

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

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.

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.

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.

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.

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

- Liquid 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

- In general, 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. Direct smears made by clinicians (or with the assistance of cytotechnologists or pathologists) may be applicable to certain specimens. This option must be discussed, prior to sampling / specimen collection, with the Attending Cytopathologist by calling the Cytology office at x 1666.

Urine and Upper Tract Brushings and Washings

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.

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.

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.

Specimen Delivery

- Cytopathology Lab specimen processing room A2-412, 9 am to 5 pm, Monday through Friday.
- Central Accessioning room A2-428 for Evenings, weekends, and holidays.

Specimen Container Pick-Up

- Cytopathology Lab Processing Room A2-412, 9 am to 5 pm Monday through Friday.
- The refrigerator in the vicinity of Central Accessioning room A2-428 - Cytolyt fixative available for Non-Gynecologic samples and FNA specimens. (if needed for evenings, weekends, and holidays)

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