Fine Needle Aspiration Biopsy
General Statement

Fine needle aspiration biopsy (FNAB) has been demonstrated reliable in assessing neoplasms, infections, and reactive lesions of salivary gland, thyroid, lymph nodes, breast, and soft tissues. Its primary advantage is low cost, minimal morbidity and rapid diagnosis (less than 24 hours). Acceptable target lesions include virtually any defined, palpable mass of the head and neck, breast, axilla, extremities, or subcutaneous skin. Fine needle aspiration biopsy is extremely safe for superficial lesions (intraabdominal and intrathoracic lesions carry an increased risk and should be performed under radiologic guidance by experienced operators).

The most frequent cause of a false negative diagnosis is a geographic miss of the lesion, an outcome with significantly increased frequency when inexperienced operators are performing the procedure. It is therefore imperative that FNAB be correlated with clinical and radiologic findings, and that discordant FNAB results be explained by repeat FNAB or another procedure.

The Berkshire Medical Center (BMC) Cytology Laboratory offers a Fine Needle Aspiration Biopsy Service from 8:00 a.m. – 4:00 p.m. Monday through Friday. The service is available on an on-call basis for patients in BMC, the Crane Center, and physician offices located in or near the BMC campus. In addition, appointments may be scheduled on a same day or next day basis for patients outside the BMC campus. To request an FNAB appointment, please call 447-2570.

One of the advantages of referring a patient to the Fine Needle Aspiration Biopsy Service is that the pathologist has broad experience with the FNAB procedure which increases the likelihood of obtaining a diagnostic specimen. In addition, rapid staining and interpretation performed at the time of biopsy ensures adequate material is obtained for a diagnosis, allows for additional biopsies if material is required for special studies, and allows for a preliminary, same day diagnosis for the majority of patients.

PRE-PROCEDURE CONSIDERATIONS
1. Target Lesion
   a. Virtually any palpable, well defined subcutaneous mass is an acceptable target lesion for FNAB.
   b. Subcutaneous lesions near vital structures such as major arteries or the pleura (as in supraclavicular or chest wall FNAB) carry increased risk of significant complications, and should be performed by experienced operators only.
   c. Ill-defined lesions such as post-radiotherapy induration or "thickenings" of the breast are often poor targets and frequently yield non-diagnostic results.
   d. "Blind" FNAB of lesions identified radiologically, but not palpable, should not be performed.
2. **Work up Algorithm**
   a. Thyroid scan should be performed prior to FNAB due to possible interference with results by the hematoma.
   b. CT/MRI scan results are not affected by FNAB.

3. **Contraindications**
   a. Vague, ill-defined lesions.
   b. Lesions too near pleura or vital structures to perform FNAB safely.
   c. FNAB should not be performed on suspected carotid body tumors due to the possibility of embolic CVA
   d. Coagulopathy
      i. For patients with major coagulopathies (i.e., hemophilia, platelet counts less than 5000, or hematologic malignancies), one may wish to consider alternative diagnostic maneuvers or therapeutic correction of the coagulopathy prior to attempting FNAB. PT/PTT and a bleeding time should be evaluated in such patients.
      ii. FNAB may be safely performed on patients on therapeutic doses (5-10 minutes) of the biopsy site after FNAB.
      iii. If the lesion is highly vascular (i.e., thyroid) or in a site (i.e. neck) that a hematoma could be life threatening, consideration should be given to alternative diagnostic maneuvers.

4. **Complications**
   a. As in phlebotomy, vasovagal reactions and fainting are by far the most common complications of FNAB.
   b. Hematomas may occur, but can be avoided by adequate tamponade of the biopsy site.
   c. Infections have been reported, but are exceedingly rare.
   d. Improper technique for lesions of the chest wall or supraclavicular region may result in pneumothorax.

**Consent**
1. FNAB of palpable subcutaneous masses is of similar risk to phlebotomy, and therefore written consent is not required.
2. It is advisable to discuss the procedure with the patient, and to document that verbal consent was obtained.
3. For FNAB of palpable lesions near vital structures such as major arteries or the pleura, written consent may be obtained, since these procedures carry risk of more significant complications. If one is inexperienced in FNAB technique, one may wish to refer the patient to a more experienced operator, or request assistance from a BMC pathologist while performing the procedure.
4. Written consent is mandatory for FNAB of radiographically detected intra-abdominal or intrathoracic lesions.

**ANESTHESIA**
1. Use of anesthesia is discouraged because:
   a. The wheal raised by the anesthetic often obscures the lesion, resulting in uncertainty as to needle placement.
   b. Use of medication (1% Xylocaine) adds potentially life-threatening allergic reactions to an otherwise minimally morbid procedure.
c. The size of the needle used to administer the anesthetic approximates the size of the biopsy needle.

2. Anesthesia is often required in a few specific situations.
   a. Extremely painful lesions (e.g., infectious lymphadenitis, neoplasms with perineural invasion).
   b. Breast lesions in which the areola must be traversed by the needle.
   c. Intraoral lesions.
   d. Pediatric patients.
   e. Extraordinarily anxious patients, with easily palpable target lesions.

3. A topical Xylocaine preparation, EMLA cream (Astra Pharmaceuticals) is available and works extremely well for FNAB, particularly in pediatric patients. Its primary limitation is that it must be applied at least one hour prior to the procedure.

PROCEDURE
1. Materials
   Exam table or chair that can be placed into Trendelenburg position
   Cameco syringe holder
   10 ml syringes
   23 or 25 gauge needles
   Alcohol swabs
   Bandaids
   Gloves
   Glass slides
   Slide folders
   Gauze 4 x 4’s
   Spray fixative or 95% ETOH in screw top jars
   CytoLyt fixative solution--sterile saline or balanced
   RPMI Flow cytometry fixative (for lymph nodes) may be obtained from BMC
   1% or 2% Xylocaine and tuberculin syringes for anesthesia as needed
   Culture swabs or transport medium (for suspected infectious lesions)

2. Position
   a. The biopsy may be performed with the patient lying or sitting. If sitting, the setting should allow rapid placement of the patient into Trendelenburg should a vasovagal reaction occur.
   b. The patient should be in a position that allows the aspirator to be in a logical, comfortable position. If the operator is in an awkward position while performing the biopsy, it is unlikely diagnostic material will be obtained.
   c. The lesion should be carefully examined prior to the biopsy to ensure it can be adequately immobilized by the index and middle fingers of the non-dominant hand. Ensuring stabilization of the mass and approaching the biopsy in a comfortable, organized manner may take longer than the biopsy itself, but is well worth the effort given the increased accuracy of needle placement and quality of material obtained.
   d. For lesions near vital structures such as pleura (axilla, supraclavicular, chest wall, breast FNA) or major arteries (carotid/femoral/axillary) it is
imperative to plan the FNA with the needle aligned parallel or away from the vital structure, **not perpendicular** to it.

3. **Technique**
The object of FNAB is to use the tip of the needle as a microscalpel to core out minute tissue fragments, with minimal contamination by peripheral blood. Suction does not contribute to the procurement of cells; rather its function is to hold the sample in the needle. Indeed, excellent samples can be obtained with no suction applied. It is highly desirable to keep the entire specimen confined to the needle alone since a sample which is drawn into the syringe usually never makes it out to the slide, and is wasted.

   a. Label 5-10 slides and specimen containers with patient’s name, date of birth and biopsy site.
   b. Clean skin with alcohol swab (use Betadine near joints or other sterile spaces that could be accidentally entered). After excluding possible allergies, anesthetize skin if indicated.
   c. Perform biopsy
      i. Immobilize the lesion with index and middle finger of the non-dominant hand.
      ii. Introduce needle attached to syringe and syringe holder into the lesion. (Do not apply negative pressure). The thumb or index finger of nondominant hand can be used to stabilize the syringe as the needle is directed into the lesion.
      iii. With needle in the lesion, apply 1-2 cc of negative pressure.
      iv. Perform biopsy using fine, back and forth oscillations of the needle (similar to an electric sewing machine) for approximately 10 seconds, 10-15 cycles of the needle, or until blood appears in the hub of the needle.
      v. Stop needle oscillations.
      vi. **Important:** Always stop biopsy when blood appears in the hub of the needle Specimen in the syringe usually clots or dries, and is lost for diagnostic purposes. Excursions should be in the same horizontal and vertical planes; do not “fan” the axis of the needle as this causes increased bleeding.
      vii. Release pressure with needle in lesion, to avoid sucking the specimen into the syringe.
   d. Remove needle from patient.
   e. Perform smears (see Part IV Smears)
   f. Perform needle rinses into sterile saline solution by drawing 1-2 ml of saline through needle into syringe and express all fluid back into saline container.
   g. Repeat rinse 2-3 times.
   h. **Repeat technique for total of 3 or more biopsies.**

4. **Common Errors in Biopsy Technique**
   a. Failure to adequately stabilize the mass, resulting in errant needle placement (geographic miss) and false negative biopsy.
   b. Use of too large needle (use 23 gauge or smaller to avoid excessive contamination by peripheral blood).
c. Operator aspirates instead of biopsies (i.e., tries to "suck out" cells). **Use staccato, sewing machine-like motion to core out tissue fragments.** Slow, saw-like motion also results in poor cell yield.

d. Aspirator continues to draw sample into syringe even after sample appears in hub of needle. Sample will clot and air dry inside syringe. Always stop when blood appears in the hub.

e. Needle is removed from patient with suction on, resulting in specimen being drawn into the syringe, dried, clotted, and lost for further evaluation.

f. Operator rinses entire specimen into CytoLyt solution. The best diagnostic sample is obtained on smeared, alcohol-fixed material. Saline or CytoLyt solution salvages specimens caught in the syringe; however, CytoLyt preparations are less than adequate for evaluation of most lesions.

g. Operator performs one pass.
   i. Multiple passes ensure that different areas of the lesion are sampled.
   ii. Multiple passes ensure against geographic misses.
   iii. Three or more passes are essential for lesions less than 1.0 cm and greater than 3.0 cm. Large lesions may have extensive necrosis, fibrous stroma and often yield scanty cellular specimens.

h. Operator drains cyst without sampling cyst wall. Cyst fluid, although abundant, seldom contains diagnostic cells. Always perform additional passes of the cyst bed, or any residual mass (See cysts, page 8).

5. **SMEARS**
   Excellent samples can be destroyed by smears which are poorly fixed or too thick, hence good smear technique is as important as good biopsy technique. Bloody specimens must be quickly and properly handled since blood impedes fixation or clots in the needle.

6. **Technique**
   a. Remove needle and draw 10 cc of air into the syringe, replace needle.
   b. Place 3-4 mm droplet on slide, 1 cm from frosted end.
      i. If more sample is available, prepare multiple slides, particularly if the specimen is bloody.
      ii. Use of a large droplet (greater than 5 mm of sample) is likely to result in a thick, poorly fixed, uninterpretable smear.
   c. With non-dominant hand, pinch slide between thumb and forefinger, with remaining three fingers supporting back of slide.
   d. With dominant hand, lay spreader slide perpendicular to specimen slide. Observe change in surface tension.
   e. With no pressure, glide spreader slide down the length of specimen slide and instantly fix (within 3 seconds) smeared slide. (You may wish to have an assistant ready with spray fixative).
   f. Spread remaining slides with spreader slide and fix as quickly as possible.
   g. Examine smeared, fixed slides for white particles which are an indication that diagnostic material is present on the slide. Smears which appear to consist of blood only are likely to be nondiagnostic.
7. Common Errors in Smear Technique
   a. Too much specimen placed on slide. Specimen droplet should not exceed 3-4 mm in diameter and should be placed 1 cm from frosted end of the slide.
      For cysts and very bloody specimens, perform two or three smears and rinse the remainder into CytoLyt solution. CytoLyt solution will lyse the red blood cells and the laboratory can attempt to salvage the sample.
   b. Operator waits too long to fix slides. Air drying completely destroys cell morphology. Once a smear is prepared, it must be fixed immediately.
   c. Excessive pressure in smearing the specimen, resulting in crush artifact and uninterpretable cell morphology.
   d. Operator puts smeared slides into CytoLyt fixative solution. CytoLyt solution is not an adequate fixative for smears. Use only spray fixative or 95% ethanol in screw top jars.
   e. Operator fails to smear slides. Specimens that are not smeared are too thick to permit adequate interpretation.
   f. Operator submits slides that are not labeled with the patient's name, date of birth and source of specimen. Unlabeled slides cannot be accepted by laboratory for interpretation.
   g. Operator rinses entire specimen into CytoLyt solution. The best diagnostic sample is obtained on smeared, alcohol-fixed material. CytoLyt solution salvages specimens caught in the syringe; however, CytoLyt preparations are less than adequate for evaluation of most lesions.

8. SPECIAL CONSIDERATIONS
   a. Lymph Nodes
      All lymph nodes, whether suspected to be reactive or lymphoma, should have material obtained for flow cytometric analysis of lymphoid markers. This should be procured in addition to standard smear preparations.

      After obtaining smear specimens, perform one or two additional passes, and rinse the entire specimen into flow cytometry fixative (RPMI). Send specimen to flow cytometry lab. Please call 447-2570 if there are questions.

   b. Suspected Infections
      1. Cultures can be successfully obtained from FNAB material.
         i. If pus is obtained, prepare 1-2 smears for cytology in the standard smear fashion. Save the remainder of the specimen in the syringe.
         ii. Remove the needle and replace with original plastic syringe cap.
         iii. Label syringe and smear with patient name, date of birth and biopsy site. Forward to Microbiology.
         iv. Prioritize the type of cultures needed as specimen quantity may be insufficient for all assays.

      2. If no fluid specimen is obtained, express a drop or two of specimen from the needle onto a sterile culture swab. Place swab into transport medium. Label with patient name, date of birth and
biopsy site and send to Microbiology. This type of preparation is unsuitable for mycobacterial cultures or gram stains.

Alternatively, rinse the needle into sterile nonbacteriostatic saline in a sterile tube. This is less optimal due to possible contamination by skin flora on the outside of the needle. (Thioglycolate broth may be used if available instead of sterile saline.)

3. Air dried smears can be sent to Microbiology for AFB/Gram stains.

c. **Cysts**
   1. Whether a benign or a cavitated, necrotic malignancy, cyst fluid does not generally contain diagnostic material.
   2. If fluid is obtained when suction is applied to the needle, do not perform the finely oscillating biopsy technique, rather, leave the needle stationary and apply enough suction to completely drain the cyst.
   3. If the syringe fills prior to total decompression of the cyst, perform multiple sticks until all fluid is completely drained.
   4. Re-examine the patient.
      i. If a residual mass persists, perform two or more FNAB using the standard oscillating technique.
      ii. If no mass persists, perform one or two "blind" FNAB of the cyst bed in an attempt to sample the wall of the cyst.
   5. Make one or two slides from the cyst fluid, and place the remainder directly into CytoLyt solution. Label slides and specimens with patient name, date of birth and biopsy site. FNAB of any residual mass or "blind" sticks of the cyst should be smeared in the method described in Part V, Smears.

9. **PATIENT INSTRUCTIONS**
   a. If significant swelling or pain occurs after the FNAB, the patient should seek medical attention.
   b. For small amount of pain, minor analgesics such as acetaminophen are adequate as needed.
   c. There are no restrictions as to bathing, exercise, medications, etc.

10. **SPECIMEN SUBMISSION**
   a. The requisition form should include the specific site where lesion is located. Breast or "Neck" is not specific enough. Examples include:
      i. Left breast at 6 o'clock, 3 cm from left nipple
      ii. Right lateral neck, 1 cm from angle of jaw
      iii. Left upper thyroid lobe
   b. All slides must be labeled with patient's name, date of birth and source of specimen.
   c. Basic patient demographic and physician's name should be included on request form.
   d. Any history of XRT, chemotherapy, recent trauma or surgery is essential, since cellular changes related to these procedures may mimic malignancy on cytology.
e. State clinical and radiographic impressions as to benign, indeterminate, or malignant. This aids us in assessing if the cytologic findings are representative of the clinical lesion, and hence, if the mass truly has been sampled.

f. Any history of prior malignancy. This is useful so we can compare the morphology of the prior tumor to determine if the new mass is metastatic or primary.

11. RESULTS
   a. If the specimen is received in the Cytology Lab prior to 9:30 a.m., the routine result will be available the same day.
   b. Specimens received later can be processed and interpreted the same day in emergency situations. The Cytology Lab should be notified by telephone as early as possible in the day so that arrangements can be made to rush the specimen.
   c. Rapid interpretations are routinely performed on all FNAB performed by BMC pathologists. Preliminary diagnoses are available within 30 minutes of the procedure.

If you experience difficulty in obtaining adequate samples or have any questions, our pathologists are pleased to speak with you. We can arrange to observe your technique and give suggestions about how to improve your diagnostic yield for FNAB. In addition, our cytopathologists are available to perform rapid assessment of adequacy in instances when prior attempts at FNAB have been non-diagnostic.

If you have any further questions regarding FNAB or wish to have a cytopathologist assist you with your procedure, please call 413-447-2570 from 8:00 a.m. to 4:00 p.m., Monday through Friday.

REFERENCES: