

Collection of Non-Gynecologic Specimens for Cytopathology Testing

- Specimen containers may be picked up in room A2-412, Cytology Lab. Fluid should be poured immediately into the container. Write patient's complete name, date of birth and/or medical record number on the container.
- All specimens must be treated as potential infectious material. Therefore use specimen transport bag. Place the completed cytology requisition in the outer pouch. Place the specimen inside the bag. Refrigerate specimen if delivery is delayed.

Collection of Sputum, Bronchial, Esophageal & Gastric Specimens

A Sputum

- A deep cough specimen should expectorate directly into a jar containing CytoLyt Fixative. Three specimens over 3 days are recommended (can be pooled). It is crucially important to get a deep cough specimen, explain to the patient the difference between sputum and saliva. Aerosol inhalants can be used to stimulate sputum production. Obtain samples before meals to avoid food contamination. Patient should thoroughly rinse their mouth before expectorating.
- Do not use 95% ethanol. Higher concentration of ethanol binds with proteinaceous material to form a hard lump, which makes it difficult to mash up and make thin-layered slides.

B Bronchial Aspirates and Washings including Bronchoalveolar Lavage (BAL)

- Bronchial secretion aspirate must be fixed immediately with an equal part of CytoLyt Fixative. Rinse aspiration tube 2 or 3 times with a small amount of CytoLyt and add these washings to the specimen container. Bronchial irrigation with saline may be used. Fix as above. Specimens must be labeled whether bronchial secretion or bronchial washing and from which side obtained.

C Bronchial Brushings

- Brush should be appropriately handled to obtain adequate sampling and removed from the bronchus with the entire bronchoscope so material is not lost from surface of the brush. For each sample the entire scope and brush should be reinserted. The entire sample and disposable brushes must be submerged in a centrifuge tube containing CytoLyt fixative and delivered to the Cytopathology lab. (A2-412). When cultures are indicated, send separate specimens to Microbiology in the correct media.

Note: If there is a suspicion that the patient may have lipoid pneumonia, the sputum or BAL should be collected fresh, unfixed for Oil Red O Fat Stain.

D Esophageal and Gastric Washings

- Esophageal and gastric washings should be mixed with an equal part of CytoLyt Fixative. Rinse aspirator with a small amount of fixative and add to the specimen container.

E Esophageal and Gastric Brushings

Esophageal and Gastric brushing are similar to bronchial brushings, above.

- The entire sample must be deposited into a centrifuge tube containing Cytolyt fixative and delivered to Cytopathology Lab (A2-412). In certain clinical circumstances, it may be appropriate to air dry slides. This option must be discussed prior to specimen collection, with the Cytology Supervisor or Attending Cytopathologist by calling the Cytology office at x 1666.

Exudates (Pleural, Pericardial and Peritoneal Fluids)

Pleural, Peritoneal, Pericardial and other Fluids

- Body cavity fluids may be sent **fresh and unfixed** provided they are promptly (1–2 hours after collection) delivered to the laboratory. All liquid body cavity fluids should be kept refrigerated at all times, unless submitting immediately to the Cytology laboratory for processing. **DO NOT Freeze**. If the specimens are refrigerated immediately they can last over a 4-day-long weekend.
- For the best cytological morphology, NEVER add any kind of fixative or anticoagulant. If a delay in processing is anticipated or refrigeration is *not possible*, mix with equal volume of Cytolyt fixative and send to the laboratory.
- *Do Not* use high concentrations of ethanol as a fixative. 70% and 95% ethanol will combine with protein in fluid to form heavy precipitation. The heavier the precipitation, the more protein and fewer cells will be on the smears.

Spinal Fluid

- It is best to process CSF in the fresh state. During normal operating hours, fresh CSF, ideally 5-10 ml can be brought directly to the laboratory for immediate processing. During nights and weekends (or if specimen is not delivered immediately to the laboratory) it should be fixed in equal volume of CytoLyt Fixative and refrigerated.
- Volume: at least 1 to 2 ml if possible. Absolute minimum: 0.5 ml.

- Due to small volume, spinal fluids are stored in small test tubes.
- CSF Delivery - Please deliver CSF specimen to the lab as soon as possible. Cells in CSF are very delicate and may degenerate if not processed immediately.

Fine Needle Aspirate (FNA) Specimens

A For FNAs performed by clinicians

- The preferred collection technique, regardless of the site of FNA, is to deposit and rinse the entire sample into a centrifuge tube containing CytoLyt fixative and deliver to the Cytopathology Lab (A2-412) for processing.

B For EUS FNA adequacy assessment requests

- At least 24 hours advance notice (excluding weekends and holidays) is required. However, the ideal ordering algorithm would be that, at the time the procedure is scheduled for outpatients, our department is notified of any possible need for adequacy.
- Requests made with less than 24 hours notice (presumably for inpatients) require approval from the director of Cytopathology or his designee and, in such cases, adequacy assessment will only be provided if the procedure and need for adequacy assessment is clinically urgent.

Requests must include:

- 1) Patient's name, MRN, and DOB.
 - 2) Expected location and approximate start time of procedure. Note that this service is only available between 9:30AM and 3PM on non-holiday weekdays.
 - 3) Clinical information is always a plus. However, any special circumstances must be noted at the time of request (e.g., R/O lymphoma) in order for pathology staff to be appropriately prepared.
- On the procedure day, a 20 minute lead time before the cytotechnologist is needed on site is required. Please call Cytopathology at x1666 or x2657. Additionally, should a procedure be cancelled or should the adequacy assessment be cancelled for another reason, please call Cytopathology to advise us of this change.

Adequacy Assessment Procedure:

- Upon the arrival of the cytotechnologist, they will be provided with an appropriately labeled Cytopathology requisition including two patient identifiers and the following:
 - 1) Site(s) to be biopsied.

- 2) Detailed clinical history including clinical impression (i.e., provisional diagnosis) and supporting data such as the patient's presentation and workup results to date (e.g., imaging findings).
- Upon withdrawn of the biopsy device from the endoscope by the physician performing the EUS FNA procedure, the nurse will make sure the needle is in the retracted position. She/he will hand the needle tip end of the device to the cytotechnologist.
 - The cytotechnologist will position it appropriately above a glass slide pre-labeled with two patient identifiers.
 - Only upon the direction of the cytotechnologist who will say "needle out" will the nurse extend the needle.
 - The cytotechnologist will direct the nurse to apply positive pressure on the attached air filled syringe by saying "push slowly".
 - When either the cytotechnologist is satisfied with the material present on the glass slide or when positive pressure is not yielding a significant amount of (or any) material, the cytotechnologist will advise the nurse to retract the needle by saying "needle in."
 - Once the needle is observed to be fully retracted within its sheath, the cytotechnologist will hand the biopsy device back to the nursing staff who will transfer any remaining sample into the appropriate fixative, usually CytoLyt. Use one appropriately labeled centrifuge tube with pooled sample from all needle passes for a single site.
 - When significant clotting of sample in the device occurs, as evidenced by the inability to expel sample or air from the needle with no more than moderate positive pressure, the sample must be expelled into specimen fixative for cell block processing instead of onto slides for smearing.
 - The cytotechnologist will not participate in specimen transfer to the centrifuge tube containing fixative, as not only is the procedure of smearing time sensitive, but the rapid transmission of the adequacy result is also of priority.
 - Air-dried, Diff-Quik stained smears (as well as alcohol fixed smears as appropriate) will be prepared by the cytotechnologist.
 - After preliminary onsite review of Diff-Quik smears, the cytotechnologist will relate to an appropriate physician a sample adequacy result as follows: "adequate", "inadequate", or "equivocal".
 - Each distinct statement of adequacy will be documented on the EUS FNA Adequacy Assessment form as well as transcribed into the final Cytopathology report.

- Cytotechnologists are prohibited from providing a preliminary impression or diagnosis.
- Upon completion of the adequacy assessment, the Cytotechnologist will take responsibility for transporting all specimen material (i.e., requisitions, slides and centrifuge tubes) to the lab.
- As soon as it's available, the patient's EUS report must be delivered or faxed to the attention of Cytopathology (x3331). At the discretion of the pathologist, case signout may be delayed until the EUS report is received.

Note: On December 9, 2014, an interdepartmental training session led by the Director of Cytopathology (Dr. Somma), all cytotechnologists, and the senior endoscopy nursing staff occurred in OR 11. Training included the distribution and review of the document "Endoscopy / Cytopathology EUS FNA Adequacy Training" version 1. Subsequent training of any additional staff who become involved in these procedures from either department will occur as needed and will be the responsibility of each individual department.

Urine and Upper Tract Brushings and Washings

A Voided Urine

- Voided urine should be obtained 3-4 hours after the patient has last urinated.
- Recommended Volume: 25-100ml.
- Collect urine in a clean container, mix well and put lid on tightly. Deliver to the doctor's office, clinic or Cytology Lab promptly.
- If a delay in transport to the lab is anticipated, refrigeration is recommended.
- If a delay of more than 24 hours is anticipated, fixation with approximately an equal volume of Cytolyt fixative is recommended to avoid degeneration.

Note: DO NOT drink water to induce urination within an hour or so. Urine collected by this method produces poor cellular content.

B Catheterized Urine

- This is the method of choice for female patients. If malignancy is suspected, voided urine from a female patient has no value in establishing tumor involvement of the bladder. Malignant cells from the vagina cannot be ruled out.
- Midstream urine is an alternative method of collecting urine if catheterization technique is not available.

- To obtain midstream urine: Wash vaginal area well. Urinate approx half of the urine from the bladder. Collect the second half of the voided urine in a clean container. Mix well.
- Catheterized urine collection is also used by the Urologist during the Cystoscopic procedures (i.e. bladder washings). Volumes may vary, in part depending how much urine is in the bladder at the time of Cystoscopy.
- Recommendations for refrigeration and fixation are as given above for voided urine specimens.

C Ureteral / Pelvic Brushings and Washings

- The entire sample from Ureteral and Pelvic brushings should be submerged in a centrifuge tube containing CytoLyt fixative. For washings, ratio of specimen to CytoLyt fixative should be close to 50/50.
- Recommendations for refrigeration and fixation are as given above for voided urine specimens.

Collection of Gynecologic Specimens for ThinPrep Pap test

Purpose

The detection of cervical cancer and its precursors as well as other Gynecologic abnormalities is the primary purpose of obtaining a cervical cell sample. It is important to obtain a specimen that is not obscured by blood, mucus, inflammatory exudates or lubricant.

The ThinPrep 2000 System is used in the processing of liquid-based Gynecologic specimens - ThinPrep Pap Test. In conjunction with the Molecular Pathology Laboratory at DMC, HPV testing is also performed on patients age 30 and older, and, reflexively, on younger patients with PAP results of ASCUS. The HPV testing results are posted separately in the Cerner System at DMC.

The Procedures

Preparation of Patient

- The patient should be tested 2 weeks after the first day of her last menstrual period and definitely not when she is menstruating. Even though ThinPrep reduces obscuring blood, clinical studies have demonstrated that excessive amounts of blood may still compromise the test and possibly lead to an unsatisfactory result.
- The patient should not use vaginal medication, vaginal contraceptives, or douches during the 48 hours before the exam.

Specimen Collection Preparation

- Lubricant jellies should not be used to lubricate the speculum.
- Remove excess mucus or other discharge present before taking the sample. This should be gently removed with ring forceps holding a folded gauze pad. The excess cervical mucus is essentially devoid of meaningful cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.
- Remove inflammatory exudates from the cervical canal before taking the sample by placing a dry 2x2 inch piece of gauze over the cervix and peeling it away after it absorbs the exudates or by using a dry proctoswab or scopette. The excess inflammatory exudates is essentially devoid of diagnostic cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.
- The cervix should not be cleaned by washing with saline or it may result in a relatively acellular specimen.
- The sample should be obtained before the application of acetic acid.

Collect Gynecologic Sample Using the 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.
- 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.
- Tighten the cap so that the torque line on the cap passes the torque line on the vial.
- Record the patient's name, date of birth and/or medical record number on the vial.
- Record the patient information and medical history on the cytology request form.
- Place the vial and requisition in a specimen bag for transport to the laboratory.

Collect Gynecologic Sample, Using the Endocervical Brush/Spatula Device

- Obtain an adequate sampling from the ectocervix using a plastic spatula with 360 degree rotation while maintaining tight contact. Rinse the spatula as quickly as

possible into the PreservCyt solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.

- Obtain an adequate sampling from the endocervix using an endocervical brush device. Insert brush into cervix until only the bottom-most fibers are exposed. Slowly rotate $\frac{1}{4}$ or $\frac{1}{2}$ turn in one direction. *Do not over rotate.*
- 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 vigorously to further release material. Discard the brush.
- Tighten the cap so that the torque line on the cap passes the torque line on the vial.
- Record the patient's name, date of birth and/or medical record number on the vial.
- Record the patient information and medical history on the cytology requisition form.
- Place the vial and requisition in a specimen bag for transport to the Laboratory.

Collection of Conventional PAP Smears

The Procedures

- Preparation of patient – the same procedures as in 01.8.1
- Print patient's name at the frosted end of the slide before collecting specimen.
- The cervical OS, transformation zone and any lesion must be sampled. Cytobrush is preferred device for sampling. After the smear is made, immediately spray the slide with spraycyte. Follow instructions on the spray container. Spray thoroughly. Smear should still be wet when spray is applied to prevent cellular deterioration.
- If spraycyte is not available, put smear in container containing 95% ethanol fixative immediately.
- Put sprayed slides in slide cardboard holders. Allow smears to dry before putting smears in slide folders to prevent cells sticking to inside cover.
- All specimens are classified as potential infectious material. Therefore use specimen transport bag.

- Record the patient information and clinical history on the Cytology requisition form. Put the cytology form in the outer pouch. Place the specimen in the inside pouch. Deliver specimen to the Lab for processing.

References

- Koss, LG, "Diagnostic Cytology and its Histopathologic Bases" 5th edition. 2006
- "Papanicolaou Technique; Approved Guideline" NCCLS Document GP15-A Vol. 14 No 8.
- ThinPrep 2000 System Operator's Manual: Chapter Four (4.1-4.6).
- Cibas, ES. "Cytology Diagnostic Principles and Clinical Correlates" 2nd edition. 2003