Cytology Specimen Collection Procedures

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- Urine Specimens
- Breast Discharge
- Direct Skin Scraping/Tzanck Prep
- Bronchial Washings/Brushings/BAL
- Body Cavity Fluids
- 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:

Medical Director of Cytopathology: 447-2598
Chief Technologist, Histopathology/Cytopathology: 447-2583
Pathology Dept.: 447-2570
Prep Area: 447-2585
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Conventional Pap Smear Procedure

Materials Needed:
- BMC Requisition form with patient information completed (attached)
- 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, preferably first and last name, 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 not to spray too closely as this may “wash” the material off the slide.

Approved by: D. Carter, MD
Medical Director: Adopted: July 2004
Approved by: J. Shaffer
Technical Director: Revised:
Prepared by: Reviewed: 1-27-10
Technologist:
**SurePath® Pap Smear**

Materials Needed:  
- BMC Requisition form with patient information completed  
- One Cervex® brush or broom  
- One SurePath collection vial

I. Label the vial with patient name. Additional information such as name of clinician or date is optional. 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 five (5) 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. Entire brush remains in the vial until processed in the Laboratory; no need to swish or swirl brush in vial.

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.

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- **Medical Director:** D. Carter, MD  
- **Technical Director:** J. Shaffer

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Sputum Specimens

Single Sputum Specimen:

Materials needed: 1 wide-mouthed plastic container with screw top
BMC requisition form with patient information completed
CytoLyt™ fixative (optional)

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.

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
CytoLyt fixative (optional)

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 patient should place each in the refrigerator (if CytoLyt is not used). If CytoLyt fixative is used, the specimens do not need to be refrigerated. Again, screw tops should be tightly fastened to prevent leakage.

III. After the three sputum samples are completed, they should be sent to the Laboratory as soon as possible.

(IV. Once in the Laboratory, these specimens will be combined and processed as one.)
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. 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.

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Breast Discharge

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

I. Write the patient’s full name 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, or take another slide and let material flow between two slides and either “pop” slides apart or pull apart at the horizontal end. Take care to ensure that the specimen is placed on the same side as the patient’s name is written. Fix immediately.

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.

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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 Thin Prep method. **The Thin Prep method is preferred.**

**Thin Prep:**

Materials Needed:  
- CytoLyt fixative  
- Cytobrush  
- BMC requisition form with patient information completed

I. Label cytolyt container with patient’s full name.

II. If the lesion is open, wipe off surface debris. If the lesion is closed, carefully break open the vesicle and clean out fluid and debris. Gently brush lesion with the cytobrush. Place cytobrush directly into CytoLyt fluid and rotate the brush in the solution 10 times while pushing against the wall of the container. Discard the brush.

III. Tightly seal the CytoLyt container and send to the Lab as soon as possible, with requisition form stating specimen site. If specimen is not able to be sent to the Lab within 24 hours, refrigerate the specimen.

**Direct Smear:**

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

I. Label slide with a #2 pencil writing the patient’s full name 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, then gently wipe away fluid and debris. Scrape the lesion and follow technique in step II above. Fix immediately.
IV. If lesion is very dry, gently moisten lesion with a cotton swab dipped in saline, and discard swab. Then use a scraper to scrape lesion and follow technique in step II. Fix immediately.

IV. Send slide(s) to Lab with requisition, providing location of lesion.
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; or container of CytoLyt fixative provided by the Cytology Department.
- Clear glass slides with frosted end.
- For fixing direct slides, cytology spray fixative or container (such as a Coplin jar) of 95% ethyl 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 and the type of specimen and site, and a requisition form or computer entry must contain specimen types and sites. If direct smears are made onto glass slides, the patient’s name 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 or may be placed into commercial liquid-based vials such as CytoLyt® fixative from Cytyc Corp. 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 CytoLyt fixative fluid. The brush may be left in the fluid if possible, or should be gently but firmly rotated in the CytoLyt 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.
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 or may be placed into CytoLyt. 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 pericardiacentesis. 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. Therefore, the pathologist must be absolutely certain before a diagnosis of malignancy is made on a body cavity fluid.

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 is 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 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 prevents 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: