JOHNSON MEMORIAL HOSPITAL LABORATORY PROCEDURE		
Procedure: Cytology Specimen Collection Procedures		Policy Number:
Function: Describe the specimen collection Requirements and associated policies.		Department: Specimen Manual
Revised by: T.Valliere		Number of Pages: 12
Effective Date: 9/19/02	Original Date 9/90	Revision Date 6/16/04
Medical Director Approval:		Date:
Laboratory Manager Approval:		Date:

I. Principle: Cytology specimens must be properly labeled, identified and collected properly for accurate results.

II. Policies and Procedures

A. **Criteria for rejection**: UNACCEPTABLE UNSATISFACTORY SUBOPTIMAL TISSUE AND CYTOLOGIC SPECIMENS

1. Tissue and/or cytologic specimens will be deemed unacceptable if they are received unlabelled, improperly labeled, if pre-made slides are broken, or if no specimen is received. The OR or the physician's office will be notified by phone upon receipt of such specimens, and the histotechnologist will document notification on the requisition slip. Unacceptable specimens will not be processed and no report will be generated.

2. Tissue and/or cytologic specimens will be deemed unsatisfactory or suboptimal if, after processing, they are acellular, scantily cellular, improperly preserved or fixed, excessively degenerated or distorted, or are not representative of the intended site. In such cases, a report will be generated which may indicate "tissue insufficient for diagnosis", "suboptimal specimen", or "less than optimal specimen" with a description of the specimen limitations.

3. Notification of unsatisfactory specimens
   a. Unsatisfactory reports are sent to physician's office and/or to the floor, just as all reports are. These reports are clearly labeled "Unsatisfactory"
   b. Unidentified or questionable specimens are returned to their source for proper identification or re-collection.

4. Criteria for Categorizing Cytology Specimens as Unsatisfactory
   a. GYN:
      Scant cellularity
      Poor fixation or preservation
      Presence of foreign material

Obscuring inflammation

a. GYN (cont.)
Obscuring blood
Excessive cytolysis or autolysis
No endocervical component
Not representative of anatomic site

b. Non Gyn: All Sources:
Inadequate amount
Inadequate fixation or preservation
Clotted fluid
Unauthorized source
Presence of interfering substances
Not representative of anatomic site, i.e.
Sputum: Absence of deep macrophages
Thyroid: Absence of follicular cells
Breast: Absence of ductal cells
Other sites as determined by the pathologist and cytotechnologist

B**. Requisitions**: The Patient Requisition should contain the following information:

1. In Patients:
   a. Patient demographic information:
      Patient's Name
      Patients' ID Number
      Doctors Names
      Date of Service
      Clinical History
      Diagnosis
      Specimen information

   b. GYN Cytology requires in addition to a. above:

      A specific cytology request form
      Specimen source: cervical, vaginal, and other
      Patient history: Prenatal, Post Partum, Post menopausal, and LMP
      Other clinical information: Hormone usage, IUD, DES Exposed (in utero), Abnormal bleeding, radiation therapy.
      Other pertinent clinical information

   c. Non GYN cytology requires in addition to a. above:

      A specific Pathology/Cytology request form:
      Clinical history
      Pre-Op and Post-Op diagnosis
      Specimen information

2. Out Patients:
   a. Patient demographic information:
      Patient's Name
      Date of Service
      Physician
      Address and telephone
      Sex
      Date of birth
      Social security number
      Race
      Marital status

   b. ICD9 or diagnosis codes

   c. Insurance information – printout from an office computer with the patient's demographics and insurance is acceptable. A copy of the insurance

   d. GYN Cytology requires in addition to a, b, c above:

      A specific cytology request form
      Specimen source: cervical, vaginal, and other
      Patient history: Prenatal, Post Partum, Post menopausal, and LMP
      Other clinical information: Hormone usage, IUD, DES Exposed (in utero), Abnormal bleeding, radiation therapy.
      Other pertinent clinical information

   e. Non GYN cytology requires in addition to a, b, c above:

      A specific Pathology/Cytology request form:
      Clinical history
      Pre-Op and Post-Op diagnosis
      Specimen information

C. **Labeling:**

1. Slides: all slides should be labeled with the patient's name and the site from which the specimen was taken. The slides should be marked **in pencil only.**

2. Body fluids and any liquid specimens: All specimen containers must be

labeled with the patient's name, the type of specimen, the patient's ID number (if an IN PATIENT), and the date.

D. Table of Collection of Cytology Specimens (also see individual procedures)

COLLECTION OF CYTOLOGY SPECIMENS

Cervical Vaginal Endocervical	one slide fixed immed. spray or 95% EtOH or Thin Prep	refrigeration unnecessary*
Urine	50-100 cc voided or cath. fresh	refrigerated
Sputum	early morning (fresh)	refrigerated
Bronchial Wash	secretions sent immediately (fresh)	refrigerated
Bronchial Brush	slide(s) fixed with sray or 95% EtOH	refrigerated unnecessary*
Gastric Wash	must be received in lab within 5 min of collection	must be processed immediately
CSF	2 cc fresh	refrigerate
Body Fluids Peritoneal Pleural, etc.	tube, specimen container or bottles (Fresh) no additives	refrigerate
Gastric Brush	slices fixed with spray or 95% EtOH	refrigeration unnecessary*
Nipple Secretions	apply slide directly to nipple-fix slide immediately	refrigeration unnecessary*
Cyst Aspiration	See Body Fluids	refrigerate
Fine Needle Aspirate	slides fixed immediately tissue in 10% formalin	refrigeration unnecessary*
Tzank Prep	slides fixed immediately	refrigeration

(for Herpes)	with spray or 95% EtOH	unnecessary*

*Refrigeration will not harm specimen

E. **Cytec ThinPrep System Sample Collection Technique Using Medscan Cytobrush Plus GT, gentle touch tip and Pap-Perfect Plastic spatula.**

With patient in lithotomy position, expose cervix using a vaginal speculum moisted with warm water. Visual mucosa and cervix for lesions, ulceration or discharge. Document findings of the examination on patient's record, at relevant clinical findings to laboratory for optimum cytological interpretation.

1. To collect specimen from the exocervix, select contoured end of plastic spatula and rotate to 360° around the entire exocervix while maintaining tight contact with exocervical surface. Remove spatula.

2. Rinse contoured end of plastic spatula in a vial of PreservCyt Solution (supplied Cytye Corporation) by swirling vigorously **ten** (10) times. Discard plastice spatula. Place cap on vial until step 4.

3. Insert Cytobrush Plus **GT** device into the endocervix until only the bottom-most Fibers are exposed. Slowly rotate one-quarter to one-half turn in one direction. Remove device. Do not over rotate. Additional rotation may cause bleeding and contaminate specimen.

4. Rinse the Cytobrush Pluse **GT** in the PreservCyt Solution by rotating the device in the solution **ten** (10) times while pushing it against the wall of the vial. Swirl the device vigorously to further release material. Discard device.

5. Tighten the PreserveCyt vial cap so that the torque line on the cap passes the Torque line on the vile.

WARNING
DO NOT use Cytobrush Plus **GT** cell collector gentle touch tip for endometrial sampling. **Use for cervical sampling only.** For endometrial sampling, use "Medscand's Endorette" cannula. The Cytobrush device is not to be used on pregnant patients, (due to insufficient clinical data).

F. **Collection of gyn specimens – Conventional vaginal/cervical sampling**

1. Vaginal material: take a cervix-scraper (round end), and collect from the vaginal pool for hormonal evaluation (MI), the lateral vaginal wall is the preferred site.

2. Cervical scrapings : from the complete squamo-columnar junction by rotating the spatula (bone end) 360° (degrees) around the external os, high up the endocarvial canal.

3. Place the entire specimen the plain glass portion of the slide and smear gently.

4. Spray generously with fixative **immediately**.

5. Cervix-scrapers or cyto-brushers are preferred over the use of swabs.

G. **V-C-E prepation (gyn) pap-pak cytology kit**

1. Open fixative ampule first.

2. Take vaginal smear from posterior fornix with spatula end of cervical scraper. If an accurate hormonal evaluation is desired, obtain the specimen by lightly scraping the mid-lateral vaginal wall. Spread material on Section "V".

3. Take cervical smear with cervical scraper (bone end) by thoroughly scraping the entire ectocervix with special emphasis on the squamo-columnar junction.

4. Take endocervical smear with "moistened" cotton applicators by rotating sightly in endocervical canal.

5. Smear endocervical material evenly on section "E". Smear cervical material evenly section "C".

6. Immediately fix preparations by flooding entire V-C-E slide with fixative.

7. Do not remove slide from **PAK**. Print patient's name on slide in pencil before sample is smeared on slide.

8. The V-C-E technique is an efficient and accurate method as it directs the clinician's attention to the cancer prone areas of the cervix, endocervix, and vaginal pooled material. Equally important in proper smear preparation is immediate cellular fixation enabling study of nuclear detail. Patient's complete gynecological history and clinician's comments are also essential for accurate cytological evaluation.
   ***NOTE:** Their kits may be used for any slides to be sent to Cytology for evaluation. If the site is other than **GYN**, please note the site on the slip.

H**. Cyto-brush technique**

1. Gently insert the cyto-brush device in the endocervix until only the bristles closest to the handle are exposed.

2. Slowly rotate one half to one full turn.

3. Remove.

4. Prepare the endocervical-smear by rolling and twisting the brush with moderate pressure across a glass slide.

5. Fix with spray fixative.

6. Discard the cyto-brush collector.

7. Contraindications:
   a. **Do Not** use for endometrial sampling.
   b. **Do Not** use for pregnant patients due to insufficient clinical data.

8. Due to the thorough sampling of the endocervical canal by the cyto-brush, there may be some minor painless spotting for a day or two following the Pap test.

9. Never re-use the brush.

10. For use by medically trained personnel only.

11. Follow CDC guidelines for the safe collection of human specimens.

12. Ref. International Cytobrush Inc., Package insert (Zelsmyr Cyto-brush Cell Collector)

I. **Collection and handling of sputum specimens**

1. The patient should be instructed to deposit in the container only those secretions coughed up from the bronchial tree in contrast to saliva which accumulates in the mouth or post-nasal accumulation in the pharynx.

2. The best time for collecting sputum is just after awakening. Forceful, vigorous coughing at this time is often very productive. Have a patient cough deeply (from the diaphragm), into a container. Encourage the patient to expectorate sputum, not saliva.

3. It is important for the patient to rid his mouth of saliva and other material by spitting or rinsing his mouth with water before attempting to bring up sputum.

4. For patients' with scanty sputum, it may take 15-30 minutes of intermittent coughing before an adequate sample can be obtained.

5. For those patients who experience difficulty, sputum coughed up at any time may be used.

6. To increase diagnostic accuracy, it is recommended to repeat the procedure each morning for 3 consecutive days. The success of sputum cytology in detecting positive cases of malignancy is directly proportional to the amount of care taken in explaining the sampling technique to the patient.

7. Sputum specimens should be brought to the laboratory as soon as possible after collection and placed in the specimen refrigerator.

8. A sputum specimen found not to contain deep dust cells will be reported out as unsatisfactory.

J. **Bronchial washings, bronchial brushings, and biopsy**

1. Washings:

1. Submit entire specimen to the laboratory in original containers. If Luken tubes are used, there will be multiple containers. Each container must be labeled with the patient's name, ID#, and type of specimen (be as specific as possible).

2. Laboratory requisitions must contain all necessary information. The number of slips required is dependent upon the orders. Many tests may be ordered on a single bronchial washing. Refer to the listing of slips required on other page.

2. Brushings:

1. Label 2 or 4 frosted end slides with the patient's name. Please write the name in pencil.

2. As soon as the brush is removed from the bronchoscope, the cellular material should be applied to the slide in a circular motion, confined to an area of approximately 2 cm in diameter.

3. Speed of application of material and immediate fixation of slides are critical steps. Failure of rapid fixation (before drying) may destroy the diagnostic value of the specimen.

4. The slides must be fixed immediately. Place slides in 95% Ethyl alcohol for fixing. Place container with slides and requisition in a biohazard bag and transport to laboratory.

5. The brushing slides require a cytology slip for each sampling site. Mark eachSlip with site. If required, the brushes may be immersed in formalin and sent for cell block.

K. **Body fluid specimens: pleural, peritoneal, pericardial, cyst fluids, aspirates**

1. Label the specimen with the patient's name, ID#, physician's name, room#, and type of specimen (be specific). Specimens for Cytology should be submitted in containers with **NO** additives; i.e. red top tubes, specimen cups, glass or plastic bottles, or in a syringe with the needle removed but capped.

2. Fill out the Body Fluid Requisition form (it is a three part form). Check off boxes for all test ordered under each laboratory dept. (i.e. Chemistry-glucose, amylase, etc.; Microbiology – AFB, C&S; Hematology – cell count, differential etc.; Cytology) Many tests may be performed on a single sample. The laboratory will divide the sample according to the orders.

3. When Cytology is ordered on a body fluid please write in any pertinent patient Information (i.e. previous disease, x-ray findings, clinical diagnosis, radiation therapy, or chemotherapy).

4.If there are any questions as to type of container, slips required etc. Please call The laboratory:
Cytology = ext. 5197
Microbiology = ext. 5201

4. Most common tubes used for body fluids:
Red top – Chemistry, Cytology, Microbiology
SST (red and black top with gel) – Chemistry only
Lavender top – Hematology
Yellow – Microbiology only
Green top – Chemistry (sometimes Hematology)

Sterile specimen cups – Cytology Microbiology
Plastic bags from thorocenthese trays – most laboratory dept.
Evacuated glass bottles – most laboratory dept.

L**. Gastric brushings for cytology**

1. Slide Preparation: As soon as the brush is removed from the endoscope:

    a. Apply the cellular material to the slide in a circular motion confined to an area of @ 2 cm in diameter. This must be done quickly.

    b. Fix the slides immediately. With any of the following;
    Drop-on liquid fixative (e.g. **CYTO-FIXER**)
    Spray Fixative (e.g. **CYTO-PREP**)
    95% ethyl alcohol to immerse slides (leave for 20 min.) The slides(s) must be wet down with the fixative. The force of the spray will not dislodge the cells form the slide.

    c. Speed of application of material and immediate fixation are critical. Failure of rapid fixation may destroy the diagnostic value of the specimen.
    d. Label each slide with the site if multiple sites.

3. The brushing slide(s) require a cytology slip - fill out site at which brush was obtained .If multiple sites are brushed, each site requires a Cytology slip.

M. **Breast smear and aspiration (breast cyst)**

1. Preparation of the slides (smear):

    a. Label slides with patient's name (in pencil only).
    b. As soon as the slide is taken, fix immediately before it dries. Spray or drop on fixatives are the most convenient.
    c. If smears of both the left and right breasts are taken, make sure that each slide I Is labeled Lt. or Rt.
    d. When slide(s) is dry, place in a slide folder and secure with a rubber band.
    e. Slides do not need to be refrigerated but refrigeration will not harm slides. The slides and requisition may be dropped off to the laboratory at any time.

N. Aspirates of Breast Cysts: The syringe may be submitted to the laboratory. Remove needle and label the syringe. Place in biohazard bag with slip and send to laboratory. If not transported immediately, refrigerate specimen.

O. Collection of Breast Smears:

1. Gently strip the subareola area and nipple using the thumb and forefinger. If secretion occurs allow only a drop the size of a pea to accumulate on the apex of the nipple.

2. Support the areola and nipple with one hand.

3. With the other hand, place a slide on the nipple, momentarily pause to allow the material to spread a bit laterally, the draw the slide quickly across the nipple.

4. Immediately fix the slide (spray or drops).

5. Repeat entire procedure until all secretions obtainable from the nipple are utilized. Numerous smears may be possible.

P. **Tzank prep – smear for herpes**

1. Write patient's name on the slide in pencil.
2. Using a wooden tongue depressor which has been soaked in water: scrape the lesion energetically, several times.
3. Place the collected, cellular material on the glass end of the frosted slide, and smear gently but thoroughly enough to remove the cellular material from the tongue depressor.

4. Spray (or soak) smear immediately with the fixative. Wet the material thoroughly with the fixative. Spray fixatives will not dislodge the cells from the slide.

5. Fill out the Cytology requisition slip:
6. Fixatives:
   CYTO-FIXER= a drop on liquid fixative
   CYTO-PREP=a spray fixative
   95% Ethyl alcohol in a slide container=must fix for 20 min.
   Any brand name of cytology fixative is appropriate. Formalin should never be used as a cytology fixative.

Q. Oral smears

1. Write patient's name on the slide in pencil.

2. Using a wooden tongue depressor which has been soaked in water; scrape the lesion energetically, several times.

3. Place the collected cellular material on the glass end of the frosted slide, and smear gently but thoroughly enough to remove the cellular material from the tongue depressor.

4. Spray (or soak) smear immediately with fixative. Wet the material thoroughly with the fixative. Spray fixatives will not dislodge the cells from the slide.

5. Fill out the Cytology requisition form.
6. Fixatives:
   CYTO-FIXER = a drop on liquid fixative
   CYTO-PREP = a spray fixative
   Any brand name of cytology fixative is appropriate. Formalin should never be used as cytology fixative.

R. **FINE NEEDLE ASPIRATION**

1. Specimen:

   a. Non-palpable lesions are usually aspirated by the radiologists. Palpable lesions are generally aspirated by surgeons or internists. When the patients are prepared in Radiology, or the ER, the Pathology or Cytology departments should be notified.

   b. When the material is aspirated from the lesion, a drop or two is expressed onto a glass slide and fixed immediately. These will be used for cytology. Usually only 4-6 slides are made. If there is any tissue, it may be put into 10% formalin (or saline) for further processing by the laboratory (e.g. Cell Block).

   c. The specimen may be taken to the laboratory and 1-2 slides stained with a Rapid H&E, to determine specimen adequacy. If the specimen is determined to be non- diagnostic, procurement of another aspirate may be warranted.

   d. The rest of the slides will be stained with a H & E stain and the tissue will be reported out as a cell block.

   NOTE: The quality of the specimen is very important so that a diagnostic conclusion may be reached. Therefore, proper handling of the specimen is crucial. The smears must not be allowed to air-dry.

   f. Fixatives:

      CYTO-FIXER = a drop of liquid fixative
      CYTO-PREP = a spray fixative
      98% Ethyl alcohol in a coplin jar = must fix for 20min.
      Any brand name of cytology fixative is appropriate. Formalin should not be used as a cytology fixative. Formalin is for cell blocks and tissue only.