

**Cytopathology Specimen Collection and Handling**

**Specimen Acceptance Policy**
If a specimen does not meet the criteria below, it will not be processed until the problem is resolved, which will create a delay in turn-around time. To avoid unnecessary delays, PLEASE follow these instructions carefully:

1. Patient’s name MUST be written on slide or specimen container AND on requisition.

2. Patient’s name MUST match name on specimen and requisition.

3. Requisition MUST state the specimen type or specimen source.

4. The following information MUST be included on requisition for Pap smears:
   - Patient’s name
   - Clinician
   - Date of birth
   - Patient’s address (for billing purposes)

5. Slides broken during transit will be processed unless more than 50% of slide is broken beyond repair. The Cytology Department will contact clinician if a slide must be returned.

6. Non-gynecologic specimens should arrive fresh from inside the hospital or from Billings Clinic outpatients. ALL other service areas MUST collect non-gynecologic specimens in 30 mL of CytoRich® Red cytology fixative (blue) or Preservcyt® solution (clear) for transport to laboratory. (Preservcyt® is used for direct lesion scrapes and nipple discharges ONLY.)

**Gynecologic Cytology (Pap Smears)**

A. **Determine if the patient has Medicare coverage:**

   1. **NO Medicare coverage:**
      a. Select/order desired tests in “Non-Medicare” patient section of the “Gynecologic Cytology Request Form,” and include an ICD-9 code.
      b. Complete requisition with all required information.

   2. **Medicare coverage:**
      a. Determine if “Screening Pap, High Risk Screening Pap, or Diagnostic Pap.”
         (1) Screening: The beneficiary has not had a screening Pap smear test during the preceding 2 years. (V76.2) Mark “Screening Pap” on requisition.
         (2) High Risk Screening: There is evidence (on the basis of her medical history or other findings) that she is of childbearing age and has an examination that indicated the presence of cervical or vaginal cancer or abnormalities during any preceding 2 years; OR that she is at high risk for developing cervical or vaginal cancer. (V15.89 - other specified personal history presenting hazards to health.) Mark “High Risk Pap Screening” on requisition.
            (a) high risk factors
            (b) early onset of sexual activity (under age of 16 years of age)
            (c) multiple partners (5 or more in a lifetime)
            (d) history of a sexually transmitted disease (including HIV infection)
            (f) fewer than 3 negative Pap smears within the previous 7 years
            (g) DES-exposed daughters of women who took DES during pregnancy
            (3) Diagnostic: Pap test is performed for a diagnostic reason. Mark “Diagnostic Pap” on requisition and provide ICD-9 code.
      b. Complete requisition with all required information.

   3. **Advanced beneficiary notice (ABN):**
      a. Present patient with ABN to sign when you reasonably believe that Medicare will deny payment.
         (1) EXAMPLE: Screening Pap more frequent than every 2 years.

**Pap Smear Collection Instructions**

A. **Conventional Method**

   1. **Patient Preparation:** Optimal time for collection of Pap smear is 2 weeks after first day of last menstrual period. Patient should be instructed not to use vaginal medications, spermicides, or douches 48 hours prior to collection. Patient should also refrain from intercourse 24 hours prior to collection.
2. **Preparation of the Cervix**: Warm water should be used to lubricate speculum. Lubricant jelly should be avoided as it often obscures cellular material making cytologic evaluation difficult or impossible. Speculum must be positioned so that outer surface of cervix appears at end of instrument, since a specimen from this area is necessary for adequate specimen collection. Remove excess blood, mucus, or inflammatory material with a cotton swab prior to taking smear.

3. **Materials (supplied):**
   a. Speculum
   b. Wooden cervical scraper
   c. Cytobrush
   d. Glass slide (frosted end)
   e. Spray fixative
   f. Slide container (plastic or cardboard)
   g. Gynecologic Cytology Request Form
   h. Label with patient’s name

4. **Specimen Collection:**
   a. Complete the “Gynecologic Cytology Request Form” with the following patient information:
      1. Age
      2. Date of birth
      3. Date of last menstrual period (LMP)
      4. Pertinent clinical information
      5. Complete name, mailing address, and billing information
   b. Label frosted end of glass slide with patient’s name in pencil.
   c. Pap smear must be obtained under direct visualization.
   d. Insert extended end of wooden scraper into cervical os. Rotate scraper with moderate pressure around cervix, using external os as a pivot (Figure 1). Do not smear at this time, rather hand scraper to an assistant to hold until collection is complete.
   e. Insert cytobrush into external os to obtain endocervical specimen (Figure 2). Brush should be inserted so that only last bristles can be seen. Rotate brush 180 degrees.
   f. Spread cellular material from cytobrush on half of slide, opposite label end, by rotating and twisting brush with moderate pressure (Figure 3).
   g. Evenly spread cellular material from wooden scraper onto glass slide closest to label (Figure 4).
   h. IMMEDIATELY FIX WITH SPRAY FIXATIVE (Figure 5).
   i. Allow slide to dry approximately 15 minutes.
   j. Place dry slide in a plastic or cardboard slide holder (Figure 6).
   k. Label slide holder with patient’s name.
   l. Send slide and properly completed “Gynecologic Cytology Request Form” to laboratory.
B. **SurePath® Method**

1. **Patient Preparation**: Optimal time for collection of Pap smear is 2 weeks after first day of last menstrual period. Patient should be instructed not to use vaginal medications, spermicides, or douches 48 hours prior to collection of smear. Patient should also refrain from intercourse 24 hours prior to collection of smear.

2. **Preparation of the Cervix**: Warm water may be used to lubricate device. Lubricant jelly should be avoided as it often obscures cellular material making cytologic evaluation difficult. Excess blood, mucus, or inflammatory material should be removed with a cotton swab prior to taking sample.

3. **Materials and Reagents**:
   a. Cervex-Brush® (broom-like device) used to collect specimen
   b. SurePath® Preservative Fluid Collection vial

4. **Storage Requirements**:
   a. Storage condition for SurePath® Preservative Fluid without cytologic specimens is up to 36 months from date of manufacture at ambient temperature (15-30° C).
   b. Storage requirements for SurePath® Preservative Fluid with cytologic specimens is 6 months at refrigerated temperatures (2-10° C) or 4 weeks at ambient temperature (15-30° C).

5. **Specimen Collection**:
   a. Contact cervix with Cervex-Brush® (broom-like) device and insert central bristles into cervical canal deep enough to allow shorter bristles to fully contact ectocervix.
   b. While maintaining gentle pressure in direction of cervix, rotate brush 5 times in a clockwise direction.
   c. Detach entire broom head from handle and place it in collection vial. Place cap on vial, making sure that cap is tightened to avoid leaking, label with patient’s information, and send to laboratory along with properly filled out requisition.

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**Hormonal Evaluation**

**A. Conventional Method**

1. Collect smear like that of a conventionally collected Pap smear EXCEPT, SCRAPE WILL ONLY BE FROM LATERAL VAGINAL WALL.
2. Fix slide immediately with spray fixative.
3. Forward specimen promptly to laboratory with a properly completed “Gynecologic Cytology Request Form” in “Request Forms” in “Special Instructions.” BE SURE TO MARK “HORMONAL EVALUATION.”

**B. ThinPrep® Method**

1. Collect specimen the same way as stated above for conventional method with exception of step #2.
2. Instead of spray fixing smear, put material directly into a vial of Preservcyt® solution.
3. Forward specimen promptly to laboratory with a properly completed “Gynecologic Cytology Request Form” in “Request Forms” in “Special Instructions.” BE SURE TO MARK “HORMONAL EVALUATION.”

**C. HPV DNA Hybrid Capture**

1. HPV DNA Hybrid Capture can be done on ThinPrep® contents per request from provider/care giver.

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**Fine-Needle Aspirations**

The following fine-needle aspiration (FNA) procedures are identified by either “palpation” or “imaging.” Palpation FNA procedures most often involve sites such as breast, thyroid, neck lesions, parotid gland, and lymph nodes. Imaging FNA procedures most often involve sites such as lung, liver, kidney, pancreas, and retroperitoneal area. Imaging procedures are always performed in CAT scan, ultrasound, or special x-ray under direction of a radiologist.

**A. Palpation—Palpable Lesions**

1. **Materials (supplied)**:
   a. Cameco® syringe pistol (optional)
   b. 20 mL disposable syringe (MUST use Luer-Lok® syringes with Cameco® syringe pistol)
   c. 22 gauge disposable needles
   d. Alcohol swabs
   e. Two glass slides (for air-dried smears)
   f. Vial of CytoRich® Red fixative (30 mL)

2. **Palpation Procedure**: Place patient in a comfortable lying or sitting position that allows lesion to be easily aspirated. Before performing aspiration, palpate mass to identify depth of target and its relation to surrounding structures.
3. **Aspiration Procedure**:
   
a. Clean skin overlying the region to be aspirated with an alcohol swab and immobilize area with thumb and index finger of one hand.

b. Attach a 20 mL disposable plastic syringe with Luer-Lok® to a 22 gauge needle and fit it into Cameco® syringe pistol. Pistol will enable one-handed withdrawal and release of syringe plunger.

c. Place needle against skin at the determined puncture site and insert it into mass with a single, quick motion without negative pressure on syringe.

d. Once needle is in the mass, retract plunger of syringe to create negative pressure in syringe and needle lumen. This will draw material into syringe.

e. Move needle back and forth several times and direct it into different areas of mass. This “fanning” motion allows for sampling a wide area of lesion.

f. Maintain constant negative pressure in syringe throughout this manipulation by keeping plunger of syringe retracted.

g. Closely observe junction of needle and syringe. At first sight of material, aspiration is complete.

h. Allow pressure in syringe to return to atmospheric pressure by gently releasing plunger. Aspirated material should remain within needle.

i. Withdraw needle from lesion and apply pressure to puncture site with a sterile gauze pad. Needle should never be removed while any negative pressure is applied to syringe.

j. After needle is removed from the mass, detach it from syringe (Figure 7), fill syringe with air, and reattach needle (Figure 8). Express a small amount of material, about “pea-size,” onto a glass slide. Cover slide with another glass slide and perform the “two-slide-pull” to spread the material (Figure 9). Allow the 2 slides to air dry. Express remaining material into a vial of CytoRich® Red fixative. Remove needle, draw fixative into syringe, reattach needle, and flush contents into vial (Figure 10).

k. Repeat previous step for each pass of aspiration, EXCEPT for the 2 air-dried slides.

l. Forward specimen promptly to laboratory with a properly completed “Reference Pathology/Non-Gynecologic Cytology Request Form” in “Request Forms” in “Special Instructions.”

**Note:**

1. Label specimen container and both glass slides with patient’s name and specimen source (aspiration site).

2. **Specimen source (aspiration site) is also required** on request form for processing.
Draw a few mL of CytoRich® Red cytology fixative into syringe through needle.

Express syringe contents back into CytoRich® Red cytology fixative vial. Repeat if necessary.
B. Imaging — Internal Lesions/Masses

1. Review roentgenogram (CAT scan, or ultrasound) for general location of lesion. Identify lesion on fluoroscope, CT scan, or ultrasound and sterilize area for needle insertion.

2. Place tip of a sterile hemostat or other radiopaque marker on area directly over lesion and inject anesthesia (1% lidocaine [Xylocaine®]) locally over the chosen entry site. Site chosen should enable needle to transverse lower half of intercostal space and avoid injury to intercostal vessels.

3. Make a small incision in skin and subcutaneous tissue with a #11 scalpel blade (optional). This facilitates free passage of needle through skin and enables better appreciation of increased resistance when needle tip encounters lesion.

4. Insert biopsy needle or cannula with stylet in place into lesion to be biopsied during patient breath-hold. After area has been penetrated by needle, ask patient not to take deep breaths. It is important to place fluoroscope so that needle is in center of image to avoid parallax problems. It is usually possible to feel needle tip entering lesion, and most operators feel this is the most useful sign. Verification of accurate needle placement can be documented by lateral fluoroscopy, CT scan, ultrasound, or by synchronous movement of needle tip and lesion during shallow respiration when using either single-plane or bi-plane fluoroscopy. When needle has entered lesion, discontinue fluoroscopy and ask patient to breathe normally.

5. When tip of needle is in its optimal position, instruct patient to hold his/her breath. Remove stylet from needle and place thumb over open needle hub to avoid air embolism.

6. Attach a 20 mL disposable syringe to the needle or introduce biopsy needle through cannula. Trigger biopsy mechanism or retract plunger to create negative pressure in syringe and needle lumen.

7. Rotate needle with attached syringe clockwise and counter-clockwise while moving it slightly forward and backward within lesion.

8. Maintain constant negative pressure throughout this manipulation by keeping plunger of syringe retracted. Unless negative pressure can be achieved, a successful aspiration is unlikely; therefore, needle is advanced or retracted until negative pressure is obtained.

9. When aspiration is completed, allow pressure in syringe to return to atmospheric pressure by gently releasing plunger. Do NOT release plunger quickly to prevent aspirated material from being blown out of needle.

10. Withdraw needle and syringe as a unit from lesion. Never remove needle while any negative pressure is applied to syringe. Such pressure would force aspirated material out of the and into syringe.

11. Aspirated material should remain within needle and not syringe. If material is seen entering syringe, discontinue negative pressure and withdraw needle.

12. If specimen is a core biopsy, please put material into a vial of PreFer® fixative supplied. Please complete a “Reference Pathology/Non-Gynecologic Cytology Request Form” in “Request Forms” in “Special Instructions” including all pertinent patient information and forward it with specimen.

**Note:** Specimen source is required on request form for processing.

13. If technical assistance is required for cytology smears or specimen adequacy check, please contact the Cytology Department at 1-866-232-2522.

C. References


2. Department of Cytopathology, Medical College of Virginia, Virginia Commonwealth University, Laboratory Procedure Manual