

Cytology

I. Hours of Operation

8:00 AM – 4:30 PM; Monday-Friday

II. Contact Section

449-4938

III. Scope of Testing

Microscopic examination of cells from body tissue and fluids to determine the presence or absence of abnormal or malignant cells, infection and other abnormalities.

IV. Tests Available

The cytological tests consist of those on gynecologic specimens and non-gynecologic specimens.

A. Gynecologic Specimens

Routine screening smears: Surepath, cervical scrape, HPV testing, cytobrush and vaginal pool smear

Lateral vaginal wall scrapings for cytohormonal evaluation

Other: smears of vaginal wall/vulvar lesions etc.

B. Non-GYN Specimens

Endometrial aspirate, brush, or wash

Endocervical canal brush

Sputum

Bronchial aspirate and wash

"Bronchial brush biopsy" (brush biopsies are always cytological specimens not surgical tissue specimens)

Body fluids: pleural, peritoneal and pericardial effusions, spinal fluid, joint cavity fluid, peritoneal wash fluid, cyst fluid, etc.

Urine, urethral brush biopsy and other genitourinary specimens (including those for cytomegalovirus, prostatic secretion, etc.)

Breast specimen

Gastrointestinal specimens: oral, esophageal, gastric, duodenal, pancreatic, biliary/colonic washes

Buccal smears for sex chromatin

Skin vesicle (Tzanck test) and eruption smears

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Fine needle aspirates of solid/cystic organs/lesions, transcutaneous/endoscopic

Other specimens for cytological studies

C. All specimens should be accompanied by a properly completed Cytology Requisition. Information required:

Patient's name is correct on both the slides/container and the requisition.

Physician's name is on the requisition.

Specimen collection date is correct.

Source of the specimen is properly marked on the requisition.

Patient's sex and date of birth is properly marked on the requisition.

Pertinent clinical information is given including:

- Last menstrual period
- Previous pap smear results
- Any results of biopsies or treatment
- Any known factor that could contribute to the
Patient's risk of developing cervical cancer

The slides are identified with the patient's name on one end of the frosted slide in pencil. All GYN smears should be immediately fixed with spray fixative. Unfixed smears are not accepted in the Cytology Laboratory for Pap stains. Fresh specimens (effusion fluid, urine, etc.) upon collection from the patient should be promptly transported.

All cytology specimens should be submitted to the Laboratory separately from portions for microbiologic, hematologic, or chemical study. Prompt handling of unfixed material is necessary to prevent degeneration of the exfoliated cells, which renders the specimen unsatisfactory. Unfixed materials should be submitted to the laboratory within minutes after being obtained. Cytology personnel may be called to assist with specimen preparation.

Please allow a 5 working-day-turnaround time for routine Pap smear reports. Cytology GYN smear results are reported using the Bethesda System of Classification.

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V. SPECIMEN COLLECTION PROCEDURES

A. GYNECOLOGIC SPECIMEN (*Cyto/Gyn*):

Routine screening smears (cervical scrapings; HPV testing; cytobrush or aspirate; vaginal pool smear), Endometrial aspirate, brush or wash.

Lateral vaginal wall scraping smear for cytohormonal evaluation.

1. General Rules in Obtaining Specimens

- a. Explain the procedure to the patient. Instruct the patient **NOT** to douche on the day the smears are to be taken. Bleeding and douching within 24 hours prior to obtaining the specimen causes a higher percentage of unsatisfactory specimens, and the patient should be advised that it might be necessary to repeat the study.
- b. Use spray fixative, which should be open and readily accessible before the specimen is obtained. Exfoliated cells dry rapidly once they are spread out on the glass slide so the slide must immediately be fixed when the smear has been made.
- c. If spray fixative is not available, slides may be immersed in 95% ethyl alcohol for 15 minutes.
- d. Talcum powder or starch should be wiped from the gloved finger before the smear is made to prevent obscuring the cells with this material.
- e. The speculum must be introduced without lubricant. If necessary, normal saline may be used to moisten the speculum.

2. Equipment and Materials Needed to Obtain Specimens from the Female Genital Tract (Conventional Method)

- a. Speculum (without lubricant) for adults, or nasal speculum for children.
- b. Cervix brush or other plastic broom for pan-cervical-endocervical sampling.
- c. Cervical spatula (Ayre scraper or similar device) for cervical or vaginal wall scrapings.
- d. Cytobrush or other endocervical brush for endocervical sampling.
It is preferable not to use the cytobrush on pregnant patients.
- e. Cotton swabs are **NOT** recommended.

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- f. Clean glass slide(s) (one end frosted), identified with the patient's name on the frosted end in pencil
- g. Gloves
- h. Saline (normal physiologic saline)
- i. Spray fixative can, or Coplin jar of 95% ethyl alcohol
- j. Slide holder(s): after the slide(s) has/have dried, place it/them (unwrapped) in cardboard slide holders

The following may also be needed for special procedures:

- k. Cervical aspiration: two cured glass or disposable plastic pipettes attached to rubber bulbs
- l. Special endometrial cannula and brush for endometrial aspiration and brush biopsies

3. Sample Collection

Note: Cytological specimens should be considered infectious until fixed with a germicidal fixative. Observe Universal Precautions when handling specimens from all patients.

Visual inspection of the lower genital tract and cervix through the speculum is a prerequisite to optimal sample collection. The speculum must be positioned in a way that the entire face of the cervix appears at the end of the instrument, since a sample from this area is necessary for adequate specimen collection. A large cotton-tipped swab is often useful for helping position the cervix.

It is important to obtain a smear not obscured by blood, mucus, or inflammatory exudate. Following correct positioning of the speculum in the vagina, if excess mucus or other discharge is present, gently remove it with ring forceps holding a folded gauze pad. Inflammatory exudate may be removed by placing a dry 2x2 gauze pad over the cervix and peeling it away after it absorbs the exudate, or by using a dry proctoswab orscopette. Do not clean the cervix by washing with saline since this may result in a relatively acellular smear.

An optimal cervical specimen includes sampling of the squamous and columnar epithelium, encompassing particularly, the transformation zone where the majority of cervical neoplasias arise. The specific sampling instrument(s) and sampling technique used should be based on a consideration of individual patient anatomy, particularly the location and configuration of the transformation zone as determined by visual inspection.

- a. **Collection Procedure using Cervix brush and a liquid preservative [direct-to-vial, e.g. SurePath; (AutoCyte)]**

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Equipment needed:

- Vial with 10 mL of proprietary liquid preservative (*AutoCyte*)-
- Cervix brush or similar device-
- Gloves-
- Requisition-
- Speculum (without lubricant) for adults, or nasal speculum for children
 1. Label the vial. Open the vial. Observe Universal precautions for collecting and handling specimens. Insert the speculum (which may be slightly moistened with water or saline, if necessary). No other lubricants should be used.
 2. Visually inspect the cervix for abnormalities. Identify the transformation zone (if visible) and direct sampling efforts to encompass this area.
 3. ***Comment: If an elevated, ulcerated, necrotic, or exudate-covered lesion is observed, arrangements should be made for a biopsy following cytology sampling.***
 4. Using the Cervix ("broom-like") brush, insert the long, central bristles into the os until the lateral bristles bend against the ectocervix. Rotate the brush for 5 full clockwise turns while avoiding excessive bleeding.
 5. Immerse the head of the brush in the liquid preservative vial. Detach the head of the brush. Close the vial tightly. Shake briefly. Submit the specimen to the laboratory with a properly completed Cytology Requisition.
 6. HPV testing can be performed on residual liquid sample (Reflex testing of new ASCUS or specifically requested)

b. Collection Procedure Using Ayre-type Spatula and Cervical Brush

1. Label the frosted end of the glass slide with the patient's name prior to the sample collection. Observe Universal Precautions for collecting and handling specimens. Insert the speculum (which may be slightly moistened with water or saline, if necessary). No other lubricants should be used.
2. Visually inspect the cervix for abnormalities. Identify the transformation zone, if visible, and direct sampling efforts to encompass this area.

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Comment: If an elevated, ulcerated, necrotic, or exudate-covered lesion is observed, arrangements should be made for a biopsy following cytology sampling.

3. Choose the contoured end of the spatula which best conforms to the anatomy of the cervix and the location of the transformation zone. Rotate the spatula 360° about the circumference of the cervical os and ectocervix, while maintaining firm contact with the epithelial surface

Comment: A clockwise rotation beginning and ending at 9 o'clock (or counterclockwise rotation from 3 o'clock to 3 o'clock) will position the spatula so the collected material is retained on the upper horizontal surface as the instrument is removed.

4. Do not smear the sample at this time unless you are going to immediately fix the specimen (see options 6c and 6d below). Hold the spatula between the fingers of the non-sampling hand (or rest it on the glass slide) with the specimen face-up, while the cervical brush material is collected without delay.
5. Insert the cervical brush into the os with gentle pressure and rotate only 90° to 180° to minimize bleeding.

Comment: Brushes have circumferential, radiating bristles that come in contact with the entire os surface upon insertion. This is in contrast to the edge of a spatula that is in contact with only a fraction of the epithelial surface at any one time. Therefore, the brush need only be rotated one quarter turn (90°) while the spatula must be rotated a full turn (360°).

6. Spread the material collected on the spatula evenly over a glass slide with a single smooth stroke motion. Roll brush across the glass slide by twirling the handle (see Figure 4).

Comment: The goal is to quickly and evenly spread the cellular material in a thin layer on the glass slide. Thin-out large clumps of material as much as possible, while avoiding excessive manipulation which can damage cells. Transfer the material from both sampling instruments to the slide within a few seconds and fix immediately, in order to avoid air-drying artifact. Three options for transferring the material to the glass slide are offered:

- a. Smear the spatula sample across the upper longitudinal half of the slide; roll the brush across the lower longitudinal half of the slide. Immediately fix the slide.

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- b. Smear the spatula sample across the slide, roll the brush material directly over the previously spread sample. Immediately fix the slide.
- c. Smear the spatula sample over the left-hand side of the slide, cover the right-hand side with cardboard and immediately spray fix. Roll the brush material onto the right-hand side of the slide and immediately spray fix.

Comment: Note that with technique 6c, the spatula specimen may be spread and fixed before obtaining the endocervical brush sample.

7. Immediately fix the specimen by either immersing the slide in 95% ethanol or coating the slide with a surface fixative. If using spray fixation, hold the container 12 inches from the slide to avoid "blasting" the cells.

c. Collection procedure using the Cervix brush, conventional smear, and a liquid preservative for residual sample (including HPV specimens)

Equipment needed:

1. All the materials for procedure b (above) plus the following:
Vial with 10 mL of proprietary liquid preservative (*AutoCyte*)
2. Label the vial. Open the vial. Observe universal precautions for collecting and handling specimens. Insert the speculum (may be slightly moistened with water or saline, if necessary). No other lubricants should be used.
3. Visually inspect the cervix for abnormalities. Identify the transformation zone (if visible) and direct sampling efforts to encompass this area.

Comment: If an elevated, ulcerated, necrotic, or exudate-covered lesion is observed, arrangements should be made for biopsy following cytology sampling.

4. Using the Cervix ("broom-like") brush, insert the long, central bristles into the os until the lateral bristles bend against the ectocervix. Rotate the brush 3-5 full, clockwise turns while avoiding excessive bleeding.
5. Spread the material collected on the brush to the labeled glass slide with a single length-wise stroke for each side of the brush. This must be done very quickly.

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6. Immediately fix the specimen by coating the slide with a surface pump-spray fixative (see types of fixatives below).
7. Immerse the head of the brush in the liquid preservative vial. Detach the head of the brush. Close the vial tightly. Shake briefly. Submit the slide and the vial to the laboratory with a properly completed cytology requisition.

d. Other Collection Instruments

1. Plastic Broom (another collection instrument) a plastic "broom-like" brush: simultaneously sample the endocervix and ectocervix. To use the "broom", the long central bristles are inserted into the os until the lateral bristles bend against the ectocervix and are rotated a total of 5 times in clockwise and counter-clockwise directions. To transfer material, both sides of the "broom" are stroked across the slide.
2. Cotton Swab: use of a cotton-tip applicator is **NOT** recommended - it usually provides less cellular samples, possibly because of trapping material in the cotton fibers.

e. Special Collection Procedures

Samples for hormonal evaluation should be obtained separately using a spatula to gently scrape the epithelium from the upper third of the lateral vaginal wall. The separate slide should be labeled as to the site. The requisition should indicate a request for hormonal evaluation and provide relevant patient information.

4. Transferring the sample(s) to the slide

- a. To transfer material from the spatula: Smear sample with a single stroke motion using moderate pressure to thin out *clumps of* cellular and mucus material. Avoid excessive force or manipulation, which will damage cells.
- b. To transfer material from the brush: roll the bristles across the slide by twirling the brush handle.
- c. To transfer material from the broom: smear sample with a painting action, using both sides of the broom.

5. Options for transferring the sample(s) to the glass slide(s)

- a. Smear the spatula sample across upper half of slide; roll brush across lower half and fix.
- b. Smear spatula sample across the slide; roll brush directly over top and fix.
- c. Spread spatula sample over left side of slide and fix while covering right side. Roll brush over right side of slide and fix.

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6. Slide Fixation

- a. Fixatives are agents used on gynecologic smears to prevent cell distortion and to maintain true morphologic structure. Distortion due to improper fixation nearly always prevents proper and accurate evaluation of the cell population.

- b. Timing

After the specimen has been spread evenly on the slide, the slide should be fixed *immediately*. The interval between application and smearing of the specimen onto the slide and subsequent fixation should be kept to a minimum.

- c. Types of Fixatives

- 1. Proprietary Liquid Preservatives

- 2. Coating Surface Fixatives: Coating fixatives (alcohol with Carbowax) are those which cover the surface of the prepared smears. Pressure spray from a commercial cytofixative spray can, electric pump, or a dropper from a dropper bottle may apply the coating fixative. When coating or spray fixatives are used, the nozzle of the spraying apparatus should be held approximately 12 inches from the slide. Holding the spray fixative container too close to the slide can result in cellular artifacts and holding the spray fixative container too far from the slide may result in drying artifacts or uneven fixation. Holding the spray fixative too close to the slide can also result in flooding the slide and washing or blowing away the cells. (Use of commercially available hair spray is a common practice in some parts of the country, but its use *is discouraged* because of the variability in ingredients. These cosmetic aerosols may result in very poor specimen preservation.)

- 3. 95% Ethanol (Wet Fixation) - 95% ethanol is widely accepted as an ideal cellular fixative for gynecologic/cytological smears. (It is advisable to add Carbowax™ to the alcohol before fixing slides to be air dried and mailed. Carbowax™ will protect cells from air drying artifact). 95% Ethanol is placed in an appropriate container and the freshly prepared smear is immersed immediately into the fixative. Fixation occurs in 5 to 30 minutes.

- 4. Air drying - Air-drying is the absence of fixation. Air-drying produces artifacts and cellular distortion and may lead to misinterpretation of smears. Air drying of a Pap smear is *not recommended*. Delayed fixation can also result in air-drying artifact.

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B. RESPIRATORY TRACT

1. Sputum

When a pulmonary tumor is suspected, a complete sputum series is recommended. The complete sputum series consists of an early morning specimen each day for three days. Do not submit 24-hour specimens.

a. Material Needed Fixative in Saccomanno sputum fixative cup.

1. Technique

a. Provide the patient with a sputum container with fixative to be available early in the morning when the patient awakes.

CAUTION: The fixative contains ethylene glycol and is hazardous if swallowed. Instruct the patient of this danger and avoid mixing the specimen cup with the patient's drinking glass, etc.

b. Instruct the patient to cough deeply "from the diaphragm" upon awakening and expectorate all sputum into the cup. Encourage the patient to expectorate deep sputum, not saliva.

c. The patient should continue the deep coughing and expectoration until at least 3 mL are collected.

d. Deliver the sputum cup to the Laboratory in the morning with the requisition.

e. Repeat the procedure each day for three days.

2. Bronchial Specimens

a. Cytological specimens may be obtained during bronchoscopy by:

1. aspiration of secretion

2. brush biopsy

3. bronchial swab or a bronchial brushing, in addition to a bronchial biopsy,

4. forceps rinse after bronchial biopsy

5. bronchoalveolar lavage

6. Transbronchial (Wang) Needle Biopsy

As the findings at bronchoscopy are usually not predictable beforehand, the operator must be prepared to obtain material by any means that proves to

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be most desirable at examination time. The surface of suspicious areas should be aspirated, brushed or swabbed, or biopsied.

If sufficient secretions are present or a lesion is not visualized, lavage the bronchus in question. Sometimes, rinsing the biopsy forceps in normal saline will provide additional material for examination. **FOR FULL DIAGNOSTIC VALUE, ALL MATERIAL SHOULD BE SUBMITTED AND IDENTIFIED AS TO SITE OF ORIGIN.**

The bronchial brush biopsy specimen is always processed as a cytological specimen in the Cytology Laboratory, whereas, the bronchial tissue biopsy is processed as a surgical tissue specimen and should be separately submitted to Surgical Pathology as such. All microbiologic, hematologic, or chemical specimens should be separated from the cytological specimens at the time of bronchoscopy and submitted separately.

All bronchoscopic materials for Cytology (bronchial aspirate, bronchial brush biopsy, bronchial swab, bronchial washing, etc.) must be delivered promptly. If a long delay is anticipated, or if it is after hours, refrigerate all materials.

7. Material Needed

- a. Bronchial aspirator, one or more
- b. Bronchial brush, one or more
- c. Five or more clean, coated, glass slides. The slides are identified with the patient's name and specimen source on one end of the frosted slide in pencil.
- d. Coplin jars of fixative (95% ethyl alcohol) or cyto spray fixative
- e. One bottle of physiologic saline and equipment for lavage
- f. Saccomanno's solution

8. Technique

- a. Bronchial Aspiration
- b. Aspirate secretions as encountered
- c. Label site of procurement, e.g., bronchial branches and laterality
- d. Set aspirate aside, properly labeled, until completion of procedures
- e. Bronchial Brush
- f. Brush the surface of suspicious areas completely.

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- g. Withdraw the brush.
- h. Quickly spread or roll it on a coated, glass slide held over an open bottle of fixative and IMMEDIATELY drop the slide into the fixative. Cytospray fixation may also be used. Immediate fixation is essential to avoid drying (which occurs rapidly after material is spread onto the slide).
- i. Label the site of procurement on the requisition - suspicious area in the left main stem bronchus.
- j. After the smear is made, the brush is placed in saline (available from the lab), and sent to the Laboratory.
- k. Repeat with other suspicious areas, using a fresh brush.

3. Bronchial Biopsy

- a. With biopsy forceps biopsy the suspicious area.
- b. Place the biopsy in a small container of 10% buffered formalin, and submit to Surgical Pathology.
- c. Rinse the biopsy forceps in a small container of saline, label, and submit to the Cytology Laboratory with the other specimens.

4. Bronchial Washing

- a. Turn patient so that bronchus in question is dependent.
- b. Fill the bronchus to the carina with normal saline.
- c. Allow the saline to stand in contact as long as possible.
- d. Aspirate the washing, label as to site of procurement and set aside until the procedure is complete.

5. Bronchoalveolar Lavage

- a. Bring all bronchoalveolar lavage specimens, in the fresh state, immediately to Cytology. If after hours, refrigerate in the fresh state and call the Pathology resident on call.
- b. After examination is completed, collect postbronchoscopy sputum IMMEDIATELY. Have the patient cough deeply and expectorate. Collect an adequate amount of sputum into a sputum cup with fixative.

6. Transbronchial (Wang) Needle Biopsy

- a. Cytology may be contacted to assist.

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- b. Refer to Section J: Fine Needle Aspiration Specimens.

C. URINARY TRACT

Urine may contain cells exfoliated from cancers of the bladder, ureter, renal pelvis, or kidneys. Cytological study of a urine specimen requires an adequate amount of specimen and, most of all, well-preserved cell materials. First morning urine specimen should not be sent for cytological studies (since the first morning urine is usually made up of degenerative exfoliated cell materials and concentrated urine waste products, which obscure the cellular detail). At least 50-100 mL of urine is required for cytology. Voided urine from males and "clean catch" urine from females is satisfactory. In cases with residual urine problems or with severe urethritis or vaginitis, the urine should be obtained by catheterization.

1. Materials Needed
 - a. Routine urinalysis container
 - b. On occasion, straight urethral catheter
2. Technique: The ideal technique for urine collection for a cytological specimen is:
 - a. Have the patient drink one glass of water every 15 minutes for 2-3 hours.
 - b. At the end of two hours, have the patient void and discard the urine.
 - c. One hour later, clean the penis or urethral area as for a clean-catch midstream specimen.
 - d. Have the patient void and save the specimen.
 - e. Send the specimen **IMMEDIATELY** to the Cytology Laboratory with a requisition.
 - f. Repeat for three (3) successive days.
 - g. If immediate delivery is not possible, add the urine to an equal volume of 50% ethanol. Label this as fixed urine.

D. BODY FLUIDS

Body fluids should be submitted FRESH, UNFIXED. Exfoliated cells deteriorate in the effusion both in and out of the body. This deterioration is very rapid in the presence of blood. If clotting occurs, diagnostic material may be trapped within the fibrin network and unavailable for satisfactory examination. The entire volume of fluid collected should be submitted to the Cytology Laboratory, except an adequate amount of specimen should first be collected in separate containers for Microbiology, Hematology, and Chemistry studies, as requested. Collect hematologic specimens for cell counts in EDTA (do **NOT** use anticoagulant for microbiologic, immunologic, and chemical specimens). Add heparin

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to the material for Cytology only after the other specimens have been collected, and only if the material may clot.

1. Materials Needed
 - a. One flask or other suitable container (recommend sterile glass or plastic screw-capped specimen cup)
 - b. One paracentesis set
 - c. Three units of heparin per mL of fluid
 - d. Separate tubes for Hematologic (EDTA), Microbiology, Immunology, and Chemistry specimens
2. Technique
 - a. Place heparin (3 units/mL of fluid anticipated) into the cytology receiving bottle.
 - b. Perform the paracentesis.
 - c. Collect separately: Hematologic (EDTA), Microbiology, Immunology, and Chemistry specimens in separate appropriate small tubes. Do not take them from heparin bottle.
 - d. Gently agitate the bottle for Cytology (the remaining fluid is collected in order to mix the heparin with the fluid).
 - e. Send specimen to the Cytology Laboratory refrigerated or on ice, with a requisition.

E. BREAST NIPPLE SECRETION

Smears of nipple secretions may be utilized in the detection of breast cancers that involve ducts. **Do not massage or squeeze the breast** too vigorously - manipulation may dislodge and spread malignant cells.

1. Materials Needed
 - a. Coated glass slides. The slides are identified with the patient's name and specimen source, including laterality, on the frosted end in pencil.
 - b. One Coplin jar of fixative (95% ethyl alcohol).
2. Technique
 - a. Open the fixative bottle and have the patient hold the bottle near the breast.

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- b. Gently express from the nipple and subareolar area any secretions that may be lying in the collecting ducts. If no secretion appears at the nipple with this gentle compression - **do not manipulate further.**
- c. Allow a drop of fluid to collect upon the nipple tip.
- d. Immobilize the breast and using the nipple, smear the material across a slide.
- e. **IMMEDIATELY** drop the slide into the fixative. The smearing of the material across the slide and dropping the slide into the fixative should be accomplished in one motion.
- f. Make as many smears as the amount of the material allows.
- g. Indicate "right" or "left" breast and give all clinical information on the requisition.

F. BUCCAL SMEAR FOR BARR BODIES (CHROMOSOMAL SEX DETERMINATION)

The cytotechnologists in the Cytology Laboratory will take buccal smears upon request.
Call 449-4938.

1. Materials Needed
 - a. One metal spatula
 - b. Glass slides (all-frosted). Identify the slide by printing the patient's name on the end with pencil.
 - c. One Coplin jar of fixative (95% ethyl alcohol)
 - d. Coverslips
 - e. Mouthwash
 - f. Sterile cotton swabs
 - g. Requisition from Nursing Station
2. Technique
 - a. Adults: have patient rinse mouth with mouthwash and then rinse with water.
 - b. Infants/children: swab the buccal mucosa with a clean cotton swab dipped in mouthwash and swab again with water to remove the mouthwash.
 - c. Firmly draw the metal spatula along the buccal surface of the cheek, collecting white cellular fluid. Scrape forward several times to collect adequate cellular material.

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- d. Quickly smear the cellular material on slides. Gently flatten the material with a coverslip and the thumb. Draw the coverslip off in a horizontal motion and **IMMEDIATELY** drop the slide into the open Coplin jar containing 95% ethyl alcohol.
- e. Usually 2-3 smears each are taken from the right and left buccal mucosa. Label slides "right" and "left", respectively.
- f. Specimens should be brought directly to the Cytology Laboratory by the cytotechnologist.

G. CEREBROSPINAL FLUID (CSF)

Cerebrospinal fluid for cytology requires a separate requisition. Initially, specimens are received and processed in the Hematology Laboratory. It is important that CSF specimens are brought to the Laboratory immediately. In most cases, a cellblock is not made from cerebrospinal fluid because of insufficient sediment.

H. GASTROINTESTINAL TRACT

Because of the nature of the gastrointestinal tract, adequate preparation of the patient is essential to obtain satisfactory specimens. If possible, Cytology procedures should be performed prior to barium examinations. Otherwise, wait at least 24 hours after barium examination before attempting a cytological study.

1. Esophageal Wash for Cytology
 - a. Materials Needed
 1. One #18 Levine tube - cut additional perforations in the terminal @ 6 cm
 2. One 100 mL syringe
 3. One 100 mL container packed in ice
 4. Two 500 mL containers packed in ice
 5. 1000 mL iced normal saline
 6. All coated slides
 7. One Coplin jar with fixative (95% ethyl alcohol)
 - b. Technique (at the option of the physician)
 1. The patient should fast for eight hours prior to the procedure and have only a "soft" supper the night before.
 2. Pass the tube through the mouth to about the 22-inch mark. Do not use lubricant.

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3. Aspirate any material present and discard. If any obstruction is present, do not force the tube. After aspiration, the area may be washed with saline that is discarded.
 4. Have the patient slowly drink 100 mL of cold saline while continuous aspiration is done. Place the aspirated fluid in a 100 mL iced container labeled "swallow".
 5. Withdraw the tube 3-5 cm and gently introduce 50 mL of cold saline and immediately aspirate it back. Place this fluid in a 500 mL iced container labeled, "lavage". Without obstruction, only about 50% of the fluid will be recovered. If the patient takes a deep breath and holds it, recovery is increased.
 6. Withdraw the tube 3-5 cm each time and repeat step 5. Pool the lavage specimens. Repeat until the last mark on the tube is 3 inches past the teeth.
 7. It may be advisable to collect gastric lavage specimens for cytology in some cases of suspected esophageal tumor.
 8. Send all of the fluid specimens **IMMEDIATELY** to the Cytology Laboratory on ice (0800-1630 M-F).
 9. After hours, send to Central Deposit in the Laboratory to be refrigerated.
2. Gastric Wash for Cytology
- a. Materials Needed
 1. One #18 Levine tube
 2. One 100 mL syringe
 3. Two 100 mL specimen containers (tubes) packed in ice
 4. One 500 mL specimen container, packed in ice
 5. 1000 mL of iced normal saline
 6. Coated slides
 7. Coplin jars with fixative (95% ethyl alcohol)
 - b. Technique (at the option of the physician)
 1. The patient should have only a "soft" supper the night before and then fast until the procedure is completed. Water is permitted.

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2. Pass the tube through the mouth. Empty the stomach and discard fasting specimens.
 3. Position the patient in a reclining position, comfortably.
 4. Introduce 50 mL of cold normal saline rapidly under pressure, aspirate the fluid and reintroduce it under pressure 2-4 times, then aspirate as much as possible from the stomach. Place the fluid in the first 100-mL specimen container.
 5. Turn the patient on one side.
 6. Repeat with another 50 mL of cold normal saline. Introduce saline under pressure several times and empty the stomach. Place the fluid in the second 100-mL specimen container.
 7. Repeat such saline washing with the patient in other positions, if necessary, until the aspirated fluid is clear.
 8. Introduce 400 mL of cold normal saline under pressure.
 9. Have patient roll in bed for a while.
 10. Aspirate and reintroduce portions of the fluid under pressure with the patient in various positions. Finally, aspirate as much of the fluid as possible and place it in the 500-mL specimen container (which is packed in ice).
 11. Send the entire fluid specimen **IMMEDIATELY** to the Cytology Laboratory on ice (0800-1630 Monday-Friday).
 12. After hours, send to Central Deposit in the Laboratory to be refrigerated.
3. Duodenal-Pancreatic-Biliary Washes for Cytology
- a. Materials Needed
 1. One double lumened Rehfuss tube
 2. One 100 mL syringe
 3. Two 100 mL specimen containers, packed in ice
 4. 1000 mL of iced, normal saline
 - b. Technique
 1. The patient is intubated with double lumened Rehfuss tube, fluoroscopically positioned.

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2. 100-150 mL of iced normal saline is introduced into the duodenal side of the tube, aspirated and discarded.
3. Another 100 mL of iced saline is introduced, aspirated, and placed in an iced specimen container.
4. Send the fluid specimen **IMMEDIATELY** to the Cytology Laboratory on ice (0800-1630 Monday-Friday).
5. After hours, send to Central Deposit to be refrigerated.

NOTE: When the Rehfuss tube is in place, duodenal washes are obtained as above. It is also possible to obtain specimens more representative of the pancreatic and biliary cytology.

- a. Administer secretin and collect washings as in 1-5. This specimen is more representative of pancreatic material.
 - b. Administer cholecystokinin and collect washings as in 1-5. This material is more representative of the biliary duct system.
4. Colon Cytology
- a. Materials needed
 1. Saline enemas
 2. Proctoscopy
 3. Ewald tube
 4. Bedpan
 5. 1000 mL of iced, normal saline
 6. Two 500 mL specimen containers packed on ice
 - b. Technique
 1. The patient should be on a clear, liquid diet for at least 24 hours and should receive acathartic the evening before.
 2. With the patient on the left side, four or five saline enemas are administered to the patient (2500-3000 mL each), or until clear. The patient should be on the right side or back before expelling each enema.
 3. Prepare the patient for proctoscopy.
 4. Pass an Ewald tube through the proctoscope into the rectum and remove the proctoscope.

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5. Introduce 1000 mL of cold, normal saline with the patient in various positions (right, left, prone, etc.).
 6. Remove the rectal tube, massage the abdomen, collect the specimen in a clean bedpan and transfer it to the iced specimen containers.
 7. Send all of the fluid specimens **IMMEDIATELY** to the Cytology Laboratory on ice (0800-1630 Monday-Friday).
 8. After hours, send to Central Deposit for refrigeration.
5. Brush Biopsy or Swab of Lesions of the Gastrointestinal Tract
- a. Follow the procedure for bronchial brush biopsy or swab to prepare Cytology slides.

I. DIRECT SCRAPINGS FOR SMEARS (MOUTH, PHARYNX, SKIN, ETC.)

1. Materials Needed
 - a. Metal spatula, non-absorbent cotton swab
 - b. Physiologic saline solution
 - c. Coated, clean glass slides. The slides are identified with the patient's name on one end of the frosted slide in pencil.
 - d. Coplin jar with fixative (95% ethyl alcohol)
2. Technique
 - a. If the lesion is moist:
 1. Open the bottle of fixative
 2. Scrape the lesion with the metal spatula
 3. Smear the materials from the blade onto a coated glass slide held over the open bottle of fixative. **IMMEDIATELY** drop the slide into the fixative.
 4. For a Tzanck test for viral inclusions in a vesicle, open the vesicle and scrape the material from the base and smear it (as in 3).
 5. Prepare the requisition with adequate clinical information.
 - b. If the lesion is dry or has a necrotic and inflammatory surface:
 1. Open the bottle of fixative.
 2. Moisten and gently remove the necrotic debris with a nonabsorbent cotton swab that has been dipped in saline solution.

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3. Discard the swab and debris.
4. Using a second nonabsorbent cotton swab that has been moistened in saline, gently rub the margins of the lesion.
5. Quickly roll and spread the swab material on an all-frosted glass slide held over the open bottle of fixative and **IMMEDIATELY** drop the slide in the fixative.

J. FINE NEEDLE ASPIRATION SPECIMENS

Fine needle aspiration may be done on any palpable mass or on deep masses with image guidance. The latter should be scheduled with the Department of Radiology, who will in turn, arrange for Cytology coverage.

For clinics in the Creighton University Medical Center complex during week days/working hours, a cytotechnologist can attend the procedure to receive the specimen, make smears, and split the specimen for various tests, as needed. The cytotechnologist must know beforehand of any other laboratory tests that will be required of the specimen.

Patients may also be referred to the FNA Clinic at Creighton University Medical Center, Department of Pathology. Please call (402) 449-4938 for further information.

For other facilities, we recommend the following procedure:

1. Materials needed:
 - a. Label 2-4 coated slides (clear polished, precleaned, with frosted ends) with the patient's name.
 - b. Have spray fixative ready for immediate fixation.
 - c. Sterile needles, 22-25 gauge (1.5" is the usual length)
 - d. Alcohol swabs
 - e. Sterile gauze pads (2x2 or 4x4)
 - f. Test tube (red top or screw cap) or other small specimen container with balanced salt solution or Saccomanno's solution
 - g. A syringe gun (e.g., Cameco, Aspir-gun etc.) is recommended.
 - h. Syringes, 10 or 20 cc, depending on syringe gun used
2. Technique
 - a. Explain the procedure to the patient.
 - b. Palpate the lesion

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- c. Remove the needle from the syringe.
- d. Draw air into the syringe.
- e. Put the needle back on the syringe.
- f. Place the needle, bevel side down, on the surface of the slide and express the needle contents onto the slide in a small drop (2-3 mm is about right). If there is more material in the needle, make another slide in preference to using a bigger drop.
- g. Using another labeled and clean slide, compress each drop into a dime- or quarter-sized spot and pull the slides straight apart.
- h. Immediately spray fix one slide, allow the other to air-dry (2 slides per pass should be adequate, no more than four should be needed).
- i. Gently flush the needle with the balanced salt solution and submit these needle washings in a red top or screw top tube or other small, labeled container.
- j. Physician must provide all pertinent clinical information.