

Cytology Specimens

Introduction

The acceptance of cytopathology as a current and valid discipline in medicine is largely due to the work of George N. Papanicolaou, MD. Papanicolaou began to publish material on the cytologic method and in 1928 suggested that this method was of value in the screening for and diagnosis of cervical cancer. The use of cytology as a diagnostic tool may be applied to any organ or fluid from the body. The specimen may be exfoliated cells in a fluid such as urine, sputum, pleural, etc. or cells that have been more forcibly removed by a scraper, brush, or needle. These specimens would include both liquid based and conventional Pap smears, specimens from endoscopic brushings, and fine needle aspirations. LCM Pathologists, P.C. will only accept specimens from physicians or other persons authorized by law to submit specimens.

Requisition

Cytology specimens must be submitted with a completely filled-out Cytology requisition for LCM Pathologists, P.C. to properly process the specimen. A separate Cytology requisition should be submitted for each source/specimen site. If concurrent biopsy material is also submitted, it should be submitted with a corresponding Histology requisition. The requisition contains an area for patient demographics of: name, age, SS #, date of specimen, doctor name, chart #, DOB, insurance and billing information. The requisition also contains an area for the source and site of specimens as well as pertinent history used to correlate the results. The history and clinical information that should be provided is as follows:

Gynecologic Specimens	Non-Gynecologic Specimens
Date of LMP	Clinical diagnosis and history
Pregnant or Post-Partum	History of cancer – type and location
History of IUD	TB, liver cirrhosis, congestive heart failure, etc.
Abnormal bleeding	Radiologic findings to date, suspected lesion
Recent intrauterine instrumentation	Any systemic disease
Radiation therapy	Dyspnea
Endometriosis	Hemoptysis
Polyps	Radiation therapy (date, reason and location)
Visible lesion(s)	Drug therapy or other medications
DES exposure in utero	Hormone therapy
Recent colposcopy or biopsy (provide diagnosis)	Exposure to carcinogens
Herpes	Tobacco use (specify)
HPV (condyloma)	Recent viral infections
Hormone therapy/ birth control pills	Unexplained, continued weight loss
Previous abnormal cytology cases should provide:	Occupation (if relevant)
Date of previous abnormal	Past abnormal cytology
Treatment	
Normal subsequent cytology cases	

Fixation Methods

Immediate fixation of cytology specimens is critical to the preservation of the cellular components. It is important that no air-drying occurs prior to fixation. If a smear is already air-dried it should not be put in alcohol fixative. Please note on the requisition if the slide(s) being submitted are fixed or air-dried. Formalin fixation is not appropriate for cytology specimens. Specimens should not be exposed to formalin or formalin fumes. This alters the cells and interferes with the staining reactions. There are several fixation techniques available, depending on the type and volume of the specimen. See specimen collection techniques and fixation procedures for specific details.

1. Spray Fixative – suitable for specimens that are submitted on a slide(s). This would include specimens such as Pap smears, FNA specimens, and endoscopic brushing specimens.
2. 95% alcohol (usually used within a Coplin jar) - suitable for specimens that are submitted on a slide(s). This would include specimens such as Pap smears, FNA specimens, and endoscopic brushing specimens. The slides should be immersed in the alcohol for a minimum of 15 minutes. Alternatively, the fixative may be pipetted onto a slide until the smear is totally saturated and then allowed to dry.
3. Saccomanno Collection Fluid – a green colored fixative for the collection of sputum.
4. CytoLyt Solution (Sioux Falls clients) - a clear fixative for the collection of fluid specimens. A 50/50 ratio of specimen to fixative is appropriate (if this unavailable use 50% alcohol).
5. 50% Alcohol – (Mankato clients) a clear fixative for the collection of fluid specimens. A 50/50 ratio of specimen to fixative is appropriate.

Quick Reference Guide to Fixation Techniques

Specimen Type	Recommended Fixation Technique	Comments
Large Volume Specimens: Abdominal and Pelvic washings Body Cavity Fluids (pleural, peritoneal) Urines Gastric/Esophageal washings	Mix with equal amounts of: <u>CytoLyt Solution for Sioux Falls clients</u> <u>or</u> <u>50% Alcohol for Mankato clients</u> Fix no more than 50 ml of specimen; submit the remainder of body cavity fluid unfixed.	Refrigerate the unfixed portion of the specimen if possible.
Small Volume Specimens: FNA (fluid – not slides) Breast fluid CSF Cyst fluid Synovial fluid Bronchial washing	Mix with equal amounts of: <u>CytoLyt Solution for Sioux Falls clients</u> <u>or</u> <u>50% Alcohol for Mankato clients</u> Use 10 ml of fixative if specimen volume is under 10 ml.	With very small amounts of fluid it may be easier to transfer the fixative into the collection device (syringe, suction collection tubes) first. Then into a suitable container to submit the specimen.
Direct Smears: Pap Smears FNA specimens Brushings Nipple Secretions	<u>95% alcohol:</u> The slides should be immersed in the alcohol for a minimum of 15 minutes. Alternatively, the fixative may be pipetted onto a slide until the smear is totally saturated and then allowed to dry. <u>or</u> <u>Spray fix:</u> Hold the bottle of spray fix 3-4 inches from the slide and disperse an even layer of fixative over the slide. Preferred method for Paps.	Pap smears should be completely dry before placing them into cardboard containers. The endoscopic brush may be submitted in 50% alcohol after the slides have been prepared.

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 patient menses. 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 a two part case is being submitted (R + L cervix, MI 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 LCM Pathologists, P.C. in a plastic transport bag with the requisition.

Liquid Based Gynecological Sources

LCM Pathologists, P.C. uses the Cytyc (Thin Prep) collection vials. Specimens may be collected with either the brush/spatula combination or the broom. **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 _ or _ 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 PreservCyt 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 form 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: Clients of Sioux Falls please use CytoLyt Solution for fixation of all non-Gyn specimens. Clients of Mankato please use 50% alcohol for fixation of non-Gyn specimens and Saccomanno fluid for sputum specimens. LCM Pathologist, P.C, 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, WHAT IS NEEDED IS A DEEP COUGH PRODUCING MATERIAL FROM THE LUNGS.
3. Expectorate (spit) the material into a container of CytoLyt solution (Sioux Falls) or Saccomanno fixative (Mankato).
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 to LCM with a completed requisition.

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

Sioux Falls Clients: Breast kits are available to facilitate the collection of breast cyst fluid and nipple secretions. The kit consists of :

- 1) two slides
- 2) cytology spray fixative
- 3) a bottle of CytoLyt Solution
- 4) cardboard slide mailer

Mankato Clients: Order the supplies needed, using the supply order form.

1. Breast Cyst Fluid
Cyst fluid (more than 0.5 ml) can be expelled directly into a labeled bottle of CytoLyt Solution or 50% alcohol without making any smeared slide preparations. Alternatively, slides can be prepared as follows: Label two slides with the patient's name and source of specimen. The material is placed on one slide and smeared by placing the second labeled slide on top and pulling the two slides apart, or 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. Place both slides into the cardboard mailer, allow the spray fixative to dry thoroughly before closing the 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). Place slide(s) into the cardboard mailer, allow the spray fixative to dry thoroughly before closing the mailer.

Submit the specimen in a transport bag with a completely 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 it is a 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 swaps
4. Sterile gloves
5. Glass microscope slides
6. Spray fixative or coplin jar filled with 95% alcohol
7. Specimen container with CytoLyt Solution or 50% alcohol
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.
 - 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. Open specimen container of CytoLyt Solution or 50% alcohol.
 - 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 swaps.
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 or 50% alcohol 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 completely filled out Cytology requisition.

Body Fluids – Large volume (Pleural, Peritoneal)

1. For Sioux Falls Clients: Submit fresh, refrigerated, unfixed specimen if you send specimens through a courier. If you send specimens by mail or there is a delay of more than 24 hours anticipated (weekend specimens), fix the specimen in CytoLyt Solution. Use equal amount of fixative and specimen to obtain proper fixation. For volumes over 100 ml, submit 50 ml in fixative and the rest unfixed. If between 30-100 ml, send specimen unfixed and refrigerated. Whenever a specimen contains a substantial amount of cellular material, a cell block is routinely performed at an additional fee. Label specimen with patient's name, physician, and specimen type. Submit with a completely filled out requisition.
2. For Mankato Clients: Submit specimens fixed in 50% alcohol. Use equal amounts of fixative and specimen for proper fixation. For volumes over 100 ml, submit 50 ml in fixative and the rest unfixed. Whenever a specimen contains a substantial amount of cellular material, a cell block is routinely performed at an additional fee. Label specimen with patient's name, physician, and specimen type. Submit with a completely filled out requisition.

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

Fix the specimen in CytoLyt Solution or 50% alcohol. Use equal amount 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 with patient's name, physician, and specimen type. Submit with a completely filled out requisition.

Body Fluids - Urine

Agitate specimen to mix contents and fix specimen in CytoLyt Solution or 50% alcohol. Use equal amount of fixative and specimen to obtain proper fixation. Label specimen with patient's name, physician, and specimen type. Submit with a completely filled out requisition. Indicate if specimen is post instrumentation/catheterized or voided.

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

Fix the specimen in CytoLyt Solution or 50% alcohol. Use equal amount 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 with patient's name, physician, and specimen type. Submit with a completely filled out requisition.

Brushings (Bronchial, Gastric, Esophageal, etc.)

Label slides with patient name and different areas (as the sample warrants). 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 (as a pap smear would be). If the brush is also to be submitted, drop into CytoLyt Solution or 50% alcohol. Label specimen with patient's name, physician, and specimen type. Submit with a completely filled out requisition.

Direct Smears of the Skin (Herpes)

Label slides with patient's name. If the lesion is extremely dry, soak under a moist towel for 10 minutes. The sample may be obtained by the use of a scraper (tongue depressor) or by scraping the slide across the lesion. If a scraper is used, transfer the material to a slide. Fix the slides immediately in 95% alcohol or spray fix. Submit the specimen in a transport bag with a completely filled out Cytology requisition.

The 2001 Bethesda Reporting System

Gynecologic Cytopathology Reports

The 2001 Bethesda System is used for reporting results of gynecological specimens. The primary interpretation is shown between asterisks on the final report and has three general categories:

1. Unsatisfactory for Evaluation: The smear does not yield diagnostic information. Additional statement(s) will state the reason that the specimen is unsatisfactory
2. Negative for Intraepithelial Lesion or Malignancy: This indicates that the findings are normal.
3. Epithelial Cell Abnormality: This category is used to indicate there are abnormal cells present. Additional statements in the body of the text will describe the severity and type of the abnormality, and may include a recommendation for further action.

Additional statements that may appear in the body of the text include: presence of organisms, presence or absence of endocervical cells (non-hysterectomy patients), obscuring elements, hormonal levels, location of testing (if not the lab where specimen is submitted). A statement of adequacy will follow these additional statements. The bottom portion of the report contains patient clinical information submitted on the requisition.

Non-Gynecologic Cytopathology Reports

The primary interpretations used for reporting are:

1. Unsatisfactory Specimen for Evaluation
2. Non-Diagnostic
3. Negative for Malignancy
4. Suspicious for Malignancy
5. Highly Suspicious for Malignancy
6. Positive for Malignancy
7. Diagnosis
8. No Category – for cases that require an explanatory text, similar to a histology report.

Additional statements about the specimen contents, cellular abnormalities, or other findings will follow the primary diagnosis when applicable. An adequacy statement will follow the additional comments. The bottom portion of the report contains patient clinical information submitted on the requisition and the gross description of the specimen.

Quality Assurance

All screening is performed by Pathologists (physicians who are certified by the American Board of Pathology) or by Cytotechnologists (registered or registry eligible by the American Society of Clinical Pathologists). A pathologist performs interpretation of all cellular abnormalities (reactive/reparative, ASCUS, dysplasia, malignancy).

Supervisory eligible personnel rescreen approximately 10% of all negative cases to confirm the original report and reduce the possibilities of false negative findings. Quality assurance monitors evaluate on an on-going basis the performance of all testing personnel as well as the performance of the overall laboratory. Continuing education and quality assurance conferences are held regularly between pathologists and cytotechnologists to discuss educational, difficult, interesting or unusual cases.

Specimen Transport of Cytology Specimens

For **all** specimens submitted on glass slides such as Pap smears, brushings, and FNAs, the patient's name should be legibly printed (pencil or indelible ink) on the frosted end of **each** glass slide. For non-gynecologic specimens, each specimen container must be labeled with the patient's name and the specimen type/site. Pap smears should be placed into plastic or cardboard slide containers and then into the plastic LCM biohazard transport bag (1 specimen/bag). Non-gynecologic specimens that are submitted as fluids should be transported in a sealable container, and placed in a plastic transport bag whenever possible. For all specimens the transport bag should be securely zipped shut and the requisition placed into the pocket on the outside of the bag. Place in area designated as the courier pickup area.

Specimen Rejection of Cytology Specimens

Cytology specimens submitted without a patient name on the specimen will be returned to the client for patient identification. We are required to verify patient identification for all specimens submitted. Specimens which cannot be processed or tested due to inadequate fixation, leaking specimen containers, slides received shattered beyond repair etc. will not be processed. A report indicating the reason for specimen rejection will be issued to the client and no charges will be made for those specimens. Every attempt will be made to prevent delay in testing or compromised results for the safety of you and your patient.

Cytology Test Listing

Test Code	Test	Specimen Requirements
104 or 1104	Pap Smear	A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for conventional pap smears for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details.
108 or 1108	Thin Layer Pap Smear	A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for thin layer pap smears for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details.
137	Sputum	A properly collected, fixed and labeled specimen. - See Specimen Collection Techniques for sputum for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details
172	Bronchial Washings	A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for washings for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details
175	Bronchial Brushing	A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for brushing specimens for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details
290	Fine Needle Aspirations	A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for FNA (solid and cystic) specimens for details. A cytology requisition that is completely and properly filled out, indicate exact specimen site. Example: FNA of thyroid not FNA of neck. See Cytology Requisition for details. Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details
110	Body Fluids	Includes large/small volume fluids, non-bronchial brushings/washings, urines, herpes smears. A properly collected, fixed and labeled specimen - See Specimen Collection Techniques for body fluids for details. A cytology requisition that is completely and properly filled out – See Cytology Requisition for details Place specimen and requisition in transport bag and place in area designated for courier pickup. – See Specimen Transport of Cytology Specimens for details
125	HPV Typing	Additional test that may be ordered on thin layer pap smears. Indicate in the available area on the requisition.
127	Chlamydia	Additional test that may be ordered on thin layer pap smears. Indicate in the “other” area of the requisition.
129	Neisseria Gonorrhoeae	Additional test that may be ordered on thin layer pap smears. Indicate in the “other” area of the requisition.