

Ultrasound-Guided Fine Needle Aspiration of the Thyroid

Tips & Techniques

INTRODUCTION

Objective

To review best practices in collecting and submitting samples to Veracyte for the Afirma® Thyroid FNA Analysis.

To provide tips and techniques for optimizing sample collection for cytological and molecular diagnostics when performing thyroid fine needle aspiration (FNA) under ultrasonographic guidance.

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PREPARING FOR THE PROCEDURE

Supplies

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Supplies required to collect patient samples for the Afirma Thyroid FNA Analysis:

Provided by Veracyte	Supplied by your practice
Afirma Requisition Form	Needles – Preferably 25g or higher; syringes (holder optional)
CytoLyt® solution tubes (and/or slide holders) for Cytopathology	Slides (if submitting in addition to or instead of CytoLyt solution tube)
FNAprotect collection tubes for the Genomic Sequencing Classifier (GSC)	Ultrasound probe cover
Nodule baggies and patient specific foil pouches	Ultrasound gel
Tube holder racks	Chloraprep, [®] Betadine [®] or similar antiseptic solution, alcohol swabs etc.

THYROID ULTRASOUND ANATOMY

Specific training in the ultrasonographic anatomy of the thyroid is essential to the safe and accurate performance of the FNA procedure, and to the optimization of sample collection for both cytology and molecular diagnostics (Figures 1 & 2). A thorough familiarity with the FNA procedure itself is also critically important.





(a)

Figure 1: (a) Transverse view of the normal thyroid. The normal thyroid has a ground-glass appearance. (T) trachea, (SM) strap muscles, (C) carotid artery, (E) esophagus, (LC) longus colli muscle, (RL) right lobe, (LL) left lobe, (I) isthmus; **(b)** surface anatomy of the thyroid.



Figure 2: (a & b) Carotid artery, longus colli muscle, right and left lobes of the thyroid, and isthmus are visible, as is the trachea. Note the close proximity of the carotid artery to the thyroid. The jugular vein is off-screen laterally and not visible.

OPTIMAL PATIENT AND PHYSICIAN POSITIONING



Figure 3: The physician is standing to the side of the patient while performing the FNA procedure. The US machine is on the left (not visible in the photograph), directly across from the physician. This setup allows the physician to see the US screen by simply lifting his/her head.

Place the patient in a supine position, with the neck slightly extended. A pillow should be placed at the level of the shoulders to achieve sufficient extension of the neck and access to the thyroid area. A folded sheet may be placed under the patient's head for comfort.

Some physicians find that the ideal position for performing the FNA procedure is on either side of the patient, rather than at the head of the bed. The ultrasound (US) machine can then be placed across from the physician, directly in his/her line of sight. This position minimizes excess movement by the physician during the procedure, since all hand movements are linear in relation to the US screen. It also allows the physician to simply look up to see the US screen rather than turn her/his head (**Figure 3**).

ANESTHESIA

Local anesthesia is recommended during the FNA procedure for the comfort of both patient and operator. A comfortable patient remains still during a procedure, thereby increasing the chance of a successful FNA.

Anesthesia as well as vasoconstriction are achieved when 1–2 ml of 1-percent lidocaine hydrochloride with epinephrine solution is injected into the skin and superficial subcutaneous tissue at the predetermined site. Sodium bicarbonate in a 1:10 ratio to lidocaine can be used to buffer the acidity inherent in lidocaine.

Ethyl chloride and other cold sprays may be used in the rare "allergic" patient, though cold-spray use is discouraged because a patient has to undergo at least four separate passes (needle sticks) per nodule.

PERFORMING THE FINE NEEDLE ASPIRATION (FNA)

The sequence of an FNA procedure

- 1. Position the patient as described above.
- 2. Clean the predetermined site with your choice of antiseptic solution. (Chloraprep does not stain patient skin or clothing.)
- 3. Place the ultrasound transducer on the patient's neck and identify the nodule, measuring in three dimensions and assessing the vascularity with color Doppler before performing the FNA.
- 4. Use a 25- to 27-gauge beveled needle for most thyroid FNAs.
- Larger needles do NOT increase specimen cellularity; they paradoxically increase the chance of nondiagnostic specimens because of the dilution caused by excess blood.
- Medium-sized needles (e.g., 23-gauge) can be used to decompress cystic lesions, followed by sampling of the solid component with a smaller needle. See page 9 for the FNA of cystic lesions.

In summary, the ultrasound-guided FNA procedure begins with identifying the nodule, then measuring it in three dimensions, followed by using color or power Doppler to assess vascularity, and finally performing the procedure itself (**Figure 4 a-d**).



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Figure 4: (a) Image of the nodule within the isthmus; (b) measurement of the nodule; (c) Doppler shows circumferential vascularity of the nodule and the surrounding great vessels; (d) FNA needle is seen within the nodule.

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Needle localization and lesion targeting

Lesions may be successfully targeted with either a parallel or a perpendicular approach. A parallel approach, however, allows the entire needle to remain visible during the procedure, and allows for the real-time localization of the needle tip (Figure 5 & 6).



(a) Parallel Approach

(b) Perpendicular Approach

Figure 5: (a) Needle is introduced parallel to the transducer; longitudinal/sagittal view; (b) needle is introduced perpendicular to the transducer; transverse/axial view.



Figure 6: (a) Needle is correctly aligned with the direction of the US beam and is fully visible on image; (b) needle is not visible and should be held motionless while (c) the transducer is gently rocked.

FNA Technique with and without aspiration

FNA techniques with aspiration (i.e., with negative-pressure suction from the syringe) and without aspiration (without suction) can be used to extract cells for cytopathology evaluation, depending on the type of lesion.

Non-aspiration technique (also called "French," "capillary action" or "Zajdela's" technique) is mostly used for non-cystic and vascular nodules (e.g., thyroid or lymph nodes):

- Wipe extra US gel from the area where the needle will be inserted.
- Advance the needle into the nodule and move it back and forth while rotating it on its axis. Do not apply suction.
- For each pass, a needle dwell time of 2–5 seconds within the nodule is recommended; three back-and-forth excursions per second maximize cellular yield while minimizing blood.
- The passes should attempt to sample different areas of the nodule. Ideally, the samples collected for the Genomic Sequencing Classifier should be similar to those collected for cytopathology.

Tip: Non-aspiration technique is useful for hypervascular nodules, which have a high probability of yielding bloody specimens and therefore inaccurate cytological analysis.

Aspiration technique (with suction) is mostly used for cystic lesions and for those circumstances when non-aspiration technique produces inadequate samples:

- Advance the needle tip into the nodule.
- Apply suction (about 3 cc, or up to 5 cc for colloid nodules) by pulling back on the syringe or the handle of the FNA gun.
- Move the needle back and forth as described above for the capillary action technique while suction is continuously applied.
- More time within the nodule does NOT result in more cells. There is an inverse relationship between aspiration time and yield (Figure 7).
- Once blood is observed entering the hub of the syringe, stop applying suction and remove the needle from the nodule.
- Repeat this procedure for four independent passes.



Figure 7: An inverse relationship exists between aspiration time and aspiration yield.

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Number of passes

While a specific number of passes is not prescribed, enough passes must be performed to obtain diagnostically useful material.

The pathologists at Thyroid Cytopathology Partners, Veracyte's independent collaborators, recommend a minimum of two dedicated passes for cytopathology assessment. The Veracyte laboratory requests two dedicated passes for the Afirma Genomic Sequencing Classifier (GSC). A total of four passes per nodule is therefore recommended for the Afirma Thyroid FNA Analysis to obtain sufficient material for cytopathology diagnosis and molecular testing.

Non-cystic vs. cystic nodules

Obtaining the sample (non-cystic)

- Use a 25- to 27-gauge needle attached to a 10-ml syringe to perform the FNA.
- A syringe holder may be used based on operator preference.
- Before aspiration, scan the transverse plane to locate the lesion.
- Color Doppler should be used to detect any large blood vessels in and around the nodule to avoid vascular injury (and subsequent hemodilution of the specimen) during the procedure.
- Insert the needle immediately adjacent to the midline of the ultrasound transducer with the parallel approach.
- Monitor the needle tip carefully throughout the procedure; perform the biopsy when the needle reaches the target.
- Continuously watch all needle movements in real time during the procedure.

Obtaining the sample (cystic)

Nodules with significant cystic contents identified at the time of ultrasound evaluation should be sampled using the aspiration technique (rather than the capillary action technique) as follows:

- Advance the needle tip into the nodule.
- Apply suction by pulling back on the syringe or the handle of the FNA gun and slowly drain the cyst's contents, continuing to pull back on the syringe plunger and moving the needle through the cystic portions of the nodule (large cysts may require additional passes to remove all contents).
- Once cystic contents have been removed, gently release the suction and remove the needle from the nodule.
- Eject cystic contents into the CytoLyt solution tube.
- Proceed with four additional passes of the remaining solid portion of the nodule as described above, using either the aspiration or non-aspiration technique.
- If there is no solid component remaining for sampling following the drainage of the cyst, a small portion of the cystic contents can be added to the FNAprotect (the FNAprotect container can only accommodate about 0.5 cc of fluid; the CytoLyt can accommodate relatively large amounts of cystic fluid).

SUBMITTING A SAMPLE TO VERACYTE FOR THE AFIRMA THYROID FNA ANALYSIS

Placing sample into Cytolyt® solution and FNAprotect tubes

After completing each pass for cytopathology, place the sample in a CytoLyt solution tube:

- 1. Disconnect the needle from the syringe.
- 2. Aspirate air (3–5 cc) into the syringe and reconnect the needle.
- 3. Point the needle down and slowly express the entire specimen into the CytoLyt solution tube.
- 4. Remove the needle from the syringe.
- 5. From the tube containing the sample, draw about 5 ml of CytoLyt solution back into the syringe; replace the needle on the syringe.
- 6. Point the needle down and slowly express the solution back into the tube.
- 7. Tighten the CytoLyt solution tube cap securely.
- 8. Invert the tube three times to ensure all solid material is mixed into the solution.

After completing each pass for the Afirma GSC, place the sample in an FNAprotect tube:

- 1. Disconnect the needle from the syringe.
- 2. Aspirate air into the syringe and reconnect the needle.
- 3. Point the needle down along the inside of the tube and slowly express the entire specimen into the FNAprotect collection tube.

Tip: To minimize bubbling, expel needle contents inside the tube but above the solution.

- 4. From the tube containing the sample, draw about 1 ml of FNAprotect reagent back into the syringe.
- 5. Point the needle down along the inside top edge of the tube and slowly express the solution back into the tube.
- 6. Tighten the FNAprotect tube cap securely.
- 7. Invert the tube three times to ensure the material is mixed into the fluid.

SPECIAL SECTION: IMAGING OPTIMIZATION

Ultrasound imaging essentials

This section describes preferred ultrasound probe selection and operator controls for optimal imaging.

Probe selection

- · Linear array probes are required for diagnostic imaging.
- A very high frequency probe (10.0 MHz or greater) is recommended for the best possible image resolution.
- Multiple frequency, broad-band probes are ideal because they allow the adjustment of frequency without having to change the probe.

Tip: Newer ultrasound systems (less than five years old) typically produce better-quality images. Although optimal ultrasound imaging and performance are dependent on the skill and experience of the operator, newer systems enable better viewing of needles and abnormalities in the performance of ultrasound-guided fine needle aspirations.

Image optimization

- *Exam "presets"* allow for the customization and storage of imaging parameters. "Thyroid," for example, is a preset.
- Operator controls optimize imaging parameters. The controls include:

1. Frequency

- Higher frequency improves axial resolution for better image quality.
- Lower frequency allows for greater penetration.

In most circumstances, use the highest possible probe frequency that allows for adequate penetration. When imaging the thyroid, set a frequency that enables you to adequately view structures that are farthest away from the probe, such as the esophagus or the longus colli muscle. Given that the thyroid is near the surface, a frequency setting of 10–12 MHz is standard for most patients. An adjustment to a lower frequency may be appropriate for patients who have deep lesions, significant goiters, or thick necks.



Figure 8: (a) The circled area becomes sharper and crisper as the frequency is increased from 5 MHz to (b) 7.5 MHz to (c) 10 MHz. The images are grainier in appearance as the frequency decreases (the differences are much more dramatic while imaging in real time).

2. Time gain compensation (TGC)

- TGC is controlled by a series of slide potentiometer knobs.
- Each TGC knob governs the brightness of a specific area of the image.
- Adjust TGC to create uniform brightness throughout the image. Structures near the top of the image, such as the isthmus, should have the same relative brightness as structures at the bottom, such as the posterior portion of the thyroid gland or the longus colli muscle.



Figure 9: Thyroid images at various gain settings: (a) too little gain; (b) gain is just right; (c) too much gain.

3. Overall gain control

- Overall gain adjusts the brightness level of the selected TGC.
- A proper gain setting will produce the greatest number of grey shades in the image.



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Figure 10: (a) Image seen when TGC is set to achieve a uniform brightness from top to bottom; (b) image seen when slide potentiometers are set opposite each other.

4. Focal Zone

- Focal zones are used to improve thyroid image quality by improving lateral resolution as determined by the relative width of the sound beam.
- Adjusting the focal zone location narrows the relative beam at a specific depth, thereby improving lateral resolution (and therefore the image) at that depth.
- A focal zone should be set at or below the region of interest.
- Selecting multiple focal zones will significantly improve an image, but will also decrease the frame rate. A selection of one or two focal zones is appropriate for thyroid imaging.





(a)

Figure 11: Focal zone (indicated by green arrow) is set (a) correctly on image; (b) too high on image.

5. Dynamic range

• Dynamic range adjusts the overall contrast quality of an image. A lower dynamic range setting gives a "black and white" appearance to the thyroid, while a higher setting will produce a wider range of gray shades in the image. This can be quite helpful when differentiating characteristics of thyroid lesions and their borders.



Figure 12: (a) Grainy, low-contrast image of thyroid tissue at a very low dynamic range setting; (b) image at an in-between dynamic range setting; (c) a smooth, high-contrast image of thyroid tissue at a very high dynamic range setting.

The ideal dynamic range setting is a matter of visual preference for the user, and it will vary somewhat from patient to patient. Although there is no perfect setting, most operators prefer something in the middle—as demonstrated above in (b)—when assessing the thyroid and its nodular characteristics. A dynamic range between 75 and 90 on most ultrasound systems is optimal.

Operator controls on some systems that allow optimization of images include the following:

6. Tissue harmonics

- A sound wave created by the reaction of sound with tissue is called "harmonic generation." This "second" wave of sound is a higher-frequency multiple of the original wave created by the ultrasound system. The system listens for the higher frequency that improves resolution.
- Tissue harmonics can be included in a thyroid preset. Its value will vary from system to system and patient to patient. It's recommended that you put tissue harmonics to use in various scenarios to determine its operational value to you.



Figure 13: The same thyroid is seen with (a) the harmonics feature turned on; and (b) the harmonics feature turned off.

7. Image compounding

- Image compounding is a processing function of the ultrasound system.
- It improves resolution in specific cases because it reduces the amount of noise in images, and therefore may help to reduce artifacts and differentiate subtle differences in tissue.



(a)



Figure 14: Image compounding may help to better differentiate subtle tissue differences in the thyroid lesion displayed, with (a) compounding turned off; and (b) compounding turned on.

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ADDITIONAL RESOURCES

Guidelines

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Liquid Based Cytology

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Ultrasound

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