Cytology Specimen Collection Procedures Outpatient

Conventional Pap Smears
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Fine Needle Aspiration

If there are any questions regarding any of the procedures included herein or with any cytology specimens, please call the Cytology Department as follows:

Director of Cytopathology: 447-2571
Cytotechnologists: 447-2568
Anatomic Pathology Manager: 447-2583
Pathology Dept.: 447-2570
Conventional Pap Smear Procedure

Materials Needed:
- BMC Requisition form with patient information completed
- Spray fixative
- One plain glass slide with a frosted end
- One cytobrush
- One spatula
- A #2 pencil
- Cardboard folder for transport

Label the slide on the frosted end with the patient’s name and date of birth using a #2 pencil.

I. Material is first obtained from the endocervix using the cytobrush as follows:

A. Place the cytobrush in the endocervical canal until only the bottom of the brush can be seen. Slowly and gently rotate the brush at least 180 degrees and at most 360 degrees (one half turn or one complete turn).

B. Place the brush on the glass slide and gently roll the brush onto the slide, making a complete turn. Material may be placed at one vertical end of the slide or along one half of the horizontal side. Care should be taken to spread the material thinly and evenly.

C. Spray immediately with fixative, holding the fixative approximately 10" from the slide, or following directions on the spray fixative.

II. Material is then obtained from the ectocervix using the spatula as follows:

A. Place the spatula at the endocervical os as pictured and rotate the scraper 360 degrees around the cervix.

B. Smear the material on the clear half of the same slide. Again, spread material as evenly and thinly as possible.

C. Spray immediately with fixative, being careful to not spray too closely as this may “wash” the material off the slide.
**SurePath® Pap Smear**

**Materials Needed:**
- BMC Requisition form with patient information completed
- One Rovers Cervix- Brush® Combi or broom
- One SurePath collection vial

I. Label the vial with patient name and date of birth. Additional information such as name of clinician or date is encouraged. Open the vial.

II. Insert the collection device into the endocervical canal and apply gentle pressure until the bristles form against the cervix.

III. Maintaining gentle pressure, hold the stem between the thumb and forefinger; rotate brush two (2) times in a CLOCKWISE direction.

IV. Place thumb against the back of the brush pad and disconnect the entire brush from the stem into the SurePath preservative vial. Brush remains in the vial through specimen processing.

V. Place the cap on the vial and tighten. Place into a specimen bag, seal the bag, and place the folded requisition form into the pocket on the specimen bag.

VI. Send bag to the Laboratory.
Sputum Specimens

Single Sputum Specimen:

Materials needed: 1 wide-mouthed plastic container with screw top
BMC requisition form with patient information completed

I. Patient is given the plastic cup and instructed to cough deeply and expectorate coughed up material into the cup. Care should be given by the patient to avoid saliva specimens. An early morning sputum is encouraged.

II. Specimen should be sealed tightly to avoid leakage. Specimen should be labeled with patient’s name and date of birth.

III. Specimen should be sent to the Laboratory as soon as possible. If any delay, sputum specimen should be refrigerated until it can be sent to the Lab.

Sputum Series:

Materials needed: Three (3) wide-mouthed plastic containers with screw top
BMC requisition form with patient information completed.

I. Patient is instructed to give 3 sputum samples as instructed above, one each day for 3 consecutive days, in the mornings.

II. After each sputum is collected, the screw top should be tightly fastened to prevent leakage.

III. Specimen should be sent to the Laboratory as soon as possible. If any delay, sputum specimen should be refrigerated until it can be sent to the lab.

IV. Each specimen will be processed separately as they arrive in the Laboratory.
Urine Samples

Materials Needed: Plastic container, able to be tightly covered. BMC requisition form with patient information completed

I. Best urine sample is the second morning urine. (First morning urine usually contains too much degeneration.)

II. Patient should be instructed to drink a few glasses of water upon rising in the morning, then give a second morning urine sample.

III. Patient should try to give a clean urine into the container, avoiding contamination from the skin around the urethra.

IV. Specimen should be sent to the Lab as soon as possible. If any delay, specimen should be refrigerated until it can be transported.

V. Label with Patient’s name and date of birth.

VI. Three successful second morning urines may be particularly helpful. Two or more of these in a series of urines may be combined in the Lab depending upon date and time of receipt.
Breast Discharge

Materials Needed:  Glass slide with frosted end  
Fixative, either spray or coplin jar of 95% alcohol  
Cardboard folder for transport  
A #2 pencil  
BMC requisition form with patient information completed  
SurePATH VIAL

I. Write the patient’s full name, date of birth and site (example; right breast) on the frosted end of the slide with a #2 pencil.

II. If secretion is evident, place glass slide against secretion and smear material across slide directly from the breast. Take care to ensure that the specimen is placed on the same side as the patient’s name is written. Fix immediately. (Spray fix or submerge in coplin jar of 95% alcohol)

III. Care should be taken to obtain secretion only onto the slide and not to scrape the skin of the nipple.

IV. If secretion is not evident, gently aid the secretion from the subareolar area to the nipple. If no secretion appears, do not massage or squeeze further. (Too vigorous manipulation is thought to loosen and possibly spread malignant cells.)

V. One smear may be sufficient, depending on the amount of material. If abundant material, more than one slide may be made.

VI. If secretion is in abundance, let secretion drip into a SurePath vial and submit to the Laboratory.
Direct Scraping/Tzanck Smears

Two methods may be used for direct skin scrapings or tzanck preps: direct smear method onto a glass slide or the SurePath method.

SurePath:

Materials Needed:  SurePath vial  
                  Cytobrush (detachable head)  
                  BMC requisition form with patient information completed

I. Label SurePath vial with patient’s full name, date of birth and specimen site.

II. A freshly unroofed vesicle is the preferred target lesion. Carefully break open the vesicle and detach its “roof.” Gently brush lesion with cytobrush. Drop detachable head of device into SurePath vial. A scabbed-over lesion is an unsuitable target for a Tzanck Scrape.

III. If the lesion is open, wipe off the surface debris. Gently brush lesion with cytobrush. Drop detachable head of device into SurePath vial. A scabbed-over lesion is an unsuitable target for a Tzanck Scrape.

IV. Tightly seal the SurePath vial and send to the Laboratory for processing, along with the requisition.

Direct Smear:

Materials Needed:  Glass slide with frosted end  
                  Spatula, scraper or tongue depressor type tool  
                  Spray fixative or coplin jar of 95% alcohol  
                  BMC requisition form with patient information completed

I. Label slide with a #2 pencil writing the patient’s full name and date of birth on the frosted end of the slide.

II. If an open lesion, scrape the lesion and spread the material gently and thinly onto the glass side (the same side as the frosted end). Fix immediately.

III. If lesion is a closed vesicle, vesicle must be opened. Carefully break open lesion and remove “roof.” Scrape the lesion and follow technique in step II above fix immediately.

IV. Send slide(s) to Lab with requisition.
Bronchial Washing/Brushing Procedure and Bronchioalveolar Lavage

INTRODUCTION

Bronchial washings and brushings are procedures that are done during flexible fiberoptic bronchoscopy (FOB) to diagnose lung disease via cytologic specimens, especially malignancy. Efforts should be made to use all techniques available, and BW and BB techniques should be done in combination with each other and with endobronchial forceps biopsy; a greater variety of specimens increases the diagnostic yield. This procedure describes how to obtain bronchial washings and bronchial brushings (BW and BB) during FOB, and includes the method of bronchioalveolar lavage (BAL), a more forceful washing that accesses the terminal bronchi and alveoli and is used commonly to aid in the diagnosis of infectious organisms, especially *Pneumocystis carinii*.

INDICATIONS

Indications for BW/BB include, but are not limited to, the following: suspected lung cancer, suspected metastatic malignancy, multiple pulmonary masses, an undiagnosed pulmonary mass, a patient who fails to respond to appropriate antibiotic treatment, a suspected infectious process, a patient with suspected lung cancer not diagnosed in at least 3 consecutive morning sputum samples, and a visible lesion seen during bronchoscopy. Indications for BAL include, but are not limited to: a suspected infectious process especially *Pneumocystis carinii*, interstitial lung disease, suspected lung cancer, and suspected metastases.

MATERIALS NEEDED

- Flexible fiberoptic bronchoscope with 3-way valve, syringe, and brushing apparatus.
- Sterile saline solution or equivalent.
- Container for specimen, such as Lukens tube or other leak-proof screw-capped plastic container.
- Clear glass slides with frosted end.
- For fixing direct slides, cytology spray fixative or container (such as a Coplin jar) of 95% alcohol.
- A #2 pencil.
- Cytology requisition form or information into computer.

PROCEDURES

All specimen containers received from the following procedures must be labeled with the patient’s name, date of birth, type of specimen and site. A requisition form or computer entry must contain specimen types and sites. If direct smears are made onto glass slides, the patient’s name, date of birth and specimen site must be written on the frosted end in pencil (pens or other writing instruments should not be used because they wash off in the slide staining process). If more than one of a particular type of specimen is obtained, such as a BW from 2 or 3 separate areas, the container and the requisition must be identified as to specific site so diagnoses can be applied to the appropriate areas.

**BW**: If the lesion is endoscopically visible after introduction of the bronchoscope into the lower respiratory tract, a washing over the surface of the lesion should be done by the instilling of 3 to 5 ml of a balanced salt solution through the bronchoscope and aspirating the resulting material via suction mechanism. The material aspirated may be suctioned directly into a container such as a Lukens tube. Also direct slides may be made with immediate fixation with cytology spray fixative or into 95% ethyl alcohol. If the lesion is not visible, a washing may be done of an area, such as the carina, the hilar...
region of a lung, a lobe, or bronchus. Be sure that the top is screwed on tightly or that any other type of container is leak proof for transport to the Laboratory. The material collected should be sent to the Lab as soon as possible; if a delay of greater than 1 hour is expected, the material should be refrigerated.

**BB:** The bronchoscope includes an apparatus by which a small brush is inserted into the FOB, and a visible lesion can be brushed. The brush is pulled back out; and either a direct smear may be made which is immediately fixed with cytology spray fixative or the specimen slide may be placed into 95% ethyl alcohol. Preferably the brush is immediately placed into PRESERVCYT fluid. The brush may be left in the fluid if possible, or should be gently but firmly rotated in the PRESERVCYT fluid container against the inside wall to remove cellular material. If no lesion is visible, brushings of the mucosa may be done, especially if there is discoloration or a change in the mucosal appearance. Be sure that the top is screwed on tightly or that any other type of container is leak proof for transport to the Laboratory. The material collected should be sent to the Lab as soon as possible; if a delay of more than 1 hour is expected, the specimen should be refrigerated.

**BAL:** The FOB is introduced into the endobronchial tree and is then advanced to a segmental bronchus and wedged in place to completely occlude the lumen. Proper wedging prevents proximal seepage of the lavage fluid which may cause irritation and cough. Sterile saline is introduced in 20-50 ml aliquots and is immediately aspirated until a return of at least 40 ml is obtained. Gentle, steady suction should be applied to the syringe plunger to avoid the collapse of the bronchial wall. Be sure that the top is screwed on tightly or that any other type of container is leak proof for transport to the Laboratory as soon as possible. The material collected may be sent to the Lab unfixed in a leak-proof, screw-capped plastic container. If a delay is expected greater than 1 hour, the specimen should be refrigerated.

References:


Body Cavity Fluids and Washings
(Pleural, Peritoneal, and Pericardial Effusions)

INTRODUCTION

Normally, microscopic amounts of fluid are located in the pericardial, peritoneal, and the two pleural body cavities. In many benign and malignant disease processes, this fluid accumulates and becomes an abnormal amount, called an effusion, which can be visualized on x-ray or grossly and which may cause symptoms, such as shortness of breath. This abnormally accumulated fluid is commonly removed for diagnostic testing and to relieve patient symptoms. Fluid is removed either by needle aspiration techniques or a surgical procedure whereby a tube is inserted into the cavity. This procedure briefly describes the methods to obtain effusions, called thoracentesis, paracentesis, and pericardiocentesis. Also included is the procedure for washings.

INDICATIONS

An abnormal accumulation of fluid in any of the four serous cavities may be removed to alleviate patient symptoms and/or may be used for diagnostic purposes. A known benign condition that commonly causes an effusion, such as congestive heart failure, COPD, or renal disease, may not require cytologic evaluation of the effusion specimen, especially if re-accumulation occurs after one or more benign diagnoses. However, an initial tapping, a suspected malignancy, or an unknown diagnosis may warrant cytologic evaluation. A malignant diagnosis in an effusion is important in the staging of the patient. A false positive diagnosis may be disastrous as it indicates a malignancy that may be inoperable and with extensive spread. It is critical that specimens are labeled correctly. Specimen containers must be labeled with the patient’s name, date of birth, type of specimen and site. A requisition form or computer entry must contain specimen type and site.

MATERIALS NEEDED

- Local anesthetic.
- Large bore needle and syringe
- If surgical specimen, scalpel, chest tube, vacuum bottle.

PROCEDURES

Specimens received from the following procedures must be placed in clean, dry, leak-proof containers. Ideally, effusions should be sampled into containers to which are added about 5 units of heparin for every cubic centimeter of aspirated fluid, or 1 ml of 1:1000 heparin solution for every 100 ml of specimen. This helps prevent bloody body fluids from clotting to a point of losing liquid properties and turning into a solid bloody proteinaceous mass.

Procedures: (Cont’d.)

All specimen containers received from the following procedures must be labeled with the patient’s name, date of birth and type of specimen and site, and a requisition form or computer entry must contain specimen type and site. Specimen containers must be labeled on the container itself, not the cover. Containers must be leak-proof and sent to the Laboratory as soon as possible. If there will be any delay, the specimen should be refrigerated. Prefixative
such as 50% ethyl alcohol should NOT be added to the specimen as this will cause precipitation of proteins in the fluid and hardens the cells which prevent them from adhering to the slides and from absorbing the stains.

1. Most effusions are removed by inserting a wide bore needle, under local anesthesia, transcutaneously into the body cavity. Suction is applied and the effusion material aspirated. Specimen container depends on the amount of fluid aspirated and the method. Material may be left in the syringe for transport to the Laboratory AFTER the needle is replaced with a plastic cap. Material may be transferred to any leak-proof container for transport to the Lab.

2. Effusions, especially pleural effusions, may be removed via the surgical placement of a chest tube which may be attached to a glass or plastic vacuum bottle. The specimen container must be determined to be leak proof prior to transport to the Lab.

3. Effusions may be removed at the time of surgery. Specimen container must be determined to be leak proof prior to transport to the Lab.

4. Also done during surgery may be a washing of a body cavity. This is especially common as a peritoneal washing done during exploratory abdominal surgery or for surgery for malignancies of the female genital tract. A physiologic saline solution (does not have to be sterile) is instilled into the various recesses of the peritoneal cavity and then withdrawn. The specimen container must be determined to be leak proof prior to transport to the Lab.

References: