis, MO..

Materials Required:

1. Place tissue in RPMI.
2. Peripheral blood and bone marrow should be place in a Sodium Heparin tube.
3. Completed Requisition Form
4. Contact the DAPS immediately at 870/930-3518.

Skin Biopsy: Immunofluorescent Studies

Currently, DAPS does not perform immunofluorescent studies on tissue or fluid specimens. If requests for immunofluorescent studies are received, we will forward them to the appropriate reference laboratory. Please submit in Michael's solution or saline if Michael's solution is not available.

Biopsy: Lymph Node

Materials Required:

1. Sterile Screw Top Container
2. Sterile Saline
3. Completed Requisition Form
4. Ice

Procedure:

1. Label the body of the container (not the lid) with the patient's name and identification.
2. Place the lymph node in the container and fill with saline. DO NOT SUBMIT IN FORMALIN.
3. Fill out requisition and include name of primary care physician or oncologist.
4. Place container in bio-hazard bag filled with ice.

Biopsy: Prosthetic Breast Implants

Materials Required:

1. Collection containers for implants (submitted without fixative) and containers for fibrous tissue capsules submitted in 10% formalin
2. Completed requisition form.

Procedure:

1. Label the specimen collection containers with the patient's name and specimen identification.
    a. The prosthetic breast implants must be submitted in separate containers without fixation.
    b. The fibrous tissue capsules may be submitted separately in 10% formalin.
2. Complete requisition form including patient name, birth date, date of service, and billing data.
3. Indicate on the requisition the presence or absence of prosthetic rupture or leakage.
4. Tightly close container lids and forward to laboratory with requisition.

Flow Cytometry

Materials Required:

1. Sodium Heparin Tube
2. RPMI
3. Completed Requisition Form

Procedure:

1. Collect blood from the patient and place into the Sodium Heparin tube.
2. Collect tissue from the patient and place into the RPMI.
3. Contact DAPS immediately for pick up (870/930-3518).

Comment:

Flow cytometry is a method for quantitating components or structural features of cells primarily by optical means. Although it makes measurements on one cell at a time, it can process thousands of cells in a few seconds. Since different cell types can be distinguished by quantitating structural features, flow cytometry can be used to count cells of different types in a mixture. Submit these samples in a sodium heparin tube, and contact the lab immediately to schedule a pick-up for this specimen.

Other Laboratory Testing

Peripheral Smear

Materials Required:

1. Stained or Unstained Peripheral Blood Smears (prefer submission of two slides).
2. Attach CBC report, if possible.
3. Completed Requisition Form.

Comment:

This test is aids in the review of various hematologic disorders including, but not limited to Leukemia, Anemia, Malaria, etc.

Laboratory Certificates and Documentation

All laboratory certificates are current and kept on file. If a clinic or provider needs copies of these certificates or physician information, please contact DAPS at 870/930-3518 to request the appropriate documentation. The most commonly used MSDS sheets, which affect clinics and providers serviced by the lab, are available.

Most Commonly Used Pathology CPT Codes

80500 Clinical Pathology Interpretation
82365 Stone Analysis
84165 Protein, Electrophoresis Fractionation and Quantitation, *Discontinued 1/1/03*
84190 Urine Protein, Electrophoresis, *Discontinued 1/1/03*
85060 Peripheral Smear
85095 Bone Marrow, Aspiration Only, *Discontinued*
85097 Bone Marrow Smear Interpretation Only
86320 Immunoelectrophoresis, serum, *Discontinued 1/1/03*
86325 Immunoelectrophoresis, other fluid types, *Discontinued 1/1/03*
86334 Immunoelectrophoresis, urine, *Discontinued 1/1/03*
87207 Tzank Stain
87491 Chlamydia Testing by PCR
87591 Gonorrhoeae Testing by PCR
87621 Hybrid Capture II HPV Test
88104 Non-Gyn Cytology Interpretation
88107 Cytopathology, fluids, washings, brushings, except vaginal or cervical
88108 Cytopathology, concentration technique
88112 Cytopathology, Selective Cellular Enhancement Technique
88141 Pathology Interpretation
88142 Thin Prep Pap Test
88147 AutoPap Conventional Smear, *Discontinued 1/1/02*
88148 AutoPap Conventional Smear, *Discontinued 1/1/02*
88164 Conventional Pap Smear
88173 FNA Evaluation
88180 Flow Cytometry, Each Cell Surface Marker
88300 Gross Only
88302 LEVEL II, Surgical Path, Gross and Micro
88304 LEVEL III, Surgical Path, Gross and Micro
88305 LEVEL IV, Surgical Path, Gross and Micro
88307 LEVEL V, Surgical Path, Gross and Micro
88309 LEVEL VI, Surgical Path, Gross and Micro
88311 Decalcification Procedures
88312 Special Stains I
88312 Special Stains, Acid
88313 Special Stains II
88321 Consultation/Report on Referred Slides
88323 Consultation/Report on Referred Material
88329 Pathology consultation during surgery
88331 Frozen Section
88332 Pathology Consultation during surgery; additional block with frozen section(s)
88342 IHC Stains
88358 Morphometric Analysis, Image Analysis – *Discontinued 1/1/04*
88361 Morphometric Analysis, Image Analysis
89060 Crystal ID