

Specimen Collection Techniques and Fixation Procedures

Conventional Gynecological Sources – Vaginal, Cervical, Endocervical Smears

For optimal gynecologic cytology, it is recommended that the cellular samples be obtained from the ectocervix and the endocervix for each case and spread on one slide. For atrophic women it is recommended that the spatula be moistened prior to taking the smear. If a specimen is submitted for hormone effect analysis (maturation index), the specimen should be taken from the upper vaginal wall, and placed on a **separate** slide. If an endometrial abnormality is suspected, a vaginal pool specimen may be submitted. The use of the endocervical brush (in non-pregnant patients) in addition to the spatula is highly recommended. Optimally, the patient should abstain from intercourse, douching, or the use vaginal contraceptives during the 24 hours prior to collection. Pap smear collection should be avoided during the menstrual phase. The following procedure should be used to help ensure an acceptable specimen:

1. Label frosted end of slide or VCE slide with the patient's name and DOB. The name should be legibly printed using a pencil or indelible ink. Do not use a grease pencil or ball point pen. If submitting a two part specimen (R + L cervix, maturation index, etc.) make sure each slide is labeled with the appropriate site information.
Note: If using unfrosted slides use a diamond point pen.
2. Ectocervical/Endocervical Specimen
 - A. Cervical Scraper Method: Insert the elongated tip of the scraper into the external os and gently rotate completely around using the tip as a pivot point. The cellular material obtained by this method will usually contain cells from the squamo-columnar junction. If this method does not prove satisfactory, we recommend the use of the cytobrush to obtain the endocervical specimen.
 - B. Cytobrush Method: After sampling the ectocervix with a spatula, gently insert the cytobrush into the endocervical canal until only the bristles closest to the handle are exposed. Slowly rotate one-half to one full turn. Remove pulling straight out.
3. Material obtained should be evenly and thinly spread on the section of the slide farthest from the frosted end. When using the cytobrush the cells should be 'unrolled or untwisted' onto the slide, not painted on which can cause air-drying and distortion of the cells.
4. **Immediately** fix the specimen. This is accomplished by holding the bottle of spray fix 3-4 inches from the slide and dispersing an even layer of fixative over the slide. Alcohol fixation may be substituted for the spray fix. Place the slide in a Coplin jar with 95% ethyl or reagent alcohol, the slide can be removed after 15 minutes.
5. Allow the specimen to dry completely and place in cardboard or plastic slide holders.
6. Submit to Sanford Health Pathology Clinic in a plastic transport bag with the requisition.

Liquid Based Gynecological Sources

Sanford Health Pathology Clinic uses Hologic (ThinPrep) collection vials. Specimens may be collected with the brush/spatula combination or the broom method. **Immediate** dispersal of the specimen into the fixative is imperative with either collection method. Vial holders (eggs) are available upon request.

Endocervical Brush/Spatula Procedure

1. Obtain an adequate sampling from the ectocervix using a plastic spatula.
2. Rinse the spatula as quickly as possible in the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
3. Obtain an adequate sampling from the endocervix using an endocervical brush device.
Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. **DO NOT OVER-ROTATE.**
4. Rinse the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the vial wall. Swirl the brush vigorously to further release material. Discard the brush.
5. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
6. Record the patient's name and ID number on the vial. Record the patient information and medical history on the cytology requisition form.
7. Place the vial and requisition in a specimen bag for transport to the laboratory.

Broom Like Device Procedure

1. Obtain an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.
2. Rinse the broom as quickly as possible into the PreservCyt Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.
3. Tighten the cap so the torque line on the cap passes the torque line on the vial.
4. Record the patient's name and ID number on the vial. Record the patient information and medical history on the cytology requisition form.
5. Place the vial and requisition in the specimen bag for transport to the laboratory.

Non Gyn Cytology Specimens

Note: Please use CytoLyt Solution for fixation of all non-Gyn specimens. Sanford Health Pathology Clinic provides specimen containers with fixative upon request.

Sputum Cytology

1. Have patient brush teeth and rinse mouth with water.
2. Cough vigorously to bring up material from deep in the lungs.
DO NOT JUST CLEAR THE THROAT OR SPIT SALIVA, A DEEP COUGH SPECIMEN PRODUCING MATERIAL FROM THE LUNGS IS REQUIRED.
3. Expectorate (spit) the material into a container of CytoLyt solution.
4. A teaspoon of material per day is adequate
5. Repeat this procedure for 3 consecutive days (same bottle of fixative may be used).
6. Label specimen bottle with patient's name, physician, and specimen type and submit with a completed requisition.

Breast Cyst and Nipple Secretion Cytology (for solid masses of the breast see FNA of Solid Masses)

Breast kits are available to facilitate the collection of nipple secretion. The kit consists of

- two glass slides
- cytology spray fixative
- cardboard slide mailer

Breast cyst fluid obtained by needle aspiration should be transferred directly into CytoLyt fixative.

1. Breast Cyst Fluid
Cyst fluid (more than 0.5 ml) can be expelled directly into a labeled bottle of CytoLyt Solution without making any smeared slide preparations. Alternatively, slides can be prepared as follows: Label two slides with the patient's name, second identifier and source of specimen. The material is transferred to one slide and smeared by placing the second labeled slide on top and pulling the two slides apart, as in a blood smear preparation. Immediately after preparation (1-3 seconds), spray the slides with spray fixative to prevent the cells from undergoing drying or degenerative changes. If spray fixative is NOT available, simply let the slides dry and note on the requisition that the slides are "AIR DRIED". Place the slides into the cardboard or plastic slide mailer.
2. Nipple Secretions
A labeled slide can be touched directly to the drop of secretion on the nipple and then immediately spray fixed. If the secretion is abundant or thick, smear the specimen by placing another labeled slide on top and pulling the two smears apart, or smearing as in a blood smear preparation. The slides should be spray fixed immediately (within 1-3 seconds). If this cannot be achieved allow the slides to air dry (noting on the requisition that the slides are "AIR DRIED"). Place slide(s) into the plastic or cardboard mailer, allow the spray fixative to dry thoroughly before closing the mailer.

Submit the specimen in a transport bag with a properly filled out cytology requisition. In addition to the required information on the requisition, it should also include: if the mass is cystic or solid, whether the specimen is an aspirate or secretion, any pertinent history, radiologic findings, and whether the mass appears clinically suspicious for malignancy.

Fine Needle Aspiration of Solid Masses (recommended technique)

The FNA utilizes the cutting action of the needle tip to obtain material, so be vigorous not timid, in aspirating solid masses. In addition to the required information on the requisition, it should also include: if the mass is cystic or solid, any pertinent history, radiologic findings, and whether the mass appears clinically suspicious for malignancy.

Material Needed

1. 22-25 gauge needles
2. 5, 10, or 20 cc syringes
3. Alcohol or Betadine swabs
4. Sterile gloves
5. Glass microscope slides
6. Spray fixative or Coplin jar filled with 95% alcohol
7. Specimen container with CytoLyt Solution.
8. Syringe holder (gun) – optional
9. Anesthesia – optional
10. Assistant

Procedure

1. Explain the procedure to the patient and get consent form signed.
2. Set up materials
 - A. Place needle on the syringe (and in the gun, if used).
 - B. Label multiple slides with patient's first and last name and second patient identifier (ex: medical record number, date of birth).
 - C. On a nearby flat surface arrange the slides to facilitate smearing and fixing.
 - D. Spray fixative or open Coplin jar of 95% alcohol in close proximity to the slides.
 - E. Have a specimen container of CytoLyt Solution ready if needed to rinse the needle. If the container is used apply pre-printed patient label to the container or label the container with patients name and second patient identifier.
 - F. Assistant ready to help by smearing and/or fixing slides.
3. Put on gloves.
4. Sterilize skin over area to be punctured using alcohol or Betadine swabs.
5. Inject local anesthesia (into skin only) if desired.
6. Fix lesion between fingers.
7. Insert needle into lesion.
8. Apply full vacuum to the needle by pulling back on the plunger.
9. Immediately make 5-10 quick, 2-5 mm in and out excursions into the lesion (do not allow the needle to exit the skin). Aspirate the lesion for 5 – 10 seconds, if however, blood gets to the needle hub it is time to stop and prepare the smears before the specimen clots in the needle.
10. **RELEASE THE VACUUM** by letting the plunger return to its equilibrium point.
11. Remove the needle from the lesion and the patient.
12. Quickly and carefully remove the needle, aspirate 5-10 cc of air into the syringe reattach the needle.
13. Expel semi-liquid aspirate onto slide (one small drop per slide).
14. The assistant should immediately smear material on the slide by placing another labeled slide onto the first slide and pulling the slides apart. To minimize crushing of the specimen, allow only capillary action to hold the slides together while pulling them apart.

15. Fix immediately (1-2 seconds) by spraying or dropping into the Coplin jar of 95% alcohol.
16. It is often helpful to have some air-dried smears as well. If adequate fixed material is obtained, 2 or 3 air-dried smears should be prepared and labeled as such.
17. Rinse any remaining material from the needle and syringe in CytoLyt Solution and submit along with the slides.
18. Repeat the entire process, performing 2-5 separate passes per lesion (depending on site and material obtained) for a total of 6-10 smears. Separate needles and syringes should be used.
19. Obtain hemostasis and bandage patient.
20. Submit the specimen in a transport bag with a properly filled out cytology requisition.

Body Fluids – Large volume (Pleural, Peritoneal)

1. Submit only fixed portions of large volume specimens. The specimen should be fixed in CytoLyt solution. Use equal amounts of fixative and specimen to obtain proper fixation. For volumes over 100 ml submit only 50 ml in fixative. A cell block is routinely attempted on all body fluids but does not always survive processing. Label specimen containers with patient's name, specimen type and second identifier. Submit with a properly filled out cytology requisition.

Body Fluids – Small volume (Breast, CSF, Synovial, etc.)

Fix the specimen in CytoLyt solution. Use equal amounts of fixative and specimen to obtain proper fixation. Excess fixative is acceptable for very small specimen amounts. Use 10 ml of fixative if specimen volume is under 10 ml. Label specimen containers with patient's name, specimen type and second identifier. Submit with a properly filled out cytology requisition.

Body Fluids - Urine

Agitate specimen to mix contents and fix specimen in CytoLyt solution. Use equal amounts of fixative and specimen to obtain proper fixation. Label specimen with patient's name, specimen type and second identifier. Submit with a properly filled out cytology requisition. Indicate if the specimen is voided or obtained by instrumentation.

Washings (Bronchial, Bladder, Gastric, Pelvic, Esophageal, etc.)

Fix the specimen in CytoLyt solution. Use equal amounts of fixative and specimen to obtain proper fixation. Excess fixative is acceptable for very small specimen amounts. Use 10 ml of fixative if specimen volume is under 10 ml. Label specimen containers with patient's name, specimen type, and second identifier. Submit with a properly filled out cytology requisition.

Brushings (Bronchial, Gastric, Esophageal, etc.)

Label slides with patient name and site of area brushed. Smear specimen from the brush onto the slide. Fix slides immediately in a Coplin jar filled with 95% alcohol for 15 minutes or if this is unavailable spray with cytology fixative. Submit the brush by placing into CytoLyt Solution or 95% alcohol. Label specimen containers with patient's name, specimen type and second identifier. Submit with a properly filled out cytology requisition.

Direct Smears of the Skin (Tzanck-Herpes)

Label slides with patient's name and lesion site. If the lesion is extremely dry, soak under a moist towel for 10 minutes. The sample may be obtained by using a scraper (tongue depressor) or by scraping or pressing the slide across/on the lesion. If a scraper is used, transfer the material to a slide. Fix the slides immediately in 95% alcohol or using spray fixative. Air dried smears are also acceptable (please note AIR DRIED on slides and requisition). Submit the specimen in a transport bag with a properly filled out cytology requisition.

Anal-rectal sample

An anal rectal sample can be collected with the patient in either the lateral recumbent or dorsal lithotomy position. If the patient is already having a gynecologic exam, lithotomy is often more convenient. The specimen can be collected before or after the gynecologic exam. For male patients, lateral recumbency is more commonly used with the patient lying on his side with knees drawn up toward his chest. A tap water moistened Dacron swab or cytobrush is used (cytobrush may be more uncomfortable for the patient). The swab or brush is inserted about 5-6 cm into the anal canal past the anal verge, into the rectal vault. Firm lateral pressure is applied to the swab/brush handle as it is rotated and slowly withdrawn from the anal canal, inscribing a cone-shaped arc. Care should be taken to ensure that the transition zone is sampled. A swab or smear of the peri-anal skin is an unsatisfactory sample. The swab or brush is then placed in the preservative vial and agitated vigorously several times to release the cellular material. Discard the collection device. If liquid based cytology fixative is not available, the swab can be smeared onto a glass slide and immediately fixed. Liquid fixative is preferred. HPV testing cannot be performed on a glass slide sample. Label the vial or smear with the patients name and second identifier. Submit the specimen in a transport bag with a properly filled out cytology requisition.