Cytology Specimen Collection and Processing

Principle
A gynecological cytology specimen (Pap smear) is an evaluation for presence of abnormal cells, which may be indicative of malignancy or other conditions requiring treatment. It is important to sample cervix or vagina well with minimal artifact and obscuring materials.

Precautions
1. Gloves should be worn when collecting and handling specimen.
2. Specimens should be taken before pelvic examination.
3. Patient should not douche or use vaginal medication for 24 hours before specimen is obtained. This should not, however, prevent obtaining a specimen. Inform patient that test may be unsatisfactory so that patient will not be unduly alarmed if a repeat PAP is later required.
4. Do not use lubricant. If necessary, speculum may be moistened with normal saline. Avoid using water, which is hypotonic and will produce cellular distortion.
5. Avoid, if possible, taking specimen during normal menses. However, if there is abnormal bleeding, obtain routine PAP and consider direct endometrial specimen.

Materials
1. Cytyc® Preservcyt® solution vial. Preservcyt® solution is a methanol-based buffered preservative solution. Store vials at 15° C to 30° C (59° F to 86° F).
2. Plastic spatula
3. Endocervical brush
4. GYN Cytology Requisition, form number 245 and Client Request Form.

These supplies can be obtained from Scott & White Reference Laboratory:
1. PreserCyt® Solution
2. Medescand Cytobrush Plus GT (endocervical brush & plastic spatula)

Note: Cotton tip swabs and wooden spatulas should not be used to obtain specimens for ThinPrep® PAP test.

Collecting the Specimen
1. Write patient’s name and medical record number on vial or place patient’s identification label on vial. This is essential to prevent a mix-up of specimens during processing.
2. Expose cervix with speculum. The cervical surface should not be wiped; wiping it will remove cell-rich adherent cervical mucus.

Cervical Scrape
Scrape external os 360° with plastic spatula and as quickly as possible place spatula into Preservcyt® solution vial, swirling spatula vigorously in vial 10 times. Never induce bleeding by scraping cervix vigorously.

Endocervical Brush
Insert brush into cervical os and rotate gently. It is recommended that brush be rotated only 180°. More rotation may cause excessive bleeding. Rinse brush as quickly as possible in Preservcyt® solution vial by rotating device in solution 10 times while pushing against Preservcyt® vial wall. Swirl brush vigorously to further release material. For thick mucoid specimens collected using the brush, to further release endocervical cells that might be entrapped in mucous; use concave side of spatula and scrape down brush bristles a few times and on different sides of brush. This can be done while holding brush in vial with left hand and using spatula to scrape with right hand.

Vaginal Scrape
For specifically desired hormonal evaluation (maturation index), gently scrape lateral wall of upper third of vagina. Rinse spatula as quickly as possible in Preservcyt® solution vial by swirling spatula vigorously in vial 10 times.
1. Tighten cap so that line on cap and line on vial meet.
2. For best results, please follow these preparation steps diligently.

Method for Submitting Specimen to the Laboratory
Clients will submit a request form and a form 245 with patient’s name, medical record number, and appropriate clinical and billing information. This includes proper diagnostic codes, specimen source, and other clinical data such as last menstrual period (LMP), previous treatment, previous abnormals, colposcopic findings, hormonal status, etc. Fill in date and time specimen is obtained. Specimen vial should be placed in a biohazard bag and completed requisition form placed in side pocket of bag. Specimens collected after 5 p.m. on weekends or holidays should be held until following workday.
Non-Gynecological Cytology Specimens

Principle
It is important that high quality diagnostic material is provided for cytopathologic examination.

Precautions
1. Gloves should be worn at all times when handling unfixed specimens in accordance with the Department of Pathology Bloodborne Pathogen Policy.
2. All NON-Gyn cytology specimens must be handled using face protection.

Procedure for Specimen Collection
All specimen containers and slides should be properly identified and labeled with patient’s name and medical record number. A completed requisition form that matches specimen identification should be submitted with specimen. Form should bear patient identification data, date and time of collection, physician/resident name, source of specimen and pertinent clinical information. Failure to properly identify specimens, or mismatches between specimens and requisition forms will result in delay of processing or rejection of specimen.

Lung
Sputum
Patient must rinse his/her mouth with water, bend horizontally at waist and press hands against abdomen (just below rib traction of diaphragm) and expectorate directly into container. Sputum specimens are collected in fresh state in sputum cups and transported to laboratory inside biohazard plastic bags. Requisition form containing demographic data, clinical information, and date and time of collection must be attached. Specimens received during night and weekends are to be placed in refrigerator and delivered to cytology the next working day. Specimens not processed within 18 hours should be fixed with 50% to 70% alcohol.

Bronchial Washing
Bronchial wash specimens are collected in fresh state in a container and transported in a biohazard plastic bag. The completed requisition form must be attached. Reference to specific site of washing should be included (eg right upper lobe). Requests for special studies (eg GMS stains, flow cytometry, etc.) should be indicated.

Bronchial Brush
Smear is made at time of endoscopy by rolling brush on a totally frosted slide or slides which are immediately immersed in a Coplin jar with 95% alcohol.

Serous Cavity Fluid
All effusions submitted for cytologic evaluation must be heparinized at time of collection to prevent coagulation. Recommended quantity of heparin (1: 10,000) is 1 mL heparin to 300 mL body cavity fluid. If patient has been bed ridden, it is advisable to gently rotate him prior to tapping fluid filled area; this is necessary to re-suspend those cells, which have settled within body cavity due to their heavy cellular density. The specimen should be brought fresh to cytology laboratory immediately following procedure during the day. Should procedure need to be performed at night or on weekends, fluid should be placed in a refrigerator. Pleur-evac containers must not be submitted to cytology laboratory.

Cerebrospinal Fluid
Spinal fluid for cytologic examination obtained during working hours (Monday through Friday from 5 a.m. to 6 p.m.) should be immediately delivered to cytology laboratory to be processed. Specimens obtained after 6 p.m. or on weekends or holidays should be mixed with an equal volume of 50% or 70% ethyl alcohol and placed in refrigerator in microbiology laboratory to be delivered the following working day.

Urine
All voided specimens should be collected as a mid-stream clean catch. First-morning specimen should be discarded. To urine specimen, add an equal part of 50% to 70% alcohol. Patient’s name should be placed on urine container. Clinical data should include whether urine is voided or instrumented. Presence or absence of a previous tumor or previous treatment should also be noted and Cytoscopy findings indicated. A form that includes requested clinical data can be duplicated. It should be attached to all requisition forms of urine specimens.

Endoscopic Brushing
Gastric, esophageal, duodenal, bile duct or colonic brushings are done on completely frosted slides. Smears are prepared quickly and placed immediately in 95% ethyl alcohol. Air drying should be avoided.
**Fine-Needle Aspiration**
Direct smears made at time of aspiration are immersed immediately in Carnoy’s fixative (provided by cytology laboratory) for approximately 5 minutes then transferred into 95% ethanol. Caution should be taken not to leave slides in Carnoy’s fixative for more than 5 minutes, because this will result in cellular distortion and artifacts. Purpose of this short immersion in Carnoy’s fixative is to lyse red blood cells and prevent obscuring of cellular detail by blood. Specimens that are not directly smeared, are to be collected in 20 mL tube of a 1:1 solution of 50% ethyl alcohol and Ringer’s solution (5 mL each). Specimen is flushed into container and sent with appropriate cytology form to laboratory for processing. Aspirates from cystic lesions can be forwarded to laboratory in syringe (after discarding needle) if they are sent immediately after procedure is completed. If that is not possible, they should be flushed into above-mentioned fixative solutions.

**Breast Fluid Secretion**
The area of greatest accumulation of secretion is found immediately below nipple and areolar area. A breast pump may be used, although material collected for cytologic evaluation is more frequently obtained through spontaneous secretions. Care should be taken not to manipulate breast unnecessarily. Material for cytologic evaluation is collected by placing a frosted-tip slide against nipple and smearing fluid quickly over slide. Immediate fixation may be accomplished if patient (or an assistant) is allowed to hold open bottle of 95% ethanol in front of breast so that slide can be immediately dropped into fixative. Air-drying should be avoided as it may render specimen non-diagnostic.