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Owner	Scott Lindrud: RMH CYTOTECHNOLOGY TECHNOLOGIST NE
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Cytology Collection - Fine Needle Aspiration Procedure - Collection and Assistance

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PURPOSE

To provide instructions for participation in FNA procedures performed at Rice Hospital. This procedure covers the processes of obtaining pathologist performed FNA samples, assisting on radiologist performed FNAs, and providing immediate adequacy assessments of the specimen by either a pathologist or cytologist (thyroid FNAs only).

RESPONSIBILITY

All cytology personnel tasked to provide assistance in FNA collection should be familiar with this procedure.

REQUIRED MATERIALS

(To be gathered prior to FNA).

A. Cart – to transport materials to FNA procedure.

1. **Cart Set up materials for FNA in XRay:**
    a. Three to Five Coplin jars containing 95% ethyl alcohol (Fixative).
    b. Line cart top with absorbent chux.

c. Three Formalin containers (for possible core biopsy samples).
d. Two Hank's Balanced Salt Solution (BSS) containers.
    i. Note: Sterile normal saline or Ringer's solution may be sued as a substitute if necessary.
e. Four Afirma FNAprotect tubes.
f. Box non-sterile slides.
g. Slide markers/pens/pencils.
h. Gloves.
i. Slide tray.
j. Requisition.
k. Goggles.

2. **Cart Set up materials for pathologist performed FNA:**
    a. Line cart top with absorbent chux and bring one extra for staining.
    b. Three to Five Coplin jars containing 95% ethyl alcohol (Fixative).
    c. Three Formalin containers (for possible core biopsy samples).
    d. Two Hank's Balanced Salt Solution (BSS) containers (for needle rinses).
    e. Four Afirma FNAprotect tubes (Thyroid FNAs Only).
    f. FNA gun.
    g. At least six 12cc syringes.
    h. Two ChloraPrep applicators
    i. 1% Lidocaine.
    j. 1% Lidocaine labels.
    k. Two filter needles.
    l. Six 18 gauge 11/2 inch unfiltered needles.
    m. Six 25 gauge 11/2 inch unfiltered needles.
    n. Six 27 gauge 11/2 inch unfiltered needles.
    o. Alcohol wipes.
    p. Five packages of sterile gauze.
    q. Cotton balls.
    r. Band aids.
    s. Adhesive paper tape.
    t. Box non-sterile slides.
    u. Marking pen for skin.
    v. Slide markers/pens/pencils.
    w. Gloves.

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x. Slide tray.
y. Requisition.
z. Paper towels.
aa. Microscope.
ab. Diff-Quik stain kit.
ac. Water bottle.
ad. Goggles.

B. Stain set up: Diff-Quik stain is used for immediate adequacy assessments (cytologists or pathologists) and/or immediate interpretations (pathologists only).
    1. Diff-Quik - Use a Coplin jar for each staining solution:
        a. Solution I (Fixative).
        b. Solution II.
        c. Solution III.
        d. Water Rinse.

PROCEDURE

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A. **FNA Notification:** When cytology staff is made aware of the FNA procedure (this may be a few days to almost no advanced notice):
    1. Obtain patient information and clinical history – include:
        a. Patient demographics.
        b. History / clinical impressions.
            i. **NOTE**: If lymphoma is suspected, Hank's BSS will need to be brought to the procedure.
            ii. Locate any current or recent patient records in the LIS. If relevant prior or current cases exist then make that information available for the case that is generated out of the FNA procedure. Include prior case slides to review and/or compare to current material when appropriate (i.e., a repeat thyroid FNA due to atypia of uncertain significance).
        c. Target organ or site of procedure to include right or left designations.
        d. Date and time of procedure.
        e. Department the procedure will be performed in (i.e., Ultrasound room, Interventional, etc.).
        f. Ordering clinician, any specialists (e.g., oncologist), and radiologist.
    2. If immediate adequacy assessment of the specimen will be needed then a cytologist or pathologist will need to attend the procedure. If an immediate evaluation is needed then a pathologist must attend.
    3. Alert appropriate people ASAP if the FNA is about to happen (no advanced notice).

4. If there is enough advanced warning (1 day or more), schedule the FNA using the corkboard in cytology prep room. Optional: Enter information in the shared cytology calendar on Outlook and send an e-mail to required cytology staff as well as the pathologist on Gross for that day.
5. Gather all required materials and set them up on the cart. Have it ready to go when radiology calls.

B. **Pathologist Performed FNA (In-Lab or designated procedure room):**

1. This type of procedure will be scheduled using a special form by registration but we will also need to place a copy of it on the corkboard.
2. When the patient arrives make sure they are registered and then take them to the procedure area.
3. Electronically order in the LIS if there is no electronic order present.
4. Label slides:
    a. Place enough slides in a row for the number of passes to be collected (2 per pass) making sure the correct side of the slide is facing up.
    b. Label the slide with the patient name, date of birth (DOB), and site of the FNA on the slide label side. Double check that the name and DOB matches your requisition and/or labels.
5. Write patient information on other collection containers (formalin jar for the needle rinse, sterile saline vial for cultures) or use preprinted labels. Ensure the patient information matches and that the site of the FNA is on all containers.
6. Establish Patient Identification: All patients undergoing an invasive procedure by laboratory personnel will be identified using an active process of communication ("Time-Out").
    a. Patient Identification and Verification: The patient (or representative) will actively participate in the verification of the patient's identification, correct procedure, site and side. If the patient is unable to respond and the representative is not present, the procedure, site/side will be verified using all relevant documents (i.e. medical records, progress notes, diagnostic studies).

        FNA Personnel (assisting personnel or the Pathologist) will verify:

        i. The patient's identity: Two identifiers MUST be used. The person assisting or pathologist will ask the patient to state their full name and date of birth. This info will be verified with the patient ID band and order.
        ii. The correct procedure(s), site, and/or side(s) by doing the following:
            a. Ask the patient or legal representative to identify the planned procedure, including laterality, digit, or level when appropriate.
            b. Review information on the patient's medical record

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and available diagnostic studies (i.e. lab results, x-rays, diagnostic tests etc.) if available.

Pathologist performing the FNA procedure will:

i. Ensure the FNA Procedure Consent Form is completed. The form will identify procedure, side/site distinction, multiple structures, and/or levels.

ii. Ensures that all necessary documents (lab results, x-rays, diagnostic tests etc.) are present prior to procedure, if needed.

iii. Marks the surgical/procedure site with his/her initials (see part b for correct site marking procedure).

iv. Actively participates in the "Time-Out" procedure prior to incision.

b. Site Marking: FNA procedures requiring marking of the site immediately prior are those involving left/right distinctions, multiple structures, and/or multiple levels. Procedures not requiring marking are those in which a single organ is involved. The pathologist performing the procedure will complete the site marking by:

i. Positioning the patient, clean, prep, and drape the FNA site(s).

ii. The patient should be involved to the greatest extent possible in the marking process by providing verbal and visual responses to the exact location of the FNA site.

iii. Mark his/her initials (NOT an "X") using a black, hypo-allergenic/ permanent marker near the FNA site, on all correct sides. For procedures which have multiple sides, right/left or bilateral, EACH individual site has to be marked by the pathologist performing the procedure.

iv. The pathologist performing the procedure will document site marking in the medical record.

7. Collection of FNA: The pathologist will obtain the specimen and give it to the tech:

a. Syringe with attached needle received:

i. Remove needle from syringe and pull back syringe plunger so 1-3 cc of air is introduced into the syringe.

ii. Re-attach needle to syringe.

iii. Holding beveled edge of needle at a 45 degree angle to the slide, express 1 drop on each slide to be smeared by provider or preparation technologist.

iv. Make smears (see step c below)

b. Needle received without syringe:

i. Pull back on the syringe plunger so 1-3 cc of air is introduced into syringe.

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ii. Attach needle hub to syringe.

iii. Holding beveled edge of needle at a 45 degree angle to the slide, express 1 drop on each slide to be smeared by provider or preparation technologist.

iv. Make smears (see step c below)

c. Prepare smears:

i. Immediately smear material on properly labeled slides by placing another slide onto first slide, letting the material spread between the slides, then pull both apart.

ii. Place one slide in the alcohol jar immediately for PAP stain and allow one to air dry for Diff-Quik stain. NOTE: With experience, one can grossly identify slides that are more likely to have cellular material on them (i.e., not blood only). Since only a few slides can be quick stained at a time, try to pick slides that look like they could have good cellular material representation.

iii. Express the remainder of aspirate within syringe into the appropriate media vial labeled with the appropriate patient information (Name, Birth date, Site, Date, etc). Appropriate media by indication:

a. Hank's (BSS) – Needle rinse. For use with cell block or cytospin preps. If lymphoma is suspected, no cell block or cytospin will be made and entire vial of Hank's will be sent for Flow cytometry, cytogenetics (if necessary)

b. FNAprotect tube (thyroid FNAs for Afrima genomic testing). See section J for detailed collection instructions for Afirma genomic testing (thyroid FNAs only).

c. Sterile Saline for possible cultures if ordered. NOTE: Optimally a separate, dedicated pass should be collected for cultures.

iv. Indicate the pass number on the slides:

a. Use a small notation in the corner of the slide label.

b. Pass number information is used to indicate which passes were immediately evaluated and the total number of passes performed (in Gross).

v. Repeat steps 7. a-c. for each pass.

vi. Once all passes have been performed and procedure is complete, return all case materials and paperwork to the cytology prep room for processing. If an immediate adequacy assessment is required, continue to step D below.

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C. **Assisting Radiologist at location of the FNA procedure (e.g., in Ultrasound)**

1. Talk to the attending staff to double check patient and specimen identification. (The Time Out procedure should be performed by the radiologist).
2. Acquire specimen label(s) from the radiology staff.
3. Set cart in a location within the room where the radiologist or technician can easily access it.
4. Label Slides:
    a. Place the enough slides in a row for the number of passes to be collected (2 per pass) making sure the correct side of the slide is facing up.
    b. Label the slide with the patient name, date of birth (DOB), and site of the FNA on the slide label side. Double check that the name and DOB matches your requisition and/or labels.
5. Write patient information on other collection containers (i.e Hanks's BSS, formalin jar. etc). Ensure the site of the FNA is on all containers.
6. Collection of FNA: The radiologist will obtain the specimen and give it to the tech:
    a. Syringe with attached needle received:
        i. Remove needle from syringe and pull back syringe plunger so 1-3 cc of air is introduced into the syringe.
        ii. Re-attach needle to syringe.
        iii. Holding beveled edge of needle at a 45 degree angle to the slide, express 1 drop on each slide to be smeared by provider or preparation technologist.
        iv. Make smears (see step c below)
    b. Needle received without syringe:
        i. Pull back on the syringe plunger so 1-3 cc of air is introduced into the syringe.
        ii. Attach needle hub to syringe.
        iii. Holding beveled edge of needle at a 45 degree angle to the slide, express 1 drop on each slide to be smeared by provider or preparation technologist.
        iv. Make smears (see step c below)
    c. Prepare smears:
        i. Immediately smear material on properly labeled slides by placing another slide onto first slide, letting the material spread between the slides, then pull both apart.
        ii. Place one slide in the alcohol jar immediately for H&E or PAP staining and allow one to air dry for Diff-Quik stain. NOTE: With experience, one can grossly identify slides that are more likely to have cellular material on them (i.e., not blood only). Since only a

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few slides can be quick stained at a time, try to pick slides that look like they could have good cellular material representation.

iii. Express the remainder of aspirate within syringe into the appropriate media vial labeled with the appropriate patient information (Name, Birth date, Site, Date, etc). Appropriate media by indication:

a. Hank's (BSS) – Needle rinse. For use with cell block or cytospin preps. If lymphoma is suspected, no cell block or cytospin will be made and entire vial of Hank's will be sent for Flow cytometry, cytogenetics (if necessary).

b. FNAprotect tube (thyroid FNAs for Afrima genomic testing). See section J for detailed collection instructions for Afirma genomic testing (thyroid FNAs only).

c. Sterile Saline for possible cultures if ordered. NOTE: Optimally a separate, dedicated pass should be collected for cultures.

iv. Indicate the pass number on the slides:

a. Use a small notation in the corner of the slide label, or if an entire alcohol jar is used (i.e., pass 3, indicate on the jar using a marker).

b. Pass number information is used to indicate which passes were immediately evaluated and the total number of passes performed (in Gross).

c. Make sure that a new pass is not confused with a new site. For example, the needle may be moved from the right thyroid to the left thyroid after pass 3. This would be pass one for the left thyroid not pass 4.

v. Repeat steps 7. a-c. for each pass.

vi. Once all passes have been performed and procedure is complete, return all case materials and paperwork to the cytology prep room for processing. If an immediate adequacy assessment is required, continue to step D below.

D. **Performing Immediate Adequacy Assessments:**

1. Diff-Quik staining: Preferred staining option for performing on-site immediate adequacy checks.

a. Dip slide in Solution I (Fixative): 10-15 dips, allow slide to drain before going into next solution.

b. Dip slide in Solution II: 10-15 dips, allow slide to drain before going into next solution.

c. Dip slide in Solution III: 10-15 dips, allow slide to drain before going into

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water rinse.

d. Water Rinse: Rinse with water until clear.

e. Wipe off water from back of slide. The slide is now ready to be place on microscope for adequacy check. Note: Do not place a cover slip on the slide unless you are going to let the slide dry and mount permanently with mounting media. Placing a cover slip on a wet mounted slide can result in the stained specimen to be removed when taking off the cover slip.

f. The pathologist (or cytologist) will make the adequacy determination and convey the information to the collecting radiologist. If adequate cellularity is not present, continue to collect material and stain additional slides until diagnostic material is identified or procedure is ended by the collecting radiologist.

2. Immediate Adequacy Assessment Documentation: After completing the adequacy assessment, document the following information on the cytology requisition:

   a. Adequacy findings for each pass. This information may be combined if the findings are the same for each pass (i.e., Passes 1-3: non-diagnostic, Pass 4-6: adequate cellularity).

   b. Initials of the person performing the adequacy check.

   c. Date of procedure.

   This information will be entered into the case by the cytologist after releasing the order or during primary screening using the provided entry fields in the LIS. This will allow the immediate adequacy assessment to display on the final report.

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E. **Cytologist Immediate Adequacy Assessments:** A pathologist will perform adequacy assessments on all possible FNA sites. At the discretion of the pathologist, a cytologist may perform immediate adequacy assessments on thyroids and other specimen sites provided training and validation have been completed prior to the procedure.

1. Thyroid Adequacy Criteria:

   a. A thyroid FNA sample may considered "adequate" if it contains a minimum of 6 groups of well visualized (i.e. well stained, undistorted, and unobstructed) follicular cells, with at least 10 cells per grouping.

   b. The 6 groups of 10 cells should preferably be on one slide, but may be spread out over the initial set of slides used to assess the adequacy.

   c. In cases with a microfollicular pattern, many of the groups may not contain 10 cells per group. In this instance, you will want to see more than 6 groups of these cells to ensure enough cells are present to render a diagnosis.

   d. If you feel you are at the minimum number of cells after the initial passes, it is recommended to request additional passes to help ensure enough diagnostic material is present to render a diagnosis.

   e. If the smears are suspicious for a lymphoma (consisting predominantly of a monomorphous population of lymphocytes without an epithelial

component), contact a pathologist for further instructions.

F. **Thyroid FNA collection for Afirma genomic testing:** Pass 2 will be a dedicated pass for Afirma testing.

1. Label FNAprotect tube: Label one tube for each nodule (see requisition section 4)
2. Collect sample
    a. Pass 1: Make two smears (air dry one and fix one in 95% alcohol) for cytopathology then rinse needle into Hanks BSS.
    b. Passes 2: Rinse the entire pass into the Afirma FNAprotect tube. ***Note:** Do not rinse bloody (no more than in the hub of the needle) specimens into the tube. Collecting staff should communicate to performing physician that pass 2 should not be aspirated (collected with a syringe attached to the needle).*
    c. Additional passes: Make two smears (air dry one and fix one in 95% alcohol) for cytopathology then rinse the needle into the Han's BSS.
    d. Repeat steps a - c for each additional nodule biopsied (collect 1 FNAprotect tube per nodule).
3. Mix sample: Invert each tube 3 times.
4. Freeze sample: Bring FNAprotect tube to the clinical lab and store it in the -20^{o}C no frost freezer in client services or in the -80^{o}C freezer in chemisty.
5. Log Afirma sample in the Afirma collection binder located in client services.
6. If the diagnosis meets the criteria for ordering Afirma, the pathologists will fill out the Afirma requisition and forward to the cytologist to send it out for testing.

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RELATED PROCEDURES

Cytology Procedure, *Cytopathology Quality Management Program*, pg 7.

REGULATORY CITATIONS

Rice Hospital Procedure, P&P No: TX-190, Universal Protocol: *Surgical/Invasive Procedure "Time-Out" Verification.*

The Bethesda System for Reporting Thyroid Cytopathology, Ali.SZ, Cibas ES, eds, Springer 2010.

Afirma Package Insert.

REFERENCE CITATIONS

Facility specific, none stated

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Approval Signatures

Step Description	Approver	Date
Medical Director / Pathologist	Leah Dvorak: Provider	7/8/2026
Medical Director / Pathologist	Steven Vanderwerf: MD	6/25/2026
Lab Manager	Julie Boehme: RMH DIR LABORATORY EX	6/23/2026
Document Owner	Scott Lindrud: RMH CYTOTECHNOLOGY TECHNOLOGIST NE	6/22/2026

Applicability

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