

**The Cytopathology Laboratory**  
**Building 10, Room 2S238**  
**Phone, (301)-480-7430**

**Regular Working Hours:** 8:30 a.m. to 5:00 p.m., Monday through Friday, excluding holidays.

**Emergencies After Working Hours:** Contact pathology resident on call through page operator (301) 496-1211 or admissions office (301) 496-3315.

**Standard Procedures**  
**Date Reviewed 11-28-2017**

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## **SECTION I: THE CYTOPATHOLOGY LABORATORY - STANDARD PROCEDURES**

### **1.1 Description of Cytopathology Service NIH-NCI**

The Cytopathology Section provides complete diagnostic service in exfoliative cytology and fine needle aspiration cytology for the Clinical Center of the National Institutes of Health. In medical practice today cytology is no longer simply a screening modality, but rather provides definitive diagnoses which direct patient care and treatment. When appropriate we utilize ancillary diagnostic techniques such as immunocytochemistry, flow cytometry, electron microscopy or molecular studies to confirm interpretations made by routine light microscopy or enhance cytologic diagnostic accuracy. The services we provide include:

- Specimen processing for protocols and diagnostics, including Immunocytochemistry.

- Full service fine needle aspiration service
- Routine case review with 24-hours turn around time.
- Rush and STAT service (within 1-2 hours).

The fine needle aspiration service is designed to afford maximum flexibility for clinicians and patients. Clinicians may request that: (1) a pathologist perform the aspiration; (2) a cytotechnologist assist the clinician in handling the specimen and assessing specimen adequacy; (3) aspirations of deep lesions be performed by the radiologist with the assistance of a cytotechnologist to evaluate the adequacy of the specimen.

The Cytopathology Section supports clinical protocols through providing diagnoses on in patient and out patient protocol patients as well as through direct support for protocol related clinical studies.

- Cytotechnologists at the NIH-NCI are not permitted to read more than 100 slides per 24 hour working day per tech (as per CLIA Regulations).

**Full service fine needle aspiration service. Some guidelines:**

- A. We prefer to perform our own aspirations, however, we do provide technical assistance for clinician-performed FNAs. **DO NOT PERFORM AN FNA WITHOUT CALLING US FIRST, AS OUR TECHNOLOGISTS WILL ASSIST YOU AND ENSURE THAT THE SPECIMEN IS PROCESSED APPROPRIATELY**
- B. Our cytotechnologist will provide assistance and adequacy assessments for FNA's. Cytology orders must be present in CRIS at the time of the procedure.
- C. Schedule FNA's in advance, preferably with 24 hours notice. If this is not possible (e.g. Patient has new lump and their airplane is leaving in 20 minutes...), we will be as flexible as we can, however, please be considerate of our doctors and technologists schedules.
- D. Case review (must be scheduled in advance). We will be as flexible as possible, however, please be considerate of our doctors daily case review responsibility which must take priority.
- E. Rush and STAT service when indicated (i.e. when necessary for immediate treatment of patient). This request **must be discussed with the Cytopathologist** who is on service.
- F. A cytopathologist will call you with the diagnosis if this is requested or if the diagnosis is unexpected.
- G. Lab hours are from 8:30 a.m. to 5:00 p.m., Monday through Friday. After 5:00 p.m., please place specimens in the refrigerator outside of Room 2S256 with the completed requisition slip. They will be processed the next morning, however, this will not delay the 24-hour turn around time. If special studies are indicated on the case, please speak to the Cytopathologist on service, who will ensure that the specimen is handled appropriately.

**ALL SPECIMENS ARE TO BE SUBMITTED WITH NO FIXATIVES ADDED AND WITH CRIS GENERATED CYTOPATHOLOGY ORDER REQUISITION.**

Please make sure that each requisition contains the appropriate clinical information as well as your name and beeper number. Specimens will not be processed without a computer generated requisition.

**II. Specimen Rejection Policy**

If a specimen does not meet all of the above criteria, it will not be processed, and the ordering physician or nurse will be notified by telephone. **Rejected specimens and the reason for rejection are entered into the ORS Occurrence Reporter System for Quality Assurance.**

**1.2 CYTOLOGY SPECIMENS RECEIVED AFTER HOURS**

**I. PRINCIPLE**

The Cytology Laboratory is open from 8:30 am to 5:00 PM Monday through Friday. Specimens that arrive before 8:30 am and after 5:00 PM Monday through Friday or on weekends and holidays are to be handled by the procedure below to ensure preservation of the specimens.

## II. PROCEDURE

- A. Delivery personnel must put all specimens with accompanying CRIS Order Requisition Form into the refrigerator outside of Room 2S256.
- B. Specimens may be held refrigerated in the clinic or ward and submitted to cytology during normal working hours (see above).

## AFTER-HOURS PROCEDURE FOR POTENTIALLY MALIGNANT CEREBROSPINAL FLUIDS

### I. PRINCIPAL

Due to the possibility of cellular degeneration in potentially malignant CSF specimens, we have set up a protocol for the processing of CSF specimens when the Cytopathology Section is closed. The intention is for the hematology section to process only cases where there is a strong clinical suspicion of involvement with a malignant neoplasm that would require excellent cellular preservation and potentially require immunoperoxidase studies for diagnosis. The protocol should be complied with as follows.

### II. PROCEDURE FOR CLINICIAN

- A. Patient as described above has CSF tap when the Cytopathology lab is closed (after 5 p.m., or weekend/holiday).
- B. Patient is considered to be high risk for CSF involvement with neoplasia by attending physician.
- C. Attending physician notifies clinical fellow of the necessity for immediate specimen processing by hematology lab.
- D. Clinical Fellow brings the **CYTOLOGY SPECIMEN ALONG WITH THE HEMATOLOGY SPECIMEN** to the **Hematology Section of Clinical Laboratory** (Building 10 / Room 2C390) for processing and requests for the hematology laboratory technologist to follow the "after hours" protocol for cytology specimens.
- E. The ordering physician puts in a Cytopathology CRIS order.

### III. HEMATOLOGY PROCEDURE FOR AFTER HOURS PROTOCOL FOR CSF SPECIMENS THAT ARE HIGH RISK FOR NEOPLASTIC INVOLVEMENT:

- A. Hematology specimen is processed in the usual manner as per hematology department procedure.
- B. Cytology specimen is utilized in its entirety as follows:
  1. Describe CSF sample - volume and color
  2. Using 9 drops per cytospin, make up to 10 cytospins on **charged slides**.
  3. Cytospins are allowed to fully air-dry (30 minutes).
  4. Cytospins are placed in the refrigerator in a sealed container with desiccant.
  5. Store remaining CSF in the refrigerator to be sent to cytopathology on the next business day.
- C. Next business day, hematology technologist informs cytology technologist of the case 301-480-7430
- D. The case is picked up by a member of the cytology section and processed as deemed appropriate by the cytopathologist.

## 1.2A SPECIMEN ACCEPTANCE AND REJECTION

### I. PRINCIPLE

Occasionally there is reason for a cytology specimen rejection. Listed below are some instances when specimens will be considered unacceptable. (These specimens will not be processed until correctly identified by the physician, registered nurse (RN) or nurse practitioner (NP) familiar with the case).

- A. **Unacceptable**
  1. Unlabeled Specimens or Slides:
  2. Labeling on the specimen does not correspond with the labeling on the CRIS order requisition.
  3. Slides broken beyond repair: (See B-4 below).
  4. Specimen received without a CRIS generated Cytopathology order requisition\*.

5. Specimen in syringes with needles attached.
6. Specimen in Pleur-Evac chest drainage system containers.
7. Excessive amounts of body fluid - only specimen up to one liter is needed. Volumes greater than one liter will not be accepted by the lab.

**B. Acceptable**

1. Patient name, hospital ID #, and CRIS order ID must be on slide or specimen container and must match the information on the CRIS order requisition;
2. Liquid specimen must be received in a sealed container placed inside a plastic bag;
3. CRIS order requisition must accompany the specimen\*.
4. Slides that have broken in transit are processed unless broken beyond repair.

\* In the event that a CRIS order does not print, it is acceptable to hand print the patient's CRIS Order ID# on the specimen label. Please also include a contact name and # in the specimen bag.

**II. Specimen Rejection Policy**

If a specimen does not meet all of the above criteria, it will not be processed, and the ordering physician or nurse will be notified by telephone. **Rejected specimens and the reason for rejection are entered into the Clinical Center Occurrence Reporter System (ORS) for quality assurance .**

**1.3 PRIORITIES OF DISPATCHING DAILY WORKLOAD**

**I. PRINCIPLE**

Both ideally and practically, all specimens are of equal importance and receive the same amount of effort in screening and diagnosis. There is variation, however, in the order in which specimens may be reviewed and consequently in the order in which they must be turned out by the cytopreparatory laboratory. This order has evolved rather naturally in response to such factors as the urgency of a submitting physician's request and the care status of the patient.

**II. PROCEDURE**

- A. The majority of the specimens submitted to the cytology laboratory have a 24-hour turnaround time.
- B. Specimens submitted prior to 3:30 p.m. will be processed and stained the same day for screening the next day.
- C. Specimens received after 3:30 p.m., unless urgent or STAT, will be refrigerated and processed the next morning.
- D. Diagnostic cases requiring immunoperoxidase received after 3:30 p.m. on Thursday should be processed that day.
- E. On Friday's an attempt is made to process all specimens that are likely to degenerate rapidly (example FNA of high grade lymphoma). If this is not possible, the sample should be refrigerated until Monday.
- F. All slides and filters will be brought to the cytotechnologists for screening by 8:30 a.m. These cases should be screened and available for the pathologist for sign-out the same day by 1:00 p.m., unless extreme nature of the workload of that day precludes this time frame.

**III. REFERENCE**

Gill, G., and Plowden, K., Laboratory Cytopathology: Techniques for Specimen Preparation. Baltimore, MD: Johns Hopkins Univ; 1975, pp. 2-2.

**SECTION II: COLLECTION, FIXATION AND SUBMISSION OF SPECIMENS**

**2.1 COLLECTION AND SUBMISSION OF CYTOLOGY SPECIMENS - GENERAL**

**I. PRINCIPLE**

The quality of the cytologic diagnosis depends in equal measure on the excellence of the clinical procedure used to secure the sample and on the laboratory procedures used to process the sample. In general, material for cytologic examination is obtained either in the form of smears prepared by the examining physician, radiologist, pathologist, cytotechnologist, at the time of the clinical examination, or in the form of fluid specimens which are forwarded to the laboratory for further processing. The procedures in this section must be followed in order to ensure a specimen of optimal quality for cytologic evaluation. Specimens are only accepted from physicians, registered nurses, physician assistants, nurse practitioners, or other persons authorized by law to submit samples to the Cytopathology Laboratory.

## II. GENERAL INFORMATION

The Cytopathology Laboratory is located in Room 2S238 (Building 10) within the Anatomic Pathology Laboratory. Normal work hours are 8:30 a.m. - 5:00 p.m., Monday through Friday. The laboratory is closed on holidays and weekends. Between the hours of 5:00 p.m. and 8:30 a.m., Monday through Friday and during the weekend and holidays, specimens can be stored in the refrigerator outside of room 2S256

## III. SUBMISSION PROCEDURES

1. Each specimen submitted to the Cytopathology Laboratory must be labeled clearly to avoid the possibility of specimen misidentification. Specimen containers should be labeled with the patient's name and Hospital Number.
2. All specimens **MUST** be accompanied by a CRIS generated Cytopathology Order Requisition. The order must be entered into CRIS by the ordering physician. Relevant clinical information is absolutely essential for deriving maximum information from a cytologic examination. Such information should be concisely stated on the test request form.

To ensure proper specimen identification, evaluation and the expeditious delivery of reports, the following information **MUST** be included:

1. Patient's name
2. Hospital number
3. Ward or clinic
4. Requesting physician's name and pager number
5. Adequate clinical data/prior history
6. Date specimen collected
7. Specific site (type) of sample

### 2.1A CYTOPATHOLOGY SPECIMEN EXCLUSIONS

#### I. GENERAL INFORMATION

The NIH Cytopathology cannot accept specimens that may contain:

- Biosafety Level 3 or 4 organisms\*, or
- infectious agents difficult to inactivate.

The NIH Cytopathology Section is designated as a **Biosafety Level 2** laboratory. It is equipped to deal with a broad range of moderate-risk infectious agents. **The primary hazards associated with level 2 agents are injection and ingestion. Infection due to aerosol transmission poses less of a risk.** Level 2 precautions are necessary when working with human blood, body fluids, or tissues where the presence of an infectious agent is unknown. Level 2 organisms include HIV, Hepatitis A, B, and C, and Toxoplasma spp.

**Biosafety Level 3 laboratories work with infectious agents that have the potential for aerosol transmission and lethal infection.** In addition to personal protective equipment, biological safety cabinets and specialized ventilation systems are needed. Level 3 organisms include Mycobacterium tuberculosis, Leishmania donovani, and Bacillus anthracis.

**Biosafety Level 4 laboratories work with infectious agents with a high risk of causing life-threatening disease, the possibility of aerosol transmission, and no known vaccine or therapy.** Level 4 agents require complete isolation. Class III biological safety cabinets, full-bodied air-supplied positive pressure safety suits, isolated facilities, specialized ventilation, and waste management systems are required. Level 4 organisms include Ebola virus, smallpox, and various hemorrhagic diseases.

## II. BIOSAFETY LEVEL 3 AND 4 ORGANISMS EXCEPTIONS

### A. PRINCIPLE

As a Biosafety Level 2 lab, the Cytopathology Section may not accept specimens having a high or known risk for containing Biosafety Level 3 or 4 infectious agents.

\*Exceptions may be made for specimens with a risk for Mycobacterium tuberculosis if malignancy is the primary concern, or for pre-made air-dried touch preps of skin biopsies with a risk for Leishmania.

### B. PROCEDURE

For specimens having a high or known risk for Mycobacterium tuberculosis, where malignancy is the primary concern:

1. Notify the Cytopathology Dept (301-480-7430)
2. Follow Cytopathology collection and submission directions in floor or [website manual](#) for the specific specimen type.
3. When entering a CRIS order, place a T.B. warning in Clinical History section. Request a Fite stain in Special Instruction section.
4. The specimen will be fixated in formalin for 24 hours and a cell block will be made. Expect a 24 to 48 hour delay in processing time.

## III. SPECIMENS CONTAINING INFECTIOUS AGENTS DIFFICULT TO INACTIVATE (Creutzfeldt-Jakob Disease - CJD)

### A. PRINCIPLE

Creutzfeldt-Jakob Disease is one of a group of incurable neurological disorders believed to result from a protein called a prion. Although these prions are not easily transmissible (unless a sample is from the brain, eye or spinal cord), they are not inactivated by ordinary methods such as formalin fixation, boiling or irradiating. **In Surgical Pathology it is required that:**

-All instruments and equipment must be soaked in 2N NaOH for 60 minutes before routine sterilizing may occur.

-Specimens must be inactivated by fixation with formalin-formic acid.

Cytopathology lab equipment cannot be decontaminated this way. Also, cytology specimens may not be fixed so that the prion is inactivated or destroyed, as described above. **Hence, cytopathology will not accept specimens thought to contain prions.**

### B. PROCEDURE

1. When possible, tissue samples for Surgical Pathology should be taken. The clinician should notify the department and follow the Laboratory of Pathology (LP) Procedure in the [online manual](#)
2. If a cerebrospinal fluid is needed for CJD evaluation, contact the Laboratory of Pathology (NCI) [Infection Control Specialist](#) at 301-496-2209.

A CSF specimen will then be sent along with a voided urine specimen to the [National Prion Disease Pathology Surveillance Center](#) along with the proper forms, through the LP Department.

## IV. REFERENCES

<http://www.cdc.gov/biosafety>  
<http://www.fss.txstate.edu/ehsrm/safetymanual/biologic/cdcnihlev.html>  
<http://home.ccr.cancer.gov/LOP/clinical/cytopath/default.asp>

1. CDC's Office of Health and Safety, Creutzfeldt-Jakob Disease (CJD) in Healthcare Settings CDC's Infection Control Practices for CJD MD public health reportable list Clinical Center's Occurrence Reporting System website National Prion Disease Pathology Surveillance Center NPDPSC test request form and contact information The World Health Organization Infection Control Guidelines for Transmissible Spongiform Encephalopathies

## **2.2 COLLECTION AND SUBMISSION OF LIQUID BASED GYNECOLOGIC SPECIMENS (Thin Prep® PAP TEST)**

### **I. PRINCIPLE**

The ThinPrep System is intended as a replacement for the conventional method of Pap smear preparation for use in screening for the presence of atypical cells, cervical cancer, or its precursor lesions (Low Grade Squamous Intraepithelial Lesions, High Grade Squamous Intraepithelial Lesions), as well as for all other cytologic lesions as defined by The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. The pap test is a screening test for cervical cancer with an inherent small false negative rate.

The ThinPrep Process Begins with the patient's gynecologic sample being collected by the clinician using a cervical sampling device, that rather than being smeared on a microscope slide, is immersed and rinsed in a vial filled with PreservCyt Solution.

At the laboratory, the PreservCyt vial is placed into a ThinPrep Processor and a gentle dispersion step breaks up blood mucus, non-diagnostic debris, and thoroughly mixes the cell sample. The cells are then collected on a TransCyt filter specifically designed to collect diagnostic cells. A thin layer of cells is then transferred to a glass slide and the slide is automatically deposited into a fixative solution.

As with conventional Pap smears, slides prepared with the ThinPrep System are examined in the context of the patient's clinical history and information provided by other diagnostic procedures such as colposcopy and biopsy, to determine patient management.

### **II. MATERIAL NEEDED**

- A. PreservCyt vial
- B. Speculum (without lubricant)
- C. Sampling Device(s)
  - 1. Cervical spatula (Ayre scraper) and cytobrush
  - 2. Plastic broom (Cervex-brush or Papette)
- D. Patient ID label or permanent marker

### **III. PROCEDURE**

- A. Label the PreservCyt vial with the patient ID label or permanent marker prior to sample collection.
- B. Insert the speculum, which may be moistened with water or saline if necessary. (No other lubricants should be used.)
- C. Visually inspect the cervix for abnormalities and identify the transformation zone, if visible, to direct sampling efforts to encompass this area.
- D. Collect the sample
  - 1. **Cervical spatula and cytobrush**
    - a. Rotate the spatula 360° about the circumference of the cervix while maintaining firm contact with the epithelial surface. IMMEDIATELY rinse the spatula in the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
    - b. Insert the cytobrush into the cervix until only the bottommost fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. IMMEDIATELY rinse the brush in the PreservCyt Solution by rotating the device in the solution while pushing against

the vial wall. Swirl the brush vigorously to further release material. Discard the brush.

- c. Tighten the cap so that the torque line on the cap passes the torque line on the vial.

## 2. **Plastic broom**

Insert the long central bristles into the os until the lateral bristles bend against the ectocervix.

- a. Rotate the broom in a clockwise direction 5 times.
  - b. IMMEDIATELY rinse the broom in the PreservCyt vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. Swirl the broom vigorously to further release material. Discard the collection device.
  - c. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
- E. Submit to cytology with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to provide all relative clinical history.

## **IV. RESULTS**

Results are reported out to include a descriptive diagnosis of the cellular sample using *The Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses* (Appendix A)

## **V. REFERENCES**

ThinPrep Processor Operator's Manual, Cytoc Corporation, Marlborough, MA, 1995 pp. 1.1-1.2.

Mayeaux, Jr., E.J., The Papanicolaou Smear, AAFP Scientific Assembly, 1994

Brosky, K. and Poprocky, L. Cytopathology Laboratory Procedure Manual, George Washington University Medical Center, 1994.

## **2.2A COLLECTION AND SUBMISSION OF GYNECOLOGIC SMEARS (CONVENTIONAL PAP SMEARS)**

### **I. PRINCIPLE**

Cervical and vaginal smears are primarily obtained as a screening procedure for precancerous, cancerous or inflammatory conditions. Ectocervical, endocervical and vaginal pool (posterior fornix) material may be placed on a single side. The pap test is a screening test for cervical cancer with an inherent small false negative rate.

### **II. MATERIAL NEEDED**

- A. Cytopathology CRIS Order Requisition Form
- B. Glass slides - one end frosted
- C. Diamond point pen or No. 2 lead pencil
- D. Cervical spatula (Ayre scraper) and cytobrush
- E. Speculum (without lubricant)
- F. Fixative: 95% ethanol (EtOH) or cytospray aerosol fixative (spray-cyte)

### **III. PROCEDURE**

- A. Print the patient's last name, first initial on the frosted end of the glass slide using the diamond point pen or No. 2 lead pencil.
- B. The speculum must be introduced with no lubricant. If necessary, normal saline may be used to moisten the speculum.



- C. Rotate cytobrush 360° in endocervical canal, then rotate spatula 360° on ectocervix.
- D. Fixation
  - 1. **Aerosol fixative:**
    - a. Unroll brush material on one-half of the glass slide and spray fix with the other half of the slide covered.
    - b. Spread spatula material on the other half of the glass slide and spray fix.
  - 2. **95% Ethanol:**
    - a. Unroll brush material on one-half of the glass slide.
    - b. Spread spatula material on the other half of the glass slide.
    - c. Immediately drop slide into 95% alcohol fixative.
- E. Submit to cytology with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to provide the following information in addition to the patient's name and identification number.
  - 1. Date of collection
  - 2. Source of specimen
  - 3. Patient's age
  - 4. Date of LMP
  - 5. Any current hormonal therapy
  - 6. Any previous atypical pap smears and/or cervical biopsies
  - 7. Any history of malignancy and subsequent surgery, chemotherapy and/or radiation therapy.

#### IV. NOTES

- A. The bottle of fixative should be opened and readily accessible before the specimen is obtained. Cells dry rapidly once they are spread out on a glass slide. The slide must be fixed IMMEDIATELY.
  - 1. Ethanol, 95% - Fix for a minimum of 15 minutes.
  - 2. Cytospray Aerosol Fixative - Immediately spray smear, holding can 10-12 inches from slide for 12 seconds, air dry, and place in cardboard folders.
- B. Bleeding and douching within the previous 24-hours are not contraindications to obtaining specimens. If possible, however, instruct the patient not to douche for at least three days prior to examination.
- C. If a lesion is visualized, a separate smear should be made and labeled appropriately.
- D. In selected cases, the clinician may want to submit a vaginal pool smear. This is done by collecting the material with the paddle end of the spatula, spreading it on a second pre-labeled glass slide and quickly fixing by either the aerosol spray or ethanol method of fixation.
- E. If a two slide case is submitted in ethanol, the slides must be kept separated by placing a paper clip on the labeled end of the slide or placed into slotted coplin jar.

#### V. RESULTS

Results are reported as a descriptive diagnosis of the cellular sample using the "Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses." See Appendix A.

#### VI. REFERENCES

Boon, M. E., M. D., Ph. D., M. I. A. C., Analysis of Five Sampling Methods for Preparation of Cervical Smears, Acta Cytologica Vol. 33 No. 6; Nov-Dec 1989

#### 2.3 COLLECTION, FIXATION AND SUBMISSION OF GYNECOLOGIC SMEARS FOR CYTOHORMONAL EVALUATION

## I. PRINCIPLE

The usefulness of vaginal hormonal cytology is based on the fact that alterations in the levels and relative concentrations of the gonadal and related adrenal steroids will be reflected in their action on the vaginal epithelium and will be evidenced in the cellular pattern of the cells shed from the vagina. Proper use of this modality requires a thorough familiarity with the normal cytologic findings in normal females throughout the various physiologic periods of life, an appreciation of the hormonal changes in the various endocrinopathies, and experience in the vaginal cytologic findings in these conditions.

## II. MATERIAL NEEDED

- A. Cytopathology CRIS Order Requisition Form
- B. Glass slides - one end frosted
- C. No. 2 lead pencil
- D. Cervical spatula (Ayre scraper) or cut tongue depressor
- E. Speculum (without lubricant)
- F. Fixative: 95% ethanol or cytospray aerosol fixative (spray-cyte)
- G. Preserv Cyt Vial, if using Thin Prep Procedure.

## III. PROCEDURE

1. Print the patient's last name and first initial on the frosted end of the glass slide using a No. 2 lead pencil.
2. The speculum must be introduced with no lubricant. If necessary, normal saline may be used to moisten the speculum.
3. Using the cervical spatula or tongue depressor, lightly scrape the mucosa of the upper third of the lateral vaginal wall and spread the material on the glass slide.
4. Fix IMMEDIATELY!
5. Submit to cytology with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to provide relevant clinical history and a detailed menstrual history.

## IV. NOTES

- A. Care must be taken to avoid contamination from the vaginal pool and the ectocervix.
- B. The bottle of 95% ethanol should be opened and readily accessible before the specimen is obtained. Cells dry rapidly once they are spread out on a glass slide. The slide must be fixed IMMEDIATELY.
  1. Ethanol, 95% - Fix for a minimum of 30 minutes.
  2. Cytospray Aerosol Fixative - Immediately spray smear, holding can 10-12 inches from slide for 12 seconds, air dry, and place in cardboard folders.

## V. RESULTS

1. The results of cytohormonal studies will be expressed in terms of a Maturation Index (MI), and whether the MI is or is not compatible with the patient's age and history.
2. Many factors may make a hormonal evaluation uninterpretable, such as inflammation, infection or the presence of all three squamous cell types. It is also imperative that the LMP be present on the request so that the results can be interpreted.

## VI. REFERENCE

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, p. 46-47.

## 2.4 COLLECTION, FIXATION AND SUBMISSION OF GYNECOLOGIC SMEARS OF PATIENTS EXPOSED TO DES IN UTERO

### I. PRINCIPLE

A synthetic compound with estrogen-like effect, diethylstilbestrol (DES) was extensively used for prevention of abortions and other complications of pregnancy during the late 1940's and early 1950's. About 20 years later, it became apparent that the use of this drug adversely affected the offspring of the patients so treated. In the female offspring, vaginal and cervical adenosis has resulted. All changes commonly observed in the endocervical epithelium may be observed in adenosis: secretory activity, squamous metaplasia and malignant transformation leading to adenocarcinoma, epidermoid carcinoma or both. Because of these risks, the detection diagnosis and follow-up of patients with adenosis is of considerable practical importance. Vaginal cytology plays an important role in this respect.

## II. MATERIAL NEEDED

- A. Cytopathology CRIS Order Requisition Form
- B. Five glass slides (one end frosted)
- C. Five cervical spatulas or one cervical spatula and four cut tongue depressors
- D. Speculum (without lubricant)
- E. Diamond point pen or No. 2 lead pencil
- F. Fixative: 95% ethanol or cytospray aerosol fixative
- G. Preserv Cyt Vial, if using Thin Prep Procedure. Note: Each vial must be labeled as per procedure III below.

## III. PROCEDURE

- A. Print the patient's last name, and first initial on the frosted end of the glass slides with the diamond point pen or No. 2 lead pencil. The slides should also be labeled with the additional information codes:
  1. CX - for cervical smear
  2. ANT - for anterior vaginal wall
  3. POST- for posterior vaginal wall
  4. RT - for right vaginal wall
  5. LT - for left vaginal wall
- B. Introduce the speculum without lubricant. If necessary, normal saline may be used to moisten the speculum.
- C. Obtain a routine cervical and endocervical smear; fix IMMEDIATELY.
- D. Obtain lateral vaginal wall smears as if for cyto hormonal study and fix IMMEDIATELY!
- E. Submit to cytology with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to provide all relevant clinical history.

## RESULTS

Results are reported out to include a descriptive diagnosis of the cellular sample using the "Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses" (Appendix A) and a statement relating to evidence of vaginal adenosis.

## V. REFERENCE

Koss, L., Diagnostic Cytology and its Histopathologic Bases, Third Edition, Philadelphia, PA: J. B. Lippincott; 1979, pp. 456-459.

## 2.5 COLLECTION AND SUBMISSION OF SPUTUM FOR CYTOLOGY - COMPLETE SPUTUM SERIES

### I. PRINCIPLE

- A. Cytologic diagnosis of pulmonary carcinoma may be made largely on detection of exfoliated carcinoma cells in sputum or bronchial secretions. The procedure has been mainly used in the diagnostic work-up of symptomatic patients or those with an X-ray abnormality of the chest.
- B. When a pulmonary lesion is suspected, a complete sputum series should be examined. The COMPLETE SPUTUM SERIES consists of a fresh, unfixed, early morning specimen each day for three days. A post-bronchoscopy specimen is considered very valuable, and may be included in the series.
- C. When TB/MDRTB is known or suspected, send sputum samples to microbiology lab for processing and interpretation (Rm. 2C385) as per procedure outlined by microbiology lab. (Phone 301-496-4433).

## II. MATERIAL NEEDED

- A. Wide-mouthed specimen cup with a water-tight lid for each day. The 120 ml. size is adequate and should be clean, dry and contain NO fixative.
- B. Cytopathology CRIS Order Requisition Form

## III. PROCEDURE I: ROUTINE

- A. Give the patient a clean sputum cup the night before and instruct the patient not to use until the following morning before breakfast.
- B. Instruct the patient that immediately upon waking, he should sit up and gargle with water, thoroughly rinsing the mouth.
- C. Ask the patient to cough deeply (from the diaphragm) and expectorate all sputum into the cup. Encourage the patient to expectorate deep SPUTUM, not saliva.
- D. The patient continues the deep coughing and expectorating until a specimen is obtained.
- E. Submit the properly labeled specimen immediately to the cytology laboratory with an accompanying Cytopathology CRIS Order Requisition Form completed to include all relevant clinical history.
- F. Repeat the procedure each day for three consecutive days.

## IV. NOTE

- A. If sputum cannot be brought immediately to the laboratory, refrigerate.
- B. When MDRTB is known or suspected, the specimen is sent to the microbiology lab (room 2C385) for processing and interpretation.

## V. REFERENCE

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 306-307.

## 2.6 COLLECTION AND SUBMISSION OF BRONCHOSCOPIC BRUSHINGS

### I. PRINCIPLE

Bronchial specimens for cytologic evaluation augment routine bronchoscopy with biopsy. Specimens may be obtained during bronchoscopy by DIRECT BRUSHINGS of suspicious areas and by BRONCHIAL WASHING. As the findings at bronchoscopy are usually not predictable beforehand, the operator must be prepared to obtain material by any means which may prove to be the most desirable at the time of examination.

### II. BRONCHIAL BRUSHINGS

- A. The brush is dropped in saline and promptly delivered to the laboratory accompanied by a Cytopathology CRIS Order Requisition Form.

**When TB/MDRTB is known or suspected**, send the specimens to microbiology lab for processing and interpretation (Rm. 2C385) as per procedure outlined by microbiology lab. (Phone 301-496-4433). However, if malignancy is the primary concern in a patient who is known or suspected of having TB contact cytopathology regarding special handling of the sample.

### III. REFERENCE

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 306-307.

## 2.7 COLLECTION AND SUBMISSION OF BRONCHOALVEOLAR LAVAGE (BAL) SPECIMENS AND BRONCHIAL WASHINGS

### I. PRINCIPLE

- A. Bronchoalveolar lavage (BAL) is a relatively simple procedure in which a high-volume saline lavage of a lung subsegment is done through a bronchoscope in order to obtain cellular and protein constituents from the pulmonary alveolar spaces. It has been estimated that existing BAL methods can sample more than one million alveoli. Among the advantages of BAL are its safety (less than 5% complication rate), the ease and rapidity with which it provides samples, and the close correlation of its results with direct histological examination of the pulmonary parenchyma.
- B. BAL is used at the National Institutes of Health for the evaluation of possible neoplastic or infectious/inflammatory disease.

### II. PROCEDURE

- A. IMMEDIATELY submit the labeled specimen to the cytology laboratory with an accompanying Cytopathology CRIS Order Requisition Form, containing all relevant clinical data.

**NOTE: When TB/MDRTB is known or suspected**, send the specimens to microbiology lab for processing and interpretation (Rm. 2C385) as per procedure outlined by microbiology lab. (Phone 301-496-4433). However, if malignancy is the primary concern in a patient who is known or suspected of having TB contact cytopathology regarding special handling of the sample.

### III. RESULTS

Results are reported out as a descriptive diagnosis including, but not confined to differential cell count (if requested), micro-organisms present, and the presence or absence of malignant cells.

### IV. REFERENCES

Kahn, F.W., and Jones, J.M., Bronchoalveolar Lavage in the Rapid Diagnosis of Lung Disease. Laboratory Management, June 1986; pp. 31-35.

Reynolds, Herbert Y., Bronchoalveolar Lavage. Am Rev Respir Dis 1987; 135: 250-263.

Linder, et al, Cytopathology of Opportunistic Infection in Bronchoalveolar Lavage. Am J Clin Pathol 1987; 88: 421-428.

## 2.8 COLLECTION AND SUBMISSION OF ESOPHAGEAL AND GASTRIC WASHINGS

### I. PRINCIPLE

The techniques of esophageal and gastric cytology are used predominantly for the investigation of patients with symptoms or signs referable to the upper gastrointestinal tract. They are, thus, primarily diagnostic procedures and

form a valuable adjunct to other techniques such as endoscopy and radiology. A combination of these various techniques results in a very high degree of diagnostic accuracy.

## **II. ESOPHAGEAL WASHINGS**

The properly labeled centrifuge tubes, in ice, should be immediately submitted to the laboratory with an accompanying Cytopathology CRIS Order Requisition Form properly completed to include all relevant clinical history.

## **III. GASTRIC WASHINGS**

The properly labeled centrifuge tubes, in ice, should be immediately submitted to the laboratory with an accompanying Cytopathology CRIS Order Requisition Form properly completed to include all relevant clinical history.

## **IV. RESULTS**

Results are reported out as a descriptive diagnosis including, but not limited to benign and reactive/cellular changes, the presence or absence of malignant cells or the presence of infectious organisms.

## **V. REFERENCE**

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 364-365.

## **2.9 COLLECTION AND SUBMISSION OF ESOPHAGEAL AND GASTRIC BRUSHINGS**

### **I. PRINCIPLE**

The techniques of esophageal and gastric cytology are used predominantly for the investigation of patients with symptoms or signs referable to the upper gastrointestinal tract. They form a valuable adjunct to other techniques such as endoscopy and radiology. A combination of these various techniques results in a very high degree of diagnostic accuracy. Esophageal and gastric brushings are collected by the endoscopist.

### **II. MATERIAL NEEDED**

- A. Cytopathology CRIS Order Requisition Form
- B. 5 to 15 ml container
- C. Physiologic saline

### **III. PROCEDURE**

- A. With the endoscopic tube in place, insert the endoscopic brush and brush the suspicious area completely.
- B. Withdraw the brush and quickly drop into enough physiologic saline to cover brush-bristles.
- C. Repeat this procedure in other suspicious areas as necessary.
- D. Submit specimen the cytology laboratory with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to include all relevant clinical history.

### **IV. RESULTS**

Results are reported out as a descriptive diagnosis, including, but not confined to benign and reactive cell changes, the presence or absence of malignant cells and the presence of infectious entities, fungal, viral, and bacteria.

### **V. REFERENCE**

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 364-365.

## **2.10 COLLECTION AND SUBMISSION OF VOIDED AND INSTRUMENTED URINE**

### **I. PRINCIPLE**

Carcinomas arising from the epithelium surfacing the urinary tract (the urothelium) desquamate readily into the urinary stream. Carcinomas (or other neoplasms) arising from deeper tissues, for example within the kidney or prostate, are unlikely to do so until they have grown to substantial size and actually disrupt the normal epithelial surface of the urinary tract. Thus, cytology of the urine sediment is most useful in the diagnosis of carcinoma of the bladder, renal pelvis, ureter and urethra. It is primarily an aid in differential diagnosis of patients who are symptomatic.

### **II. MATERIAL NEEDED**

- A. Cytopathology CRIS Order Requisition Form
- B. Urine specimen cup

### **III. PROCEDURE**

#### **A. VOIDED URINE SPECIMENS**

1. Patient should be instructed in clean catch urine technique by nursing staff or clinician. Have patient void, drink fluids and then send next clean catch voided urine to Cytology. A minimum of 30 ml. is requested, maximum of 120 ml.
2. Do not add fixative to specimen.
3. Refrigerate the specimen until it can be delivered to the laboratory. Ideally, specimen should be sent to the cytology laboratory within an hour of collection.

#### **B. INSTRUMENTED URINE SPECIMENS**

1. Instrumented urine specimens should be collected in electrolyte balanced solution only.
2. Refrigerate the specimen until it can be delivered to the laboratory. Ideally, specimen should be sent to the cytology laboratory within an hour of collection.

### **IV. RESULTS**

Results are reported out as a descriptive diagnosis including, but not confined to benign or reactive cellular changes, and the presence or absence of malignant cells.

### **V. REFERENCE**

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 419-423.

## **2.11 COLLECTION AND SUBMISSION OF BODY CAVITY FLUIDS**

### **I. PRINCIPLE**

The primary purpose of cytologic examination of fluids is to rule out metastatic or primary cancer. Occasionally, other diagnostic conclusions can be reached. Pleural, pericardial, peritoneal and joint fluid should be submitted FRESH and UNFIXED, HEPARINIZED or NON-HEPARINIZED. These recommendations are made in order to provide well-preserved, representative diagnostic material. 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 be unavailable for satisfactory evaluation. (This can be overcome, however, by submitting clots for cell block preparation.) Fixation interferes with technical processing.

## **II. MATERIAL NEEDED**

- A. Cytopathology CRIS Order Requisition Form
- B. Paracentesis set-up
- C. Sterile collection bottle or bag
- D. 10 units of heparin per 100 ml. of fluid (optional)

## **III. PROCEDURE - PLEURAL AND ASCITIC FLUIDS**

- A. Ideally specimen should be sent to the laboratory within 1 hour after being collected.
- B. The addition of 10 units of heparin/100 ml of fluid is desirable but not essential.
- C. Specimen should be refrigerated until time to be delivered to the laboratory.

## **IV. NOTES**

- A. If unable to deliver specimen to cytology laboratory in a timely manner, or if specimen is collected after hours and processing will be delayed, refrigerate the specimen.
- B. If at all possible, paracentesis should be performed when the cytology laboratory is processing routine specimens (8:30 a.m. through 3:30 p.m., Monday through Friday).
- C. Do not send excessive amounts of body fluid - only specimen up to one liter is needed. Volumes greater than one liter will not be accepted by the lab.

## **V. RESULTS**

Results are reported out as a descriptive diagnosis including, but not limited to benign or reactive cellular changes, and the presence or absence of malignant cells.

## **VI. REFERENCE**

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 472-475.

## **2.12 COLLECTION AND SUBMISSION OF CEREBROSPINAL FLUID**

### **I. PRINCIPLE**

CNS cytology is not a screening procedure but a tool for evaluating conditions for which a tissue correlation is not usually available. Subsequently, such specimens may provide the only diagnosis upon which therapy and prognosis are based. Patients for whom CNS cytology is performed either have symptoms related to a CNS disease or are candidates for CNS involvement by metastatic disease. Given the diagnostic problems confronting both the clinician and cytopathologist, the use of a pragmatic approach is advocated, in which the patient's history, age and test results are used along with the cytologic patterns to eliminate or indicate certain diagnostic choices.

### **II. MATERIAL NEEDED**

- A. Cytopathology CRIS Order Requisition Form
- B. Spinal collection tubes
- C. Spinal needle

### **III. PROCEDURE**

- A. Perform tap.



- B. Place only a small amount of fluid in first tube (to clear blood).
- C. Obtain as much cerebrospinal fluid in the second tube as clinical judgment allows.
- D. IMMEDIATELY submit properly labeled second tube to the cytology laboratory with an accompanying Cytopathology CRIS Order Requisition Form, properly completed to provide all relevant clinical data.

#### IV. NOTES

- A. Refrigerate specimen if unable to deliver it to cytology laboratory in a timely manner, or in any circumstance in which processing will be delayed (i.e., submitted after normal working hours).
- B. If bacteriologic studies are also indicated, a separate specimen must be submitted to microbiology with the proper CRIS generated request sheet.

#### V. RESULTS

Results are reported out as a descriptive diagnosis including, but not confined to micro-organisms present, and the presence or absence of malignant cells.

#### VI. REFERENCE

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth

### 2.13 COLLECTION AND SUBMISSION OF BODY CAVITY WASHINGS

#### I. PRINCIPLE

Examining cells in peritoneal fluid obtained by PELVIC WASHING is a useful method for staging metastatic disease.

#### II. MATERIALS NEEDED

- A. Cytopathology CRIS Order Requisition Form
- B. Specimen container
- C. Physiologic saline

#### III. PROCEDURE

- A. Irrigate site with physiologic saline.
- B. Collect all fluid in specimen container.
- C. IMMEDIATELY submit properly labeled specimen to the cytology laboratory accompanied by a Cytopathology CRIS Order Requisition Form properly completed to include all relevant clinical history.

#### IV. NOTES

- A. Refrigerate specimen if unable to submit it to cytology laboratory in a timely manner, or if undue delay in processing is anticipated (i.e., specimen submitted after normal working hours).
- B. Clinicians may prefer to submit separate samples to include washings of the following sites:
  - 1. Right pericolic gutter
  - 2. Left pericolic gutter
  - 3. Cul-de-sac
  - 4. Sub-diaphragmatic
- C. This technique may be adapted to other body sites (i.e., endoscopic washings, sinus washings) to obtain material for cytologic evaluation.

#### V. RESULTS

Results are reported out to include, but not confined to benign and reactive cell changes, and the presence or absence of malignant cells.

## **VI. REFERENCE**

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 222-234.

## **2.14 COLLECTION AND SUBMISSION OF MISCELLANEOUS FLUIDS (CYST FLUIDS, JOINT FLUIDS)**

### **I. PRINCIPLE**

Fluids aspirated from breast or other cysts, joints or fluids obtained by other means, should be submitted for cytologic evaluation to rule out the possibility of malignancy, either primary or metastatic or infectious etiology.

### **II. MATERIAL**

- A. Cytopathology CRIS Order Requisition Form
- B. Syringe
- C. Specimen container

### **III. PROCEDURE**

- A. Collect as much fluid as possible with the syringe.
- B. Transfer the fluid to a specimen container labelled with the patient's name and ID#.
- C. Immediately submit the unfixed specimen to the cytology laboratory, accompanied by a Cytopathology CRIS Order Requisition Form properly completed to include all relevant clinical history.

### **IV. NOTE**

- A. Refrigerate specimen if unable to submit it to the cytology laboratory or if undue delay in processing is anticipated (i.e., specimen submitted after normal specimen processing hours 8:00 a.m. - 3:30 p.m., Monday through Friday).

### **V. RESULTS**

Results are reported out as a descriptive diagnosis including, but not confined to benign or reactive cellular changes, and the presence or absence of malignant cells.

## **VI. REFERENCE**

Laboratory Manual (Ward Manual), NNMC, Bethesda, MD; 1988-89

## **2.15 COLLECTION AND SUBMISSION OF NIPPLE DISCHARGE SPECIMENS**

### **I. PRINCIPLE**

Breast carcinoma is the leading cause of cancer death in American women. Any breast secretion, except normal milk, is abnormal. The causes vary from benign or malignant breast diseases, pituitary tumors, alteration of hypothalamic function by tranquilizer intake and others. Cytologic evaluation of breast secretion is simple and the accuracy is high, although the sensitivity is low.

## II. MATERIAL NEEDED

- A. Cytopathology CRIS Order Requisition Form
- B. No. 2 lead pencil
- C. Glass slides (one end frosted)
- D. Fixative - 95% ethanol or cytospray aerosol fixative
- E. Cardboard slide holder or coplan jar

## III. PROCEDURE

- A. Label the slides on the frosted end with the patient's last name, first initial, using the No. 2 lead pencil.
- B. If using 95% ethanol as fixative, open the bottle and have the patient hold it near the breast.
- C. Gently express only the nipple and subareolar area for any secretions which may be lying in the collecting ducts. If no secretion appears at the nipple with this gentle compression, DO NOT manipulate further.
- D. Allow a "pea size" drop of fluid to collect upon the nipple tip.
- E. Immobilize the breast and, using the nipple, smear the material across a glass slide.
- F. IMMEDIATELY drop the slide into the fixative. Time is of the essence here. The smearing of the material across the slide and the dropping of the slide into the fixative should be accomplished in one motion.
- G. Make as many smears as the amount of material allows.
- H. Submit to the cytology laboratory accompanied by a Cytopathology CRIS Order Requisition Form properly completed to provide all clinical history.

## IV. NOTES

- A. If cytospray aerosol fixative is used, IMMEDIATELY spray smear, holding can 10-12 inches from slide for 12 seconds, air dry, and then place in a cardboard slide holder for submission.
- B. DO NOT massage or squeeze the breast. Too vigorous manipulation may dislodge and spread malignant cells.

## V. REFERENCES

Anon., Clinical Cytopathology: Techniques for Specimen Preparation, Baltimore, MD: Johns Hopkins Univ; pp. 32-33.

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 268-274.

## 2.16 COLLECTION AND SUBMISSION OF TZANCK SMEARS

### I. PRINCIPLE

The use of cutaneous cytology for diagnosis of vesiculobullous skin disorders is well established. Although useful in a variety of diseases, cutaneous cytology is most helpful in confirming the diagnosis of herpes simplex. This frequently involves patients with the recent onset of a vesiculobullous eruption.

### II. MATERIAL NEEDED

- A. Cytopathology CRIS Order Requisition Form
- B. Glass slide(s) - (one end frosted)
- C. No. 2 lead pencil
- D. #15 surgical blade
- E. Alcohol swab
- F. Coplin jar containing 95% ethanol

### III. PROCEDURE

- A. Label the glass slide(s) on the frosted end with the patient's last name, first initial, using the No. 2 lead pencil.
- B. Select lesion to be sampled. An intact vesicle yields the best results, although pustules and encrusted ulcers may be employed if necessary.
- C. Cleanse designated lesion with alcohol swab. Allow to dry.
- D. With a #15 surgical blade, tangentially unroof the lesion at the edge
- E. Scrape the floor of the lesion with the blade taking care not to generate hemorrhage.
- F. Quickly spread the scrapings once across the glass slide, which is held over the open coplin jar of alcohol.
- G. IMMEDIATELY immerse the slide in 95% alcohol.
- H. Submit alcohol-fixed slide(s) to the cytology laboratory accompanied by a Cytopathology CRIS Order Requisition Form properly completed to include all relevant clinical history.

### IV. NOTE

95% ethanol (95% EtOH) is the fixative of choice for this procedure. If unavailable, cytospray aerosol fixative may be substituted by IMMEDIATELY spraying smear, holding can 10-12 inches from slide, for 12 seconds. Allow to air dry and place in a cardboard slide holder for submission.

### V. RESULTS

**Results are reported out as:**

- A. Cellular changes consistent with herpes, or
- B. No evidence of herpes.

### VI. REFERENCE

Vidmar, D., Plain but Practical - Tzanck Smears. Navy Medicine, July-August 1987, pp. 26-28.

## 2.17 COLLECTION, FIXATION AND SUBMISSION OF FINE NEEDLE ASPIRATION (FNA) SPECIMENS

### I. PRINCIPLE

Fine needle aspiration cytology was originally described and tested in the 1930's at the Memorial Hospital for Cancer in New York City. The method, which was significantly modified in Sweden by the use of thin needles and by other technical refinements, has been popular in Europe for many years. With the introduction of various imaging modalities, such as computed tomography and ultrasound, the scope of diagnostic aspirates became virtually unlimited, and it may now be stated that nearly all space-occupying lesions in the human body are accessible to sampling by aspiration.

Fine needle aspiration cytology has become a highly respected and widely used diagnostic tool at the National Institutes of Health. In many instances, needle aspirations provide the sole means for establishing a patient's therapeutic course. The initial steps of this procedure, including localization of the lesion, insertion of the needle into the field, and mechanical aspiration of the sample, require the medical expertise of a practiced clinician. The actual preparation of the cytologic smears, however, demands the knowledge and acumen of a competent cytotechnologist. The following guidelines describe the cytopreparatory steps necessary for obtaining quality diagnostic samples. The recommended methods produce cytomorphology that can be reproduced, recognized and interpreted by qualified personnel.

### II. MATERIAL NEEDED

- A. **Supplied by clinic:**
  1. Cytopathology CRIS Order Requisition Form
  2. Alcohol prep pads

3. Syringes - 10cc and 20cc
  4. 2"x2" gauze pads
  5. Gloves
  6. Patient labels
- B. Supplied by Cytopathology Lab:**
1. Syringe holders - 10cc and 20cc
  2. Needles - 23G x1" and 25G x1"
  3. Glass slides - one end frosted
  4. No. 2 lead pencil
  5. Coplin jar containing 95% ethanol or Carnoy=s Solution
  6. Slide tray
  7. Centrifuge tube(s) containing sterile physiologic saline or RPMI Medium 1640 liquid
  8. Sterile gauze pads
  9. Microscope
  10. Diff-quick stains
  11. See Section 3.0 for needle handling and infection control

### III. PROCEDURE

- A. Call Cytology (301-480-7430) to request a cytotechnologist to assist with the procedure.
- B. The physician performing the procedure will identify the patient by checking two identifiers: the patient's name and medical record number or birthdate. If the patient cannot respond, a wristband may be checked or the patient's attending nurse/physician may be asked. The fna site should also be confirmed by the patient's attending physician or nurse. The physician will then explain the procedure, including its' risks and its' benefits to the patient, and obtain a verbal consent from the patient.
- C. Cleanse the area with the alcohol prep pad and allow to dry.
- D. The cytotechnologist will label glass slides on the label end with the patient's last name, first initial and medical record number or birth date, along with the anatomic site, using a No. 2 lead pencil or permanent marker. Tubes for needle rinsings will be labeled with patient's last name, first initial, medical record number and aspiration site using a permanent marker. Cytotechnologist will also uncap fixative at this time.
- E. Insert syringe into appropriate syringe holder and attach needle. Cytotechnologist uncaps fixative at this time.
- F. Uncap needle and perform the aspiration either by aspiration or nonaspiration technique as follows:
  1. **ASPIRATION**
    - a. Immobilize the nodule between two fingers and quickly insert the needle into the lesion with the plunger in the resting state.
    - b. Retract the plunger, creating suction in the needle.
    - c. Use backward and forward movements under constant suction, keeping the needle tip in the lesion.
    - d. RELEASE the plunger to prevent aspiration of the sample into the syringe BEFORE removing the needle from the lesion.
    - e. Withdraw the needle from the lesion.
    - f. Apply pressure to the lesion with a sterile gauze pad to avoid hematoma.
  2. **NONASPIRATION** (good for vascular lesions such as thyroid).
    - a. Immobilize the nodule and insert the needle, using a needle and syringe in which the suction in the syringe is broken before insertion of needle into lesion.
    - b. Use a quick back and forth motion to move the needle in the lesion.
    - c. Remove the needle from the lesion and apply pressure to the lesion.
- G. The cytotechnologist will distribute material thinly and evenly between two slides and IMMEDIATELY immerse one slide in fixative and allow the other slide to air dry.
- H. The pathologist performs an immediate assessment of adequacy on a diff-quick stained slide prepared by the cytotech. If a clinician is performing the procedure, the immediate assessment of adequacy is performed by the cytotechnologist.
- I. The slide that has been air-dried is then diff-quick stained and evaluated on site.
- J. Rinse the needle with sterile physiologic saline or RPMI liquid. The rinsings are used to prepare cell block filter, and/or cytospin preparations in the laboratory.
- K. Repeat steps C through H as needed i.e. for diagnosis and/or special studies.
- L. The cytotechnologist will return to the laboratory with the smears and needle rinsings for further processing.

#### IV. NOTES

- A. In order to maintain an efficient and timely service to clinicians utilizing FNA procedures, the Cytopathology Department requests that procedures be scheduled at least 24-hours in advance, to be performed between the hours of 8:30 a.m. and 3:30 p.m. Cytotechnologists are available only until 3:30 p.m.
- B. The Cytopathology CRIS Order Requisition Form MUST contain all relevant clinical history. This is ESSENTIAL for optimal diagnostic evaluation.
- C. **It is contraindicated** to prepare slides for review on site from a fine needle aspiration (FNA) on a suspected or known TB/MDR-TB patient without a Class II hood and proper fixation. When performing an FNA procedure, if a malignancy is to be ruled out the specimen is put directly into preservative-free normal saline and brought to Cytopathology for handling. If infection is the primary concern the sample should be sent directly to microbiology (Room 2C385) for processing and evaluation.

**The following are examples to be included with the clinical history:**

1. Site of aspirated lesion
2. Known primary malignancy - site and differentiation
3. Previous radiation and/or chemotherapy
4. Any other pertinent history
5. Imaging and clinical characteristics of lesion, particularly breast.

#### V. RESULTS

Results are reported out as a descriptive diagnosis including, but not confined to the presence or absence of malignant cells.

#### VI. REFERENCE

Koss, L., Woyke, S., and Olszewski, W., Aspiration Biopsy-Cytologic Interpretation and Histologic Bases, New York, NY: Iguku-Shoin; 1984, pp. 3-5.

Wied, G., Koss, L., and Reagan, J., Compendium on Diagnostic Cytology, Fifth edition, Chicago, IL: International Academy of Cytology; 1983, pp. 506-511.

The Uniform Approach to Breast Fine Needle Aspiration Biopsy. Diagn Cytopathol. 16:295-311, 1997.

#### 2.18 IMAGE GUIDED FINE NEEDLE ASPIRATION (FNA) SPECIMENS

- Image guided Fine Needle Aspiration biopsies are performed in Radiology and must be scheduled through the Radiology Department.
- A Cytotechnologist will assist the radiologist to prepare slides and to assess adequacy of the sample at the request of the radiologist.
- A Cytopathologist will be available for consult if needed.
- A Cytopathology non-gyn CRIS Order must be available on the patient at the time of the procedure.
- A Cytotechnologist cannot assist the radiologist unless there is a current CRIS order available.

#### I. NOTES

- A. In order to maintain an efficient and timely service to clinicians utilizing FNA procedures, the Cytopathology Department requests that procedures be scheduled at least 24-hours in advance, to be performed between the hours of 8:30 a.m. and 3:30 p.m.
- B. The Cytopathology CRIS Order Requisition Form MUST contain all relevant clinical history. This is ESSENTIAL for optimal diagnostic evaluation.

- C. **It is contraindicated** to prepare slides for review on site from a fine needle aspiration (FNA) on a suspected or known TB/MDR-TB patient without a Class II hood and proper fixation. When performing an FNA procedure, if a malignancy is to be ruled out the specimen is put directly into preservative-free normal saline and brought to Cytopathology for handling. If infection is the primary concern the sample should be sent directly to microbiology (Room 2C385) for processing and evaluation.

**The following are examples to be included with the clinical history:**

1. Site of aspirated lesion
2. Known primary malignancy - site and differentiation
3. Previous radiation and/or chemotherapy
4. Any other pertinent history
5. Imaging and clinical characteristics of lesion, particularly breast.