Ultrasound-guided Fine Needle Aspiration
Cytological Examination of Thyroid Nodules: A Practical Guideline (2019 edition)

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Fine-needle aspiration (FNA) is an important method of pathological diagnosis of thyroid nodules before treatment. The quality and quantity of specimen can directly affect the sensitivity and accuracy of pathological diagnosis results. At present, high-frequency ultrasound is not only the first choice of thyroid nodule examination and initial diagnosis, but also a reliable technique to guide FNA to achieve minimally invasive pathological diagnosis. The characteristics of thyroid nodules’ tissue structure (displayed as ultrasonographic features), the performance of biopsy instruments (including puncture needle and ultrasonography equipment for guiding puncture), and the operators’ skill level of performing biopsy (including those of puncture operators and cell smear operators) are directly related to the good outcomes of thyroid nodule biopsy, which are the key points of performing a high-quality thyroid nodule FNA and also the focus of the compilation group of this guideline.

The purpose of compiling this guideline is to emphasize the clinical practice experience and operation skills with multi-dimensional and synthetic thinking, to select appropriate puncture instruments, use puncture techniques skillfully, and handle biopsy materials properly, based on the characteristics of ultrasonic images. This guideline is exclusively used for academic exchange and reference, not for legal use, and it will be updated as appropriate.

1 Levels of Evidence and Grading of Recommendation

The contents of this guideline are supported by literature references, or based on the mature clinical practice experience of the task force members. According to the practice, different levels of evidence are given corresponding recommendation strength (Table 1).

2 Indications and Contraindications[1-2]

To determine the indications and contraindications of thyroid nodule FNA, three aspects should be considered, namely, the necessity, feasibility, and risk of puncture. FNA indications can be established when the nodule has the necessity and feasibility of puncture, and the risk is not high or the risk can be effectively prevented and resolved after evaluation. FNA contraindications can be established if puncture is necessary but not feasible, or if the risks are extremely high but effective preventive or resolving measures are lacking.
2.1 Indications

2.1.1 According to relevant international practice guidelines, it is necessary to obtain pathological diagnosis evidence of thyroid nodules through quick and minimally invasive methods, before treatment, during treatment, and even during the follow-up after treatment.

2.1.2 In the highly suspected cases of papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), anaplastic thyroid carcinoma (ATC), or other malignant tumors according to ultrasonic imaging, the pathological characteristics need to be confirmed before surgical resection or ultrasound-guided percutaneous ablation treatment[3].

2.1.3 There is no obvious ultrasonic and/or clinical malignant evidence at the initial stage of thyroid nodule examination, but ultrasonic follow-up observation shows signs of dynamic increase of solid area, dynamic increase of blood flow signal, gravel calcification, or intermittent annular calcification foci, etc.

2.1.4 Although ultrasonic imaging tends to diagnosis the patient as benign thyroid nodules, the patient demands pathological diagnosis conclusion.

2.1.5 It is necessary to further detect thyroid tumor genes by FNA.

2.1.6 Ultrasonic imaging shows that cervical lymph nodes are highly suspected of malignancy or cannot be excluded the possibility of malignancy.

**Expert consensus:** The terms fully respect the subjective and objective judgments of clinicians, ultrasound doctors, and patients, and emphasize the operators’ self-evaluation of experience and puncture skills. Regarding the nodules with a maximum diameter less than 5 mm at special location that are difficult to puncture, FNA can be implemented by or under the guidance of doctors with rich experience in fine needle puncture, or by consulting experts outside the hospital.

**Grading of recommendation:** B. Recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

Table 1  Levels of evidence and grading of recommendation

| Level of evidence | Grading of recommendation |
|-------------------|---------------------------|
| A                 | Conduction of FNA is strongly recommended as confirmed evidence has affirmed it can improve clinical outcomes, and benefit is greater than harm. |
| B                 | Conduction of FNA is recommended as good evidence has affirmed it can improve clinical outcomes, and benefit is greater than harm. |
| C                 | Intervention is recommended based on expert consensus. |
| D                 | Intervention is strongly opposed to recommendation based on expert consensus. |
| E                 | Intervention is strongly opposed to recommendation for good evidence. It cannot improve clinical outcomes, or harm is greater than benefit. |
| F                 | Intervention is strongly opposed to recommendation for confirmed evidence. It cannot improve clinical outcomes, or harm is greater than benefit. |
| I                 | Intervention is not recommended or not routinely recommended due to insufficient, lacking, or contradictory evidence for recommending or opposing. It, therefore, is not possible to weight the benefit against the harm. |

2.2 Contraindications

2.2.1 Patients have serious bleeding tendency, e.g., complicated with uncured coagulation dysfunction diseases, or the time period of stopping anticoagulant therapy shorter than that specified in the drug instructions (even if the test report shows that the coagulation function index is normal).

2.2.2 Patients have uncorrected high coagulation tendency and potential risk of internal jugular vein thrombosis.

2.2.3 Patients have unstable carotid plaque, and the plaque is at risk of falling off.

2.2.4 Patients cannot effectively cooperate with puncture due to consciousness disorder, neck extension disorder, or continuous severe cough.

2.2.5 The ultrasonographic features of thyroid nodules are highly similar to follicular nodules’, and the pathologists in the hospital lack diagnostic experience in cytological specimen of follicular nodules.

2.2.6 For nodules with a maximum diameter less than 5 mm, the operator cannot be sure that the target lesion can be accurately punctured.

2.2.7 The nodule to be punctured cannot be clearly detected by ultrasonography.

**Expert consensus:** The terms and conditions take fully into account the risks from the patients, the puncture methods, the skills of operator, and the risks of the pathologist's diagnostic ability.

**Grading of recommendation:** F. Intervention is strongly opposed to recommendation for confirmed evidence.
It cannot improve clinical outcomes, or harm is greater than benefit.

3 Operating Procedure

3.1 Preparation before puncture

3.1.1 Inquire about relevant medical history
Focus on whether the patient has a history of lidocaine allergy, ischemic heart disease, high blood coagulation (lower limb venous thrombosis, cerebral infarction, etc.), or hemorrhagic diseases, and whether the patient has used aspirin, Plavix (Clopidogrel Hydrogen Sulphate), warfarin, compound Danshen (Radix Salviae Miltiorrhizae) dripping pills, and other anticoagulants or Traditional Chinese Medicine for activating blood and resolving stasis over a long period [4]. Puncture can only be performed at least 7 days after correcting coagulation dysfunction or stopping anticoagulant drugs.

3.1.2 Evaluation of ultrasound images
Color Doppler ultrasonic diagnostic apparatus with a high-resolution linear array probe is usually selected for routine scanning. A higher frequency probe (e.g., above 10 MHz) should be selected for micro nodule puncture. Before the operation, the left and right lobes and isthmus of thyroid gland should be scanned from top to bottom to determine whether the target nodule to be punctured really originates from thyroid gland [5-8] or ectopic thyroid gland [9-10]; to determine the number, location, blood supply, and important adjacent structures of thyroid nodules; and to acquire and store dynamic and/or static images of the target nodule. At the same time, bilateral carotid artery examination should be carefully performed to determine whether there is plaque. If there is, the nature of the plaque and the risk of plaque falling off should be to evaluated.

3.1.3 Evaluation of puncture needle
According to the ultrasonic features of nodules, puncture biopsy needles of different lengths (5 cm~10 cm) and different outside diameter (22 G~25 G) should be prepared (Fig. 1), as well as auxiliary instruments for negative pressure aspiration (Fig. 2), etc. Base on some operators’ working experience, the richer blood supply the nodules have, the thinner needle should be chosen. In those cases, a lower composition ratio of blood components to nodule cells in the specimen, i.e., less blood components, has less influence on the observation of smear under microscope. On the contrary, the specimen collected by thicker puncture needles will have a higher composition ratio of blood components to nodular cells, i.e., more blood components, which will greatly interfere with the interpretation of smears [11-13]. The operator can choose a suitable puncture needle according to the blood supply status and conditions of the nodule. In the case of multiple nodules, each nodule should be punctured by one needle. It is not recommended to puncture multiple nodules with one needle, to avoid samples mixing up and interfering the accuracy of cytological diagnosis.

3.1.4 Specimen preparation
Prepare glass slides (to be smeared by the on-site operator) or liquid-based cell preservation solution containers (to preserve specimen, and then the specimen will be smeared by pathologists) according to the requirements of pathologists in the hospital.

3.1.5 Informed consent
The operator shall fully inform the patient or his/her family about the value, risk, expected results, points for attention during and after the operation. If the specimen needs to be sent to the pathology department of another
hospital for diagnosis, they will also be clearly informed. If contrast-enhanced ultrasound examination is required during the puncture, the value and risk of contrast-enhanced ultrasound must be informed. All contents must obtain the patient's full willingness and consent, and both doctors and patients sign standardized and effective informed consent forms.

**Expert consensus:** The terms and conditions embody the core requirements that puncture operators must personally participate in the preparation before puncture, including standardized ultrasound scanning, image files saving, and acquiring informed consent of patients.

**Grading of recommendation:** A. Strong recommendation.

Evidence confirms that it can improve clinical outcomes, and the advantages outweigh the disadvantages.

### 3.2 Puncture operation

#### 3.2.1 Patient Position and Puncture Operator Position

**3.2.1.1 Patients position:** In a routine puncture operation, patient should maintain the supine position with neck slightly hyperextended, and the neck and the area on chest above the line between the nipples should be fully exposed. For the patient complicated with cardiopulmonary diseases, electrocardiogram, blood pressure, and blood oxygen should be monitored and oxygen inhalation should be performed (Fig. 3).

![Figure 3](image)

**Figure 3** Intra-operative monitoring and oxygen supply. (A) Electrocardiogram monitoring electrode; (B) The infrared blood oxygen monitoring sensor, which can work on fingertips as well as toes; (C) Real-time monitoring displays of electrocardiogram, blood oxygen saturation, and blood pressure; (D) Nasal oxygen tube continuously supplying oxygen.

#### 3.2.1.2 Standing position of the puncture operator:

*Two standing positions can be adopted*[14]: ①"Head-side orientation", that is, the puncture operator stands on the right or left side of the patient's neck and faces the patient's head side (Fig. 4). The standing position is the same as that of routine thyroid ultrasound examination. The advantage is that the orientation of puncture sonograms is consistent with that of conventional ultrasonogram, which is helpful for puncture operators to accurately distinguish the upper, lower, left, and right positions of thyroid nodules, and is not easy to cause identification errors. The disadvantage is that the puncture operator stands on the right or left side of the patient's neck, which is relatively far away from the thyroid gland, therefore, it is easy to cause muscles fatigue of puncture operator's upper arm and waist. ②"Foot-side orientation", that is, the puncture operator stands on the head side of the patient and faces the patient's foot side (Fig. 5). This standing position is opposite to that of routine thyroid ultrasound examination. The advantage is that the puncture operator stands on the head side of the patient, and the puncture operation is nearby the thyroid gland as the adaptable position. It is convenient for the operator to effectively control the insertion direction and depth, and the upper arm and waist muscles are not easy to become fatigue. The disadvantage is that the orientation of puncture sonogram is opposite to that of conventional examinations, which can easily cause mistakes in the orientation of thyroid nodule to be punctured and lead to wrong identification of nodule. However, an intensive and targeted adaptation training can improve the ability to orient the sonogram at this standing position.
Expert consensus: "Foot-side orientation" is preferred, and the operator can take the sitting position to puncture, which can keep the operator’s body steady and the puncture operation steady and reduce fatigue. The errors in the orientation of puncture sonogram should be prevented.

Grading of recommendation: C. Intervention is recommended based on expert consensus.

3.2.2 Disinfection and anesthesia

3.2.2.1 Disinfection of probe:

(1) Fumigation disinfection: prepare a closed box, place the ultrasonic probe in the upper case of the box, and pour formalin solution and put potassium permanganate powder into the lower case. When formalin solution and potassium permanganate powder mixing, atomized gas generates immediately, which then fills the box and disinfects the probe. As the gas has strong irritation to respiratory tract and conjunctiva and has carcinogenic risk, this method is no longer recommended for disinfection.

(2) Soaking disinfection: soak the probe in disinfectant solution (e.g., glutaraldehyde solution). Since this method takes a long time and has low efficiency, it cannot meet the need of continuous puncture for multiple patients within a short period of time, it is rarely used now.

(3) Isolation sleeve: put the probe and its cable into a sterilized thin plastic sleeve for package isolation, then inject a small amount of physiological saline into the sleeve to expel the air between the sleeve and the piezoelectric crystals of the probe, and finally wrap the end of the sleeve tightly to the probe (Fig. 6). This simple and practical method is one of the commonly used methods. There is an even more convenient method with isolation sleeve that is to wrap the probe with a sterilization glove. Since the glove can only wrap the probe, the probe cable cannot be isolated (Fig. 7). The exposure may cause the contamination of the operative field, which does not meet the strict requirements of aseptic operation.

3.2.2.2 Disinfection of the operative field: The puncture operator wears a sterile mask, hat, and gloves, disinfects the operative skin according to the disinfection requirements of thyroid surgery. The minimum boundary of the disinfection region is around 7 to 10 cm [15] (Fig. 8) away from the puncture site. After disinfection, place a sterile towel (Fig. 9).

The sterile towel should completely cover the head, neck, and chest of the patient, and the opening should fully expose the region to be punctured.
Expert consensus: The process FNA for thyroid nodules must strictly comply with the requirements of disinfection, isolation, and aseptic operation.

Grading of recommendation: A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.

3.2.2.3 Local anesthesia: inject 5 mL of 1% lidocaine solution into puncture point from skin to subcutaneous layer to reduce pain and discomfort caused by the puncture.

Expert consensus: Local anesthesia should be applied except for the patients who are allergic to commonly used local anesthetic or those who refuse to use anesthetics. The ultrasound guidance on the injection can optimize puncture path, improve the analgesic effect on the parathyroid capsule, and prevent injecting anesthetic into blood vessels.

Grading of recommendation: C. Intervention is recommended based on expert consensus.

3.3 Ultrasound guidance

Ultrasound guidance and monitoring the whole process is the basic requirement for a safe and successful thyroid nodule FNA. According to whether needle-guide is used in the puncture process, ultrasonic guidance is divided into two types, namely, needle-guide method and free-hand method [16-17].

3.3.1 Needle-guide method (Fig. 10)

This method uses a needle-guide (also known as puncture rack), which can not only help beginners to stably control the needle insertion direction and depth, but also improve the puncture accuracy. Its disadvantage is that as the puncture angle is limited, the operator cannot flexibly adjust the direction of insertion according to the real condition.

3.3.2 Free-hand method[17](Fig. 11)

The method does not use the needle-guide. Therefore, the probe is in a relatively free state and the puncture needle is not bound to the needle-guide. Its advantages are as follows: firstly, according to the target’s position and its changing of position, the operator can freely and delicately adjust the angle and direction of puncture;
during the puncture process, the operator can adopt an "S" shape insertion flexibly to avoid the serious interference on the original approach. Secondly, when the thyroid position changes greatly due to sudden swallowing or coughing, this method can effectively avoid organ laceration caused by the puncture. The operator is required to undergo specialized learning and training until they master the skills.

**Expert consensus:** For those who have rich experience in puncture operation, the first choice is free-hand method; while the beginners should choose the needle-guide method.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

### 3.4 Puncture approach

The puncture approach or puncture path refers to the path through which the puncture needle enters the skin and subcutaneous tissue under the guidance of ultrasound to reach the puncture target. Different puncture approaches have different puncture outcomes and levels of risk. The operator should plan a safe and effective puncture approach according to the target and its surrounding anatomical structure, and make full use of the spatial interactive relationship between acoustic beam emitted from probe and puncture needle. Before the puncture needle reaching the target and after the needle entering the target, the puncture operator needs to ensure that the puncture needle tip is always clearly displayed on the ultrasonogram, and confirm the puncture approach has avoided the dangerous regions, the puncture needle accurately hits the target, and make sure the needle does not penetrate the target or even injure the important tissues behind the target.

#### 3.4.1 Probe’s end approach

This approach refers to insert needle laterally at one end of the probe (Fig. 12), which can clearly display the needle tip and shaft during the whole puncture process. The puncture operation is safe. With accurate display of the needle tip position and effective obtain of specimens, it can better improve the puncture operator’s confidence. Probe’s end approach, therefore, is the applicable for all punctures.

![Figure 12 Probe’s end approach. (A) In the end view, the puncture needle is located on the central line of the probe crystal; (B) In the side view, the puncture needle is located in the central plane of the probe; (C) Needle tip and shaft are clearly displayed on ultrasonogram.](image)

#### 3.4.2 Probe’s side approach

This approach refers to puncture needle insertion at the lateral side of the middle point of the probe (Fig. 13). Sometimes the display of puncture needle is unclear with this approach. This puncture approach is not suitable for the following situations: ① the puncture target is small, especially less than 5 mm; ② the puncture target position is too superficial or too deep; ③ Some important structures, e.g., common carotid artery, internal jugular vein, trachea, etc., are deeply below the puncture target.

**Expert consensus:** It is the key issue to perform a safe, accurate, and effective FNA with a clear and continuous display of real-time position of needle tip during the whole procedure. As the probe’s end approach has multiple advantages, it should be the first choice.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

### 3.5 Puncture aspiration

In FNA, the specimen is sucked into the needle cavity by the suction of negative pressure. After inserting the fine needle into the target lesion and pulling out the needle core, the capillary action in the needle cavity is generated in a natural negative pressure state as the inside pressure is lower than the outside atmospheric pressure, and the negative pressure in the needle cavity is even greater when the needle is withdrawn. As for the nodules with dense texture or calcification, the natural negative pressure is not great enough to suck out enough specimen. Therefore, a suction device that can generate greater negative pressure is required to be connected to the needle holder of the puncture needle, e.g., syringe. In the process of manually withdrawing the syringe to continuously raise the negative pressure in the needle cavity, more specimens can be obtained by lifting and
thrusting the puncture needle. Therefore, FNA can be divided into non-suction aspiration method (natural negative pressure aspiration) and suction method (manual pressurization aspiration). The results of comparing the two methods on the sufficiency of sampling, the satisfaction of specimen, and the diagnostic efficacy are still controversial [18-20].

3.5.1 Non-suction aspiration method

The puncture needle is not connected with a syringe. After the puncture needle entering the nodule, the operator can lift and thrust the puncture needle or combine with rotating the needle back and forth. When some aspirates are found in the puncture needle holder, the operation can be stopped and the puncture needle can be withdrawn (Fig. 14). The advantage of this method is that there are relatively few blood components in the specimens, which is conducive to cytological pathological examination.

3.5.2 Suction aspiration method

The puncture needle is connected to a syringe. After the puncture needle entering the nodule, the syringe should be kept in a continuous suction state, and the puncture operator has to lift and thrust the puncture needle for at least 3-5 times[13]. The operation will be stopped and the puncture needle will be withdrawn when some aspirates are found in the puncture needle holder(Fig. 15). The advantage of this method is that enough specimen can be obtained, while the disadvantage is that blood components are easily mixed in the specimen, and direct smear examination on that specimen may interfere with cytological pathological examination.

**Expert consensus:** What sampling method should be adopted needs comprehensive analysis of the ultrasonographic characteristics in concerns of blood supply, stiffness, with or without calcification and etc. of the nodules, and also fully taking the experience and habitual practice of the puncture operator.
into consideration. For the nodules with abundant blood supply, "non-suction aspiration method" is recommended. While for the nodules with insufficient blood supply, dense and hard texture, or complicated by coarse calcification, "suction aspiration method" is recommended.

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

### 3.6 Range and speed of puncture

When lifting and thrusting the puncture needle, the needle tip should move in a large enough range, rather than at a fast speed.

#### 3.6.1 The puncture range should be large enough

The needle tip in the target nodule must be moved back and forth in a large range. The most ideal range is from the proximal puncture point to the distal puncture point of the target (Fig. 16), to obtain the largest number of samples on the path of the puncture.

**Figure 15** Suction aspiration method of FNA. (A) One-person operation; (B) Two-person cooperative operation.

**Figure 16** The large range puncture through the target nodules. (A) Diagram of the proximal puncture point of the target nodule (p-proximal point, TN-thyroid nodule, TH-thyroid, CCA-common carotid artery); (B) Diagram of the distal puncture point of the target nodule (d-distal point); (C) Ultrasonogram of the proximal puncture point; (D) Ultrasonogram of the distal puncture point.

#### 3.6.2 Not seeking fast puncture

In the target nodule, it is inadvisable to fast lift and thrust the needle tip within a small range, or even the needle tip fast tremble at the same point (Fig. 17). After sampling, the puncture needle shouldn’t be withdrawn from the nodule too fast.

#### 3.6.3 Not moving needle unless seeing needle tip

When lifting and thrusting the puncture needle, the image of needle tip on the sonogram must be kept clear and visible. The puncture operator should always follow the principle of "not moving needle unless seeing needle tip", especially "not advancing needle unless seeing the very needle tip".

**Expert consensus:** The large range of puncture through the whole nodule can maximize the amount of specimen obtained in a single puncture operation, and a clear display of the real-time position of the needle tip is the prerequisite for effective and safe puncture.

**Grading of recommendation:** A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.
3.7 Times of punctures

The effectiveness and authenticity of FNA pathological diagnosis depend on accurate and high-quality sampling and sufficient amount of specimen. The quality and quantity of specimen are determined not only by negative pressure suction, but also by the difference of tissue characteristics in different regions within the nodule. As the density of distribution, the severity of necrosis, and the colloid contents of tumor cells in different regions may be various, the puncture sampling should encompass the widest area of the target lesion, so as to avoid the limitation of quality and quantity of sampling that may affect the accuracy of diagnosis. To increase the number of punctures can effectively reduce the incidence of insufficient punctured specimen. It is generally believed that the puncture should not be less than 3 times, as 3 times punctures can obtain enough specimen and the diagnostic coincidence rate is high. If necessary, the times of puncture on a nodule can be as many as 11 [21-23]. There are two ways to increase the amount of specimen as follows.

3.7.1 One-time puncture with multiple point sampling

For large nodules, the "9+X needle tracks" puncture mode based on "one needle puncture" is recommended, which can obtain most comprehensive specimen from the target nodule. This "one needle puncture" is to puncture needle into the target nodule, keep the needle within the thyroid gland capsule, and lift and thrust the needle tip for multiple times. On this basis, the "9+X needle tracks" puncture mode can be performed.

According to the "9+X needle tracks" puncture mode, the target nodule is divided into three subareas, i.e., the upper, the middle, and the lower subareas, on the longitudinal section of the thyroid ultrasonogram; and then each subarea is divided into three transverse sections, i.e., the anterior, the middle, and the posterior sections. The same needle is used to successively and continuously puncture and obtain specimens from the above-mentioned nine zones, so the specimens from the nine needle tracks can be collected in one-time puncture. "X" refers to the unfixed number of punctures. If there are some special ultrasonic manifestations in the targeted lesion, e.g., micro-calcification, abnormally rich or no blood flow signal, and large elastic ultrasonic strain ratio, the targeted biopsy can be additionally performed to the target lesion, and the aspirate should be smeared on another slide. (Fig. 18).

With comprehensive and balanced sample collection, sufficiency and optimized quality of sample, and high correspondence between the featured ultrasonographic image and the target nodule, the "9+X needle tracks" puncture mode reflects the current standardization and rigor of FNA.

3.7.2 Multiple times puncture

When the puncture needle enters and exits the target once, it is counted as one-time puncture. If the puncture needle enters and exits the target for many times, that is multiple times puncture, which can eventually increase the total amount of specimen.

Obviously, multiple times puncture can increase and enhance the mechanical damage of tissue on the needle track, e.g., thyroid capsule and thyroid parenchyma, and it can also increase the incidence of bleeding complications.

**Expert consensus:** Sufficient and up-to-standard specimen must be confirmed on-the-spot, to avoid a second time puncture operation due to insufficient specimen. The "9+X needle tracks" puncture mode can not only obtain comprehensive and sufficient specimen, but also reduce the damage caused by multiple punctures. It is the first choice for larger nodules.

**Grading of recommendation:** A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.

3.8 Flexible application of liquid isolation method[24]

For the target nodules adjacent to the trachea, especially at 7–9 o'clock point (or at 2–4 o'clock point in the contralateral gland), the puncture direction should be interior towards exterior (Fig. 19).
At the same time, the operator can use liquid isolation to shift the target lesion towards lateral side of body and increase the insertion angle of the needle in the end-side puncture approach, so as to avoid the tracheal wall blocking the puncture needle tip and improve the display of the puncture needle tip. For the lesions located at 2~4 o'clock point (or at 7~9 o'clock point in the contralateral gland) adjacent to the common carotid artery, it is also recommended to adopt the "interior towards exterior" puncture approach. Similarly, the operator can also use
the liquid isolation to shift the common carotid artery towards lateral side of body, to increase the distance between the target lesion and the common carotid artery (Fig. 20), to avoid accidental injury to the common carotid artery.

**Expert consensus:** Liquid isolation can shift the position of thyroid nodules or adjacent structures around thyroid, to optimize puncture route and create a safe space for operation.

**Grading of recommendation:** A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.

**Figure 19**  Liquid isolation can optimize the puncture path of FNA. (A) The target nodule (indicated by the up arrow) is close to thyroid cartilage. The puncture needle is more likely to injure the anterior capsular branch of superior thyroid artery and cartilage through the R1 approach, and the common carotid artery through the R2 approach; (B) With liquid isolation, the distance between the target nodule (indicated by the up arrow) and thyroid cartilage and that between the target nodule and common carotid artery (CCA) are all increased. No matter through the R1 or the R2 route, the puncture needle can avoid injuring the anterior capsular branch of super thyroid artery, cartilage, and the common carotid artery. R1, puncture route 1, also known as the "interior towards exterior" puncture approach, i.e., puncture needle from the midline of neck towards the lateral side of body. R2, puncture route 2, also known as the "exterior towards interior" puncture approach, i.e., puncture from the lateral side of body to the midline of neck via the sternocleidomastoid muscle.

**Figure 20**  Liquid isolation can improve the safety of FNA. (A) The target nodule (indicated by the up arrow) is close to the common carotid artery (CCA). No matter through the R1 or the R2 route, the puncture needle is more likely to injure the common carotid artery; (B) With liquid isolation, the distance between the target nodule (indicated by the up arrow) and the common carotid artery (CCA) increases, the puncture needle can avoid injuring the common carotid artery.

4 Specimen Preparation and Evaluation

4.1 Specimen preparation

4.1.1 Instant cytological smear

4.1.1.1 **Spray specimen correctly:** Take a clean and dry syringe, suck air into it, connect it to the puncture needle that was withdrawn from body, and quickly spray the specimen onto a clean glass slide (Fig. 21). The syringe must not contain any water to avoid diluting or damaging the specimen. The slide must be labeled with the information of patient’s identification and sampling site in advance. It should be clean and no oil stain or dirt, so as to avoid the specimen dropping off from the slide, or the dirt affecting the observation under the microscope.

**Expert consensus:** The glass slide should be labeled with the identification information of the patient and the specimen, so as to avoid identification errors due to mixing up. The slide should be kept dry and clean to prevent specimen dropping off and avoid difficult or even impossible diagnosis.

**Figure 21**  Spraying the specimen onto a glass slide
**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

4.1.1.2 **Makes Cell Smear Correctly:** The fresher the specimen is, the more accurate the cytological assessment is; the smear, therefore, should be made immediately after puncture.

(1) Sliding smear: Place the specimen at one end of the slide, and take another slide to push the specimen gently at an angle of about 30 degrees (Fig. 22). When preparing smear, the operation should be gentle to prevent cells from being squeezed and damaged due to excessive force (Fig. 23). The smear should be homogenous and of moderate thickness, so that the sample shows a flat, monolayer, and complete cellular morphology under the microscope.

(2) Dispensing smear: Use the syringe needle to disperse the specimen gently on the glass slide (Fig. 24) and keep the smear thickness as homogenous as possible. For larger granules, press the glass slide gently to disperse and flatten it. It is not allowed to poke and dispense the specimen by needle tip, as it may damage cells.

**Expert consensus:** It is necessary to keep the integrity of native structure of the specimen as much as possible and reduce cell damage, to avoiding affect the quality of diagnosis.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

4.1.2 **Thinprep cytologic test (TCT)**

Inject the specimen in the needle cavity into a liquid-based cell preservation liquid container labeled with the patient identification and sampling site in advance (Fig. 25), and deliver it to the pathologist for the following preparation and evaluation. The operator should check whether the granular specimens in the storage liquid container are sufficient or not. If insufficient, another puncture should be performed.

**Expert consensus:** TCT technology can avoid the interference of red blood cells and significantly improve the microscopic appearance of nuclear features of thyroid carcinoma.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

4.1.3 **Preparation of eluent for puncture residues**

After the specimen being taken out from the needle cavity by conventional methods, there may still be some residual cell or tissue fluid on the inner wall of the needle cavity. Flush the needle cavity with 1~3 mL of physiological saline or specific reagent, and place it in a test tube to prepare FNA eluent. Detection of relevant molecules or genes in the eluate of the puncture residues can help analyze the origin of the target nodule and determine the type of its malignant tumor [25].

**Expert consensus:** The preparation of eluent can make full use of specimen obtained by FNA, reflecting more diagnostic means can be introduced to FNA diagnosis.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

4.1.4 **Handle blood-rich specimens properly**

If the specimen contains more blood components (Fig. 26), the following measures can be taken to reduce the interference of red blood cells on the microscopic observation of specimen. ① Scatter the specimen to...
multiple slides to avoid the single smear with too thick specimen, to reduce the coverage of red blood cells on other cells. ②Suction aspiration and non-suction aspiration methods can be flexibly switched depending on the real condition during the puncture process. If the specimen collected by suction aspiration puncture method contains more blood-rich components, non-suction aspiration puncture should be adopted in the second puncture; if the specimen collected by non-suction aspiration method is less, suction aspiration puncture should be adopted in the second puncture. ③Thinprep cytologic test can eliminate the influence of red blood cells and improve the observation outcome of thyroid nodule cells.

Figure 26  Excessive blood-rich components in the specimen. (A) The specimen is sufficient according to naked-eye observation, but the contents are mainly blood-rich components; (B) There are too many red blood cells under light microscope (×10).

Expert consensus: For those nodules with rich blood supply, it is difficult to avoid blood-rich components during specimen collection. Therefore, should try to reduce the interference of red blood cells on the observation of smear under microscope.

Grading of recommendation: C. Intervention is recommended based on expert consensus.

4.2 Specimen evaluation

4.2.1 On-the-spot instant cytological evaluation

On-the-spot instant cytological evaluation is a set of techniques including smearing, staining, microscopic observation, and evaluating of specimens on-the-spot done by a well-trained cytologist or cytopathologist [26-27]. Its value is the instant evaluation of specimen quality and quantity. If the specimen is not satisfactory, the cytologist or cytopathologist can provide guidance to the second attempt of puncture on site, so as to improve the diagnostic efficiency of FNA by reducing repeated FNA in the future. In addition, smearing, staining, and analyzing smears on the spot can take the advantage of the freshness of specimen, to assure the diagnostic quality. Its disadvantage is that the puncture process has to be prolonged due to waiting for the evaluation result and its cost is higher [28-30].

Expert consensus: The hospital with the capability of instant cytological evaluation can carry it out.

Grading of recommendation: C. Intervention is recommended based on expert consensus.

4.2.2 Predict the satisfaction of specimen by observing the abundance of granules with naked-eye observation

If there is no condition for on-the-spot instant cytological evaluation, it is of high feasibility and a certain actual effect to train non-cytological doctors to perform on-the-spot macroscopic evaluation on the specimen smeared on glass slides or in liquid-based preservation solution. If the specimen is thick according to on-the-spot naked-eye observation, it indicates that the specimen is with sufficient cells and the diagnostic efficiency of microscopic cytology will be higher (Fig. 27). On the contrary, if the specimen is thin, it indicates that the quantity of cells may be insufficient and that will affect the cytological diagnostic efficiency (Fig. 28). If there is abundant granules in the specimen, it indicates there will be a large number of cells under microscope and an effective diagnosis is easy to achieve, and the proportion of positive results is higher (Fig. 29). There will be opposite outcomes if the specimen is with less or no granules. Although on-the-spot naked-eye observation cannot replace microscopic analysis, it can adjust the times of puncture reasonably and enhance the confidence of the operator [31].

Expert consensus: Make the most of the technical capability of pathologists or specially trained medical technicians, who can make a preliminarily judgement whether the specimen is with abundant granules by naked-eye observation on the spot and decide whether the puncture should be concluded. However, the naked-eye observation findings should not be equivalently related to the pathological properties.

Grading of recommendation: C. Intervention is recommended based on expert consensus.

5 Points for Attention after Puncture

After puncture, the matters needing attention should be explained in detail to the patient and his/her family. The patient should press the skin puncture point for
at least 10 min and avoid strenuous neck movements. After outpatient observation for 30 min and ultrasound recheck, if the puncture site has no bleeding, the patient can leave the hospital. After the patient leaving the hospital, if the symptoms occur, e.g. neck swelling and persistent pain, the patient should seek medical examination and treatment in time.

**Expert opinion:** The main complication of FNA is local hemorrhage. Compression hemostasis is simple and effective, and the efficacy is exact. A small number of patients have the risk of delayed active hemorrhage, which should be paid great attention to.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

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**Figure 27** Thick specimen with abundant granules. (A) The specimen is sufficient with many tumor tissue granules and less blood-rich components by naked-eye observation; (B) Under light microscope, there are many tumor cells and less red blood cells (×10).

**Figure 28** Thin specimen with less blood-rich components and less granules. (A) The specimen is insufficient in quantity and with thin texture by naked-eye observation; (B) Under light microscope, the specimen is thin and with less cells (×10).

**Figure 29** Sufficient specimen with abundant granules of tumor tissue. (A) The specimen is with abundant granules and less blood-rich components according to naked-eye observation; (B) Under light microscope, the nucleus of papillary carcinoma is definite (×10).

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### 6 Complications and Management

#### 6.1 Hemorrhage

Hemorrhage is the most common complication in FNA, with an incidence of 8.6% [32]. Hematoma can occur in the perithyroidal space, thyroid parenchyma, or the muscles on the puncture route, usually with mild symptoms and rapid absorption. In the case that the patient has no obvious dyspnea, local compression can be performed for 20 min as measures without any other treatment. If the wall of carotid is injured by puncture and intramural hematoma occurs, local compression lasting for 1~2h is required to stop bleeding, so as to prevent hemorrhage from spreading widely along the arterial wall. In rare cases, if there is a large hematoma or pseudoaneurysm [33-34], the patient should stay in hospital for observation and receive further treatments. The injury to feeding artery of thyroid nodule induced by FNA can also lead to pseudoaneurysm, which requires surgical intervention [35].
6.2 Infection

Infection of skin puncture site or needle track caused by FNA is relatively rare. Slight infection need not be treated or only treated with oral antibiotics. If the infection is obvious or with abscess, it needs surgical treatment.

6.3 Implantation along needle track

The incidence of tumor cell implantation along needle track after FNA is extremely low, and the incidence reported in the literature is about 1.2/10^6 [36].

6.4 Shock

Very few patients may suffer from "shock" during puncture operation, and most of them are faint during puncture. In this situation, the operation should be ceased immediately. The patient should lie in supine position and inhale oxygen; and the vital signs, e.g., heart rate and blood pressure, should be monitored intently. If necessary, the operator should promptly contact with the emergency department, the anesthesia department, and other relevant departments, for emergency consultations and further treatment.

6.5 Others

It is rare that FNA causes the damages of trachea, esophagus, recurrent laryngeal nerve, and other tissues.

**Expert consensus:** Although FNA cause only minor trauma, fewer complications, and mild symptoms after operation, the principle of aseptic operation should be abided by. The operators should always keep the safety awareness, make an emergency response plan to prevent serious complications.

**Grading of recommendation:** C. Intervention is recommended based on expert consensus.

7 Cytological Evaluation Results and Suggestion on Handling the Results

At present, the widely used thyroid cytology reporting systems include the consensus of the Italian Endocrine Association, the Royal Society of Pathologists, and the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), among which there are some minor differences [37-40].

**Expert consensus:** To adopt the general classification of TBSRTC in diagnosis [40-41]

**Grading of recommendation:** A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.

7.1 Specimens is not satisfactory or underdiagnosis

In this situation, it is suggested that ultrasound follow-up or repeated FNA should be performed every 6~12 months. If ultrasound examination discloses any suspicious malignant feature in the target nodule, especially the solid components of the nodule increase in a short period of time, a second-time FNA within 3 months is recommended to perform [42-45]. If the maximum diameter of the nodule is less than 5 mm or the cystic lesion is more than 50%; the non-diagnostic rate of the re-puncture is still high, and ultrasound follow-up is recommended [46].

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

7.2 Benign lesions

For the benign lesions, generally, no further diagnosis and treatment is required, only ultrasound examination follow-up is recommended at intervals of 12 to 24 months. When the nodule is larger than 4 cm, ultrasound-guided core-needle biopsy (CNB) or surgical treatment is recommended. When the solid components of nodules increase by more than 50% or nodules appear with suspected malignant ultrasonographic features, a second-time FNA or FNA combining with gene detection is recommended.

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

7.3 Inconclusive atypical cell lesions or inconclusive follicular lesions

It is suggested to perform a second-time FNA. If the results are still inconclusive after multiple times FNA, the frequency of follow-up ultrasound examination should be increased according to the suspicious degree by ultrasound findings. For those cases with obvious malignant features on ultrasonograms; it is recommended to perform gene detection combining with FNA, or perform ultrasound-guided CNB, or even diagnostic surgery after evaluation.

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

7.4 Follicular tumor or suspected follicular tumor

For the follicular tumor or suspected follicular tumor, it is difficult to make definite diagnosis of benignity or malignancy according the results of ultrasonography and FNA. Surgical treatment is generally recommended instead of repeated FNA [47] or gene detection combined with FNA.

**Grading of Recommendation:** C. Recommendation: based on expert opinions.
7.5 Malignant or suspected malignant nodules

It is generally recommended to adopt active treatment strategy. Regarding the suspected malignant nodules, the treatment decisions should the clinical risk factors, ultrasonic risk classification, and gene detection results take into consideration.

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

8 Detection of Molecular Markers in FNA Eluent

FNA puncture eluent can be used to detect molecular markers specifically expressed by thyroid cancer cells. At present, the usual molecular markers include RET/PTC, RAS, PAX8/PPARγ, and BRAF, which are related to the occurrence of thyroid cancer [48-54]. Among them, BRAF mutation and RET/PTC rearrangement are highly correlated with papillary thyroid carcinoma. RAS mutation and PAX8/PPARγ rearrangement may be related to thyroid follicular carcinoma. Calcitonin may be determined for medullary thyroid carcinoma [55-58]. Recently, in a group of cases from Shanghai, the sensitivity and accuracy of preoperative FNA alone were 76% and 79%, respectively. And if combined with preoperative BRAF gene detection, the sensitivity and accuracy of preoperative FNA can rise to 92% and 91%, respectively [58].

**Expert consensus:** The detection of molecular markers can effectively improve the sensitivity and accuracy of diagnosis, especially in the cases with cytological negative, the undiagnosable cases, and the cases with atypical results. Positive expression of molecular markers will be helpful for diagnosis.

**Grading of recommendation:** B. Intervention is recommended for good evidence. It can improve clinical outcomes, and benefit is greater than harm.

9 Qualification and Training [59]

9.1 Qualification

9.1.1 The medical institute shall have the permission of ultrasound diagnosis and treatment in its authorized medical practice category and hold the license of that.

9.1.2 The ultrasound department shall have the ultrasound intervention in its scope of medical service. The technical management of ultrasound-guided fine needle aspiration shall abide by the relevant regulations and management requirements of the superior authority or organization.

9.1.3 The puncture operator shall hold the "Physician Qualification Certificate" and "Physician Practice Certificate" of the People's Republic of China, with the practice scope of “Medical imaging and radiotherapy”. If the puncture operator with the certificate of other practice scopes, he/she shall take part in relevant training and obtain Color Doppler Flow Imaging (CDFI) physician qualification certificate for large-scale medical equipment.

9.2 Training

9.2.1 To carry out FNA of thyroid nodules, the operator shall take part in the training program of interventional ultrasound and/or thyroid ultrasound approved by the administrative departments on national, provincial, or municipal levels, and pass the examination.

9.2.2 The operator shall have rich experience in interventional ultrasound, the academic title of deputy chief physician or above, one year or more of clinical tutoring experience, and taken part in 50 or more cases of fine needle puncture of thyroid nodules.

9.2.3 The nurse assisting the thyroid puncture shall have some clinical working experience, take part in the specialized training on interventional ultrasound, and pass the examination.

**Expert consensus:** Interventional ultrasound technology is within the scope of surgical operation. In order to reduce medical errors and malpractice and ensure medical safety, the qualification and training system must be strictly implemented before carrying out FNA cytological exam on thyroid nodules.

**Grading of recommendation:** A. Intervention is strongly recommended for confirmed evidence. It can improve clinical outcomes, and benefit is greater than harm.

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