Appendix F

Cytology
Specimen Collection and Processing

Gynecological Cytology Specimens (Pap smear)

ThinPrep Pap test

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

Precautions
1. Gloves should be worn when collecting and handling the specimen.
2. Specimens should be taken before pelvic examination.
3. The patient should not douche or use vaginal medication for 24 hours before the specimen is obtained. This should not, however, prevent obtaining a specimen. Inform the patient that the test may be unsatisfactory so that she will not unduly be alarmed if a repeat PAP is later required.
4. Do not use lubricant. If necessary, the 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. Cytac Preservcyt solution vial. Preservcyt solution is a methanol-based buffered preservative solution. Store the vials at 15° – 30° C. (59° – 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 the Scott & White Reference Lab:
- PreserCyt Solution
- 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 the patient’s name and medical record number on the vial or place the patient’s identification label on the vial. This is essential to prevent a mix-up of the specimens during processing.
2. Expose cervix with the speculum. The cervical surface should not be wiped; wiping it will remove the cell-rich adherent cervical mucus.

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

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

For specifically desired hormonal evaluation (maturation index), gently scrape lateral wall of upper third of vagina. Rinse the spatula as quickly as possible in the preservcyt solution vial by swirling the spatula vigorously in the vial 10 times.

1. Tighten the cap so that the line on the cap and the line on the 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 the patient's name, medical record number, and the appropriate clinical and billing information. This included proper diagnostic codes, specimen source and other clinical data such as last menstrual period (LMP), previous treatment, previous abnormalities, colposcopic findings, hormonal status, etc. Fill in the date and time the specimen is obtained. The specimen vial should be placed in a biohazard bag and the completed requisition form placed in the side pocket of the bag. Specimens collected after 5:00 PM, on weekends or holidays should be held until the following workday.

Non-Gynecological Cytology Specimens

Principle

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

Precautions:

Gloves should be worn at all times when handling unfixed specimens in accordance with the Department of Pathology Bloodborne Pathogen Policy. 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 the patient’s name and medical record number.

A completed requisition form that matches the specimen identification should be submitted with the specimen. The 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 the specimen.

LUNG

Sputum

The patient must rinse his/her mouth with water, bend horizontally to the waist and press his hands against the abdomen (just below rib traction of diaphragm) and expectorate directly into the container.

Sputum specimens are collected in the fresh state in sputum cups and transported to the laboratory inside biohazard plastic bags. The requisition form containing the demographic data, clinical information, date and time of collection must be attached. Specimens received during the night and weekends are to be placed in the 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

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

Bronchial Brush

The smear is made at the time of endoscopy by rolling the brush on a totally frosted slide or slides which are immediately immersed in a Coplin jar with 95% alcohol.
FLUID FROM SEROUS CAVITY

All effusions submitted for cytologic evaluation must be heparinized at the time of collection to prevent coagulation. The recommended quantity of heparin (1:10,000) is 1 ml heparin to 300 ml body cavity fluid. If the patient has been bed ridden, it is advisable to gently rotate him prior to tapping the fluid filled area; this is necessary to re-suspend those cells, which have settled within the body cavity due to their heavy cellular density.

The specimen should be brought fresh to the cytology lab immediately following the procedure during the day. Should the procedure need to be performed at night or on weekends, the fluid should be placed in a refrigerator. Pleur-evac containers must not be submitted to the cytology lab.

CEREBROSPINAL FLUIDS

Spinal fluid for cytologic examination obtained during working hours (M-F from 5:00 AM to 6:00 PM) should be immediately delivered to the cytology lab to be processed. Specimens obtained after 6:00 PM or on weekends/holidays should be mixed with an equal volume of 50% or 70% ethyl alcohol and placed in the refrigerator in the microbiology lab to be delivered the following working day.

URINE

All voided specimens should be collected as a mid-stream clean catch. The first morning specimen should be discarded.

To the urine specimen, add an equal part of 50 to 70% alcohol. The patient's name should be placed on the urine container. The clinical data should include whether the urine is voided or instrumented. The presence or absence of a previous tumor or previous treatment should also be noted and the cytoscopy findings indicated. A form that included the requested clinical data has been attached to this page and can be duplicated. It should be attached to all requisition forms of urine specimens.

ENDOSCOPIC BRUSHINGS

Gastric, esophageal, duodenal, bile duct or colonic brushings are done on completely frosted slides. The smears are prepared quickly and placed immediately in 95% ethyl alcohol. Air-drying should be avoided.

FINE NEEDLE ASPIRATION

Direct smears made at the time of aspiration are immersed immediately in Carnoy's fixative (provided by the cytology lab) for approximately 5 minutes then transferred into 95% ethanol. Caution should be taken not to leave the slides in the Carnoy's fixative for more than 5 minutes, because this will result in cellular distortion and artifacts. The purpose of this short immersion in Carnoy's fixative is to lyse the 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). The sample is flushed into the container and sent with the appropriate cytology form to the lab for processing. Aspirates from cystic lesions can be forwarded to the lab in the syringe (after discarding the needle) if they are sent immediately after the procedure is completed. If that is not possible, they should be flushed into the above-mentioned fixative solutions.

BREAST FLUID SECRETION

The area of greatest accumulation of secretion is found immediately below the nipple and the 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 the breast unnecessarily. The material for cytologic evaluation is collected by placing a frosted-tip slide against the nipple and smearing the fluid quickly over the slide. Immediate fixation may be accomplished if the patient (or an assistant) is allowed to hold the open bottle of 95% ethanol in front of the breast so that the slide can be immediately dropped into the fixative. Air-drying should be avoided as it may render the specimen non-diagnostic.