Rapid on-site Evaluation (ROSE)

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Introduction

Fine-needle aspiration cytology (FNAC) has been used as a tool to obtain specimens for the morphological diagnosis of numerous lesions in a variety of locations for more than 80 years. The expanded utilization of biopsied material in tailoring personalized therapy has heightened the importance of specimen acquisition and increased the need for obtaining adequate specimens. Rapid on-site evaluation (ROSE) is laboratory service to assess the cytomorphological features of FNA smears and provide real-time feedback.

Endoscopy-guided FNAC (EUS-FNAC) is a highly skill-led procedure, the accuracy of which is directly related to the experience of the aspirator

What is ROSE?

- ROSE → real-time" evaluation of procured material.
- Cytotechnologist has function:
  - ✓ Check the cellular content and adequacy of FNAC smears.
  - ✓ Inform the surgeon/ operator of need to obtain additional samples
  - ✓ Provide preliminary diagnosis
  - ✓ Avoid repeat procedure

Cytotechnologist is checking for cellularity of FNAC smears.

Purpose of ROSE

- Determine the diagnostic adequacy and viable yield of cytology samples in real time.
- Allow correct sample triage and optimal preservation of the material.
- Ensure complete diagnosis and patient eligibility for personalized targeted therapies can be established in one minimally invasive procedure.

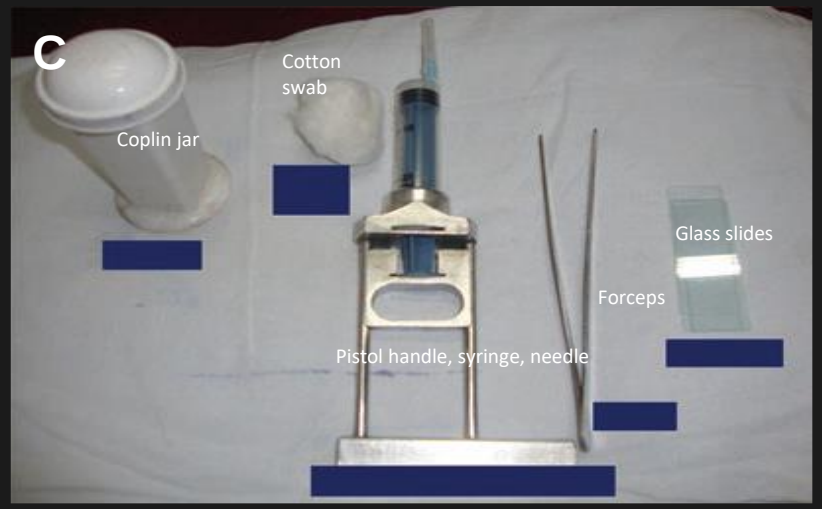
Basic instrument for screening.

ROSE applicable sites

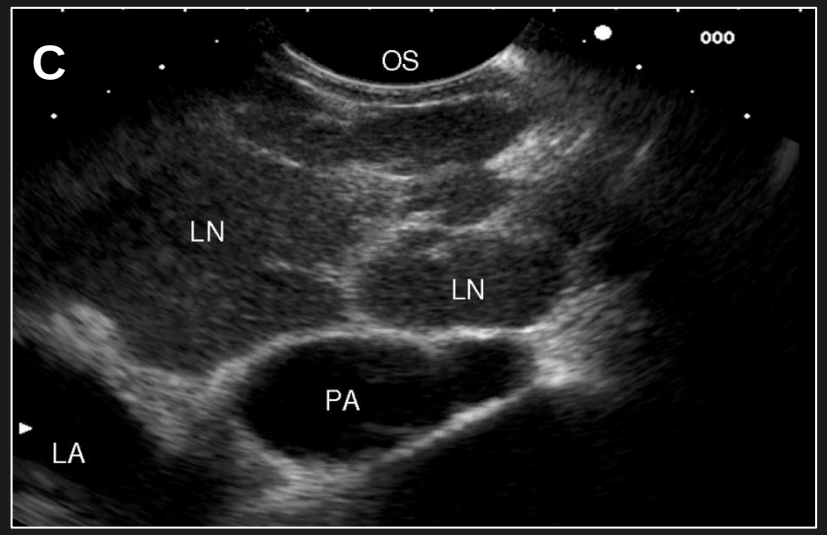
Endobronchial ultrasound FNA	Endoscopic ultrasound-guided FNA	Ultrasound-guided FNA
• Bronchus	• Pancreas • Liver • Spleen • Adrenal • Gastrointestinal tract • Lymph nodes	• Percutaneous lung mass • Head, neck & salivary glands • Palpable mass

Consumables and equipment

Consumables	Equipment	Solution
Personnel protective equipment	Microscope	CytoLyt solution
Frosted glass slides	Pen & pencil	Diff-Quick stain
Slide boxes and mailers	Coplin jar	95% alcohol
Patient request & consent form	Aspiration gun	Sterile saline
Basic dressing set		Formalin
Cotton swab		
5 ml/ 10 ml syringe		
23 G/ 21 G needle		

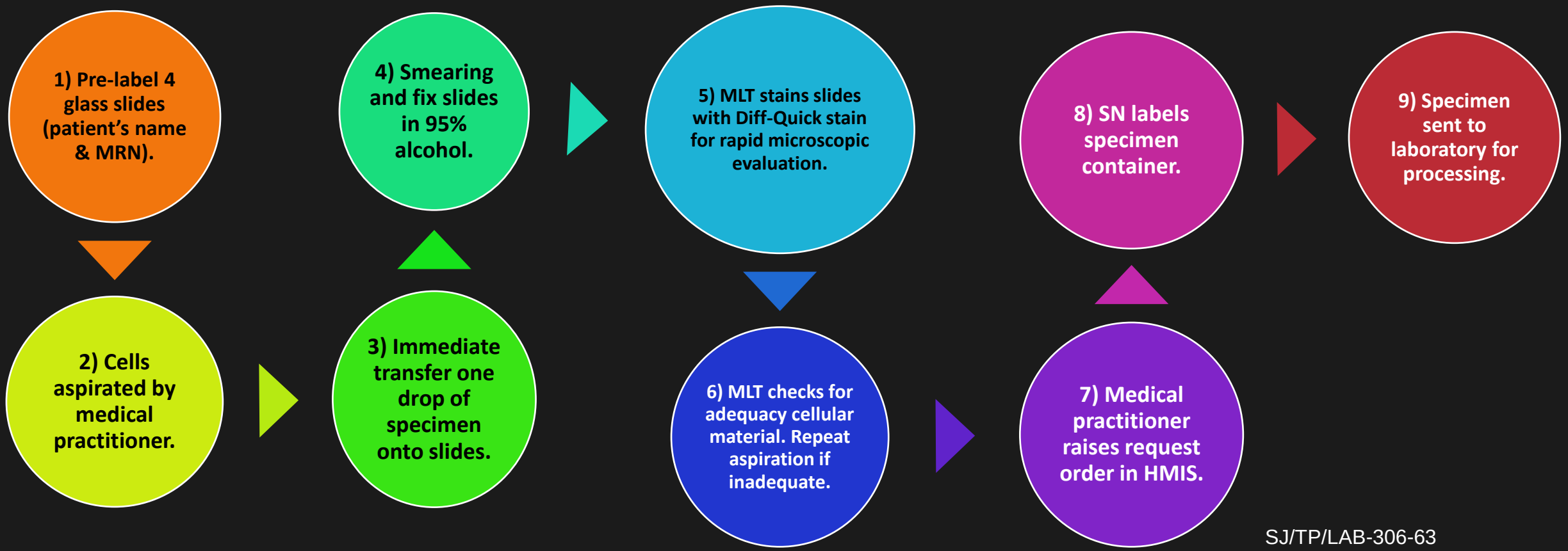


(**A**) FNA procedure without aspiration (capillary technique): The needle tip is passed through the skin, inserted into the lesion, and moved back and forth to dislodge cells. (**B**) FNA of cutaneous basal cell carcinoma performed by a pathologist. Syringe attached to a Cameco syringe holder: vacuum in the syringe helps to dislodge cells and to obtain a cellular specimen. (**C**) Essential equipment to do FNA.



(**A**) Endoscopic ultrasound-guided fine needle aspiration procedure techniques to obtain samples via slow-pull technique. (**B**) Initial designs of the endoscopic ultrasound-guided fine needle aspiration needle (Vilmann-Hancke needle). (**C**) Endoscopic ultrasound image in a patient with sarcoidosis demonstrating multiple, well demarcated lymph nodes (LN) located between the esophagus (OS), left atrium (LA) and pulmonary artery (PA).

ROSE process



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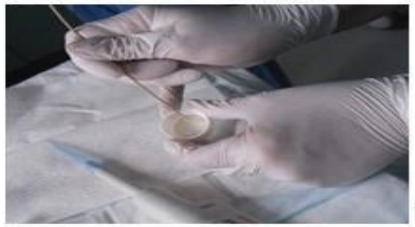
Cytopathology Rapid On-Site Evaluation of FNA/ EUS-FNA

Schematic instructions for performing for ROSE SSA technique.

1. Whole sample is expelled into a vial lid using a stylet

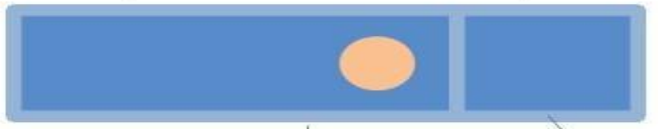


2. Mix sample

Macroscopic

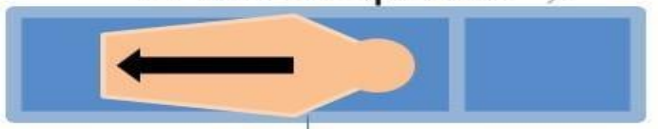
Verbally feed back to clinician quantity of sample obtained for that pass

3. Place representative drop onto slide



Glass Microscope Slide

4. Direct spread



5. Stain air dried slide (Quick MGG)

6. Microscopy assessment *using criteria in figure 4*

Microscopic

Verbally feed back to clinician adequacy for that pass

7. Needle flushed with saline into Vial

8. Transfer remaining sample into vial containing saline

For pancreatic lesions that require CEA and Amylase - A separate fresh sample is sent for analysis without saline

Vial

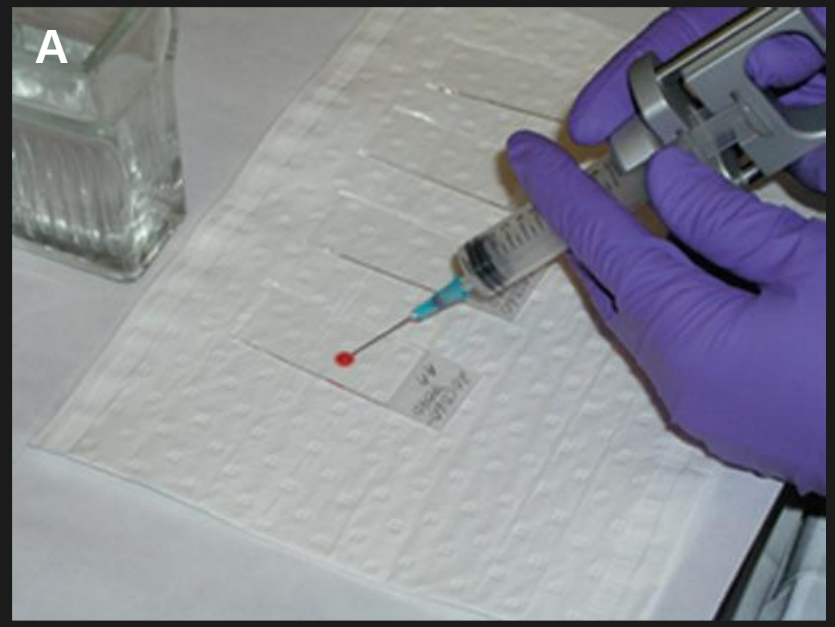
Saline

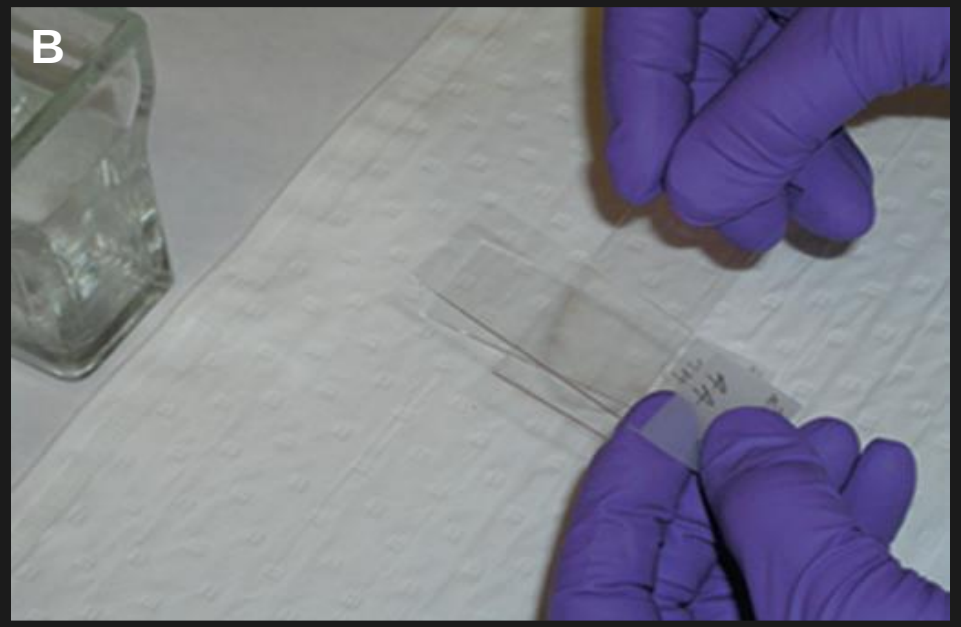
9. *At the end of the procedure* - Make aliquots for ancillary tests where required

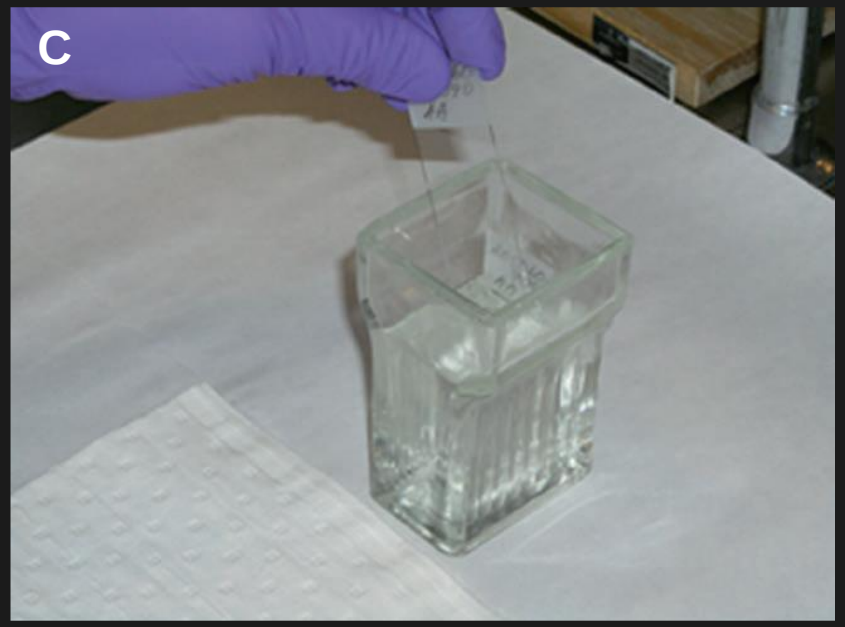
Saline

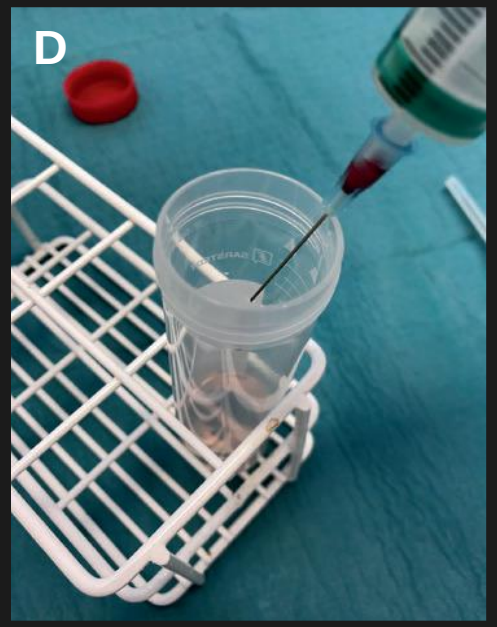
Flow Cytometry / microbiology

10. Add Cytolyt preservative *(Same amount of cytolyt to volume of sample)*

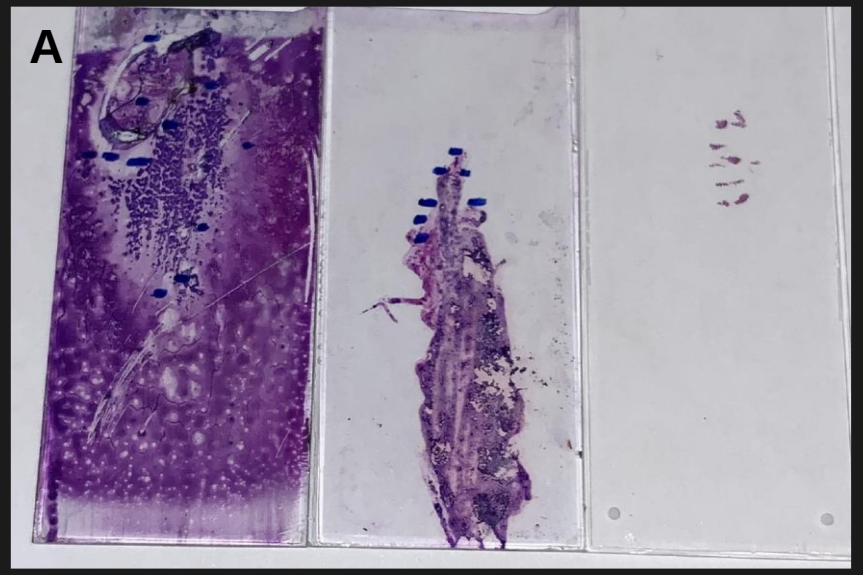


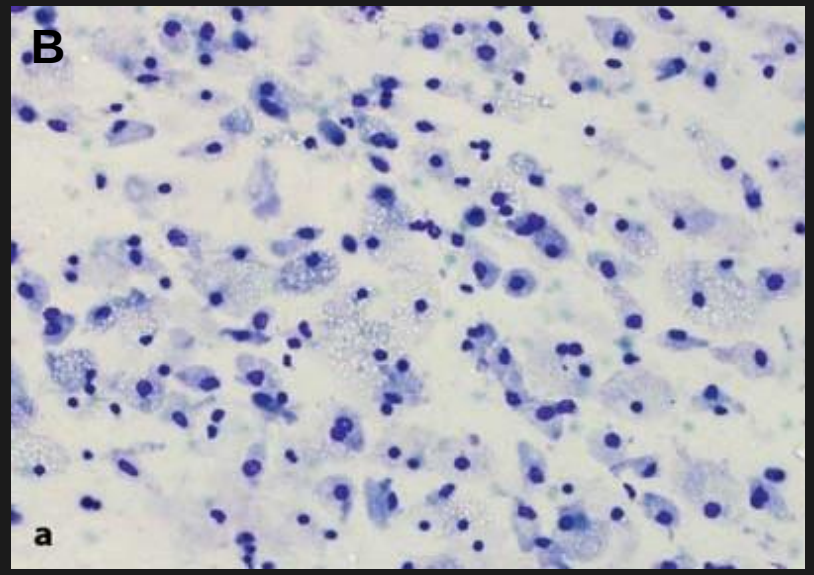


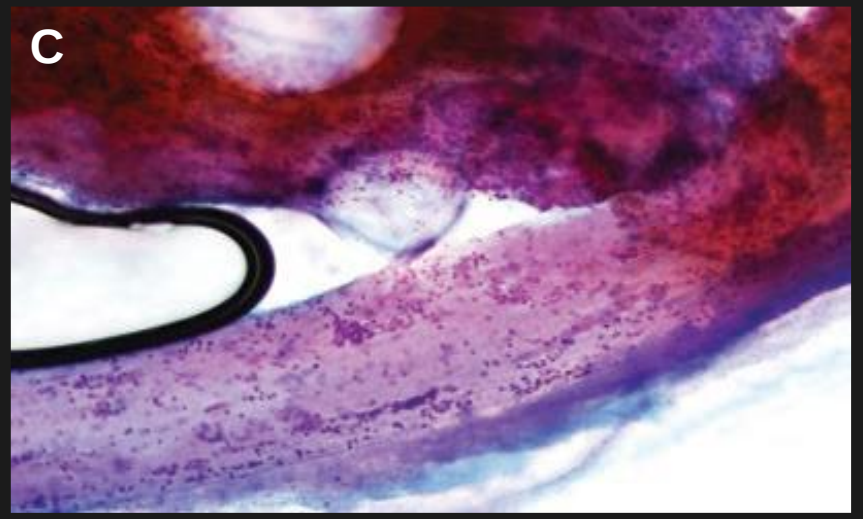




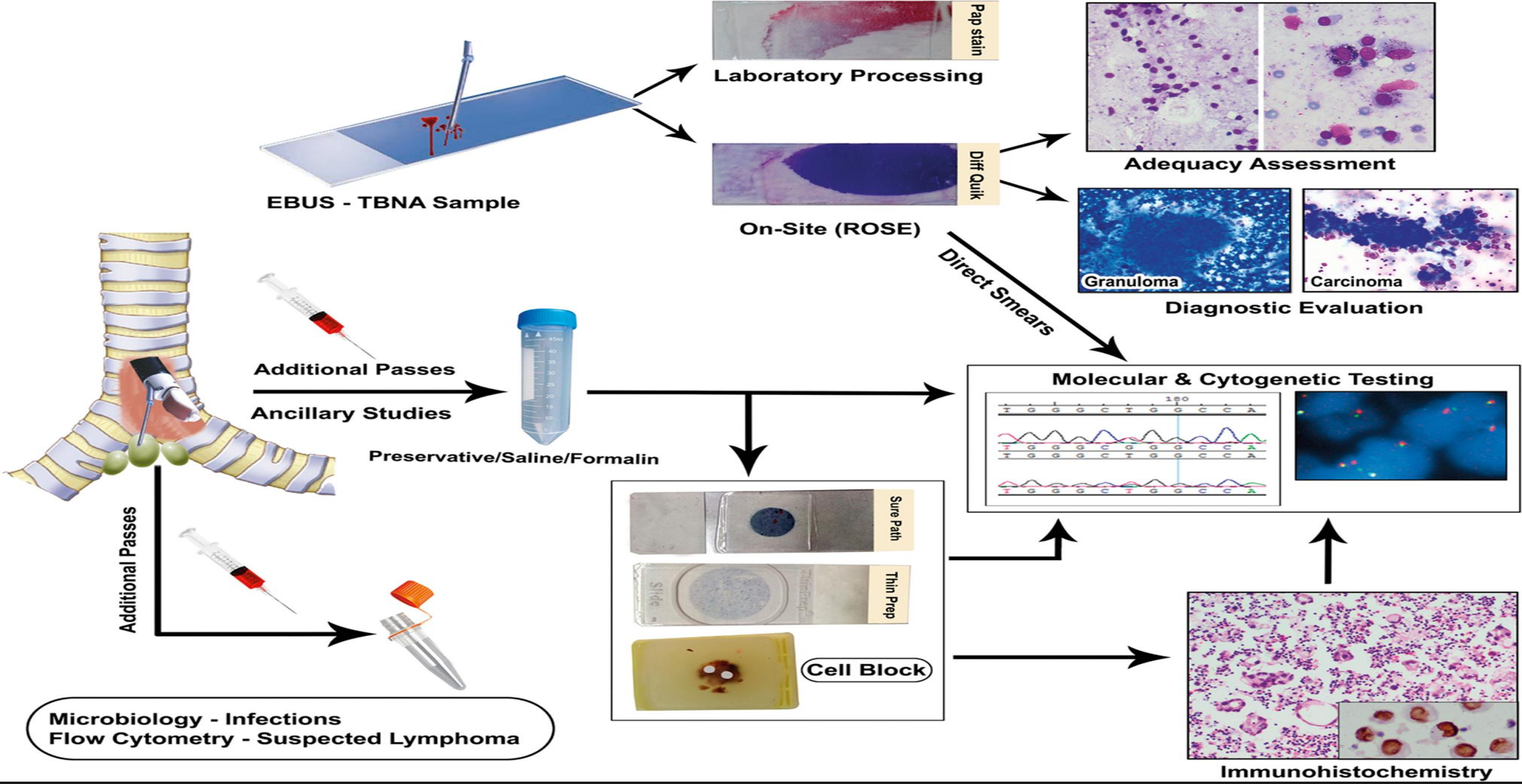
(**A**) Air is aspirated and the material is expelled on glass slides. (**B**) The material is gently but rapidly smeared on the slides. (**C**) Slides with smears are immediately dipped in fixative or left to air dry. (**D**) For liquid based preparation the aspirate is collected directly in specially developed liquid fixative.





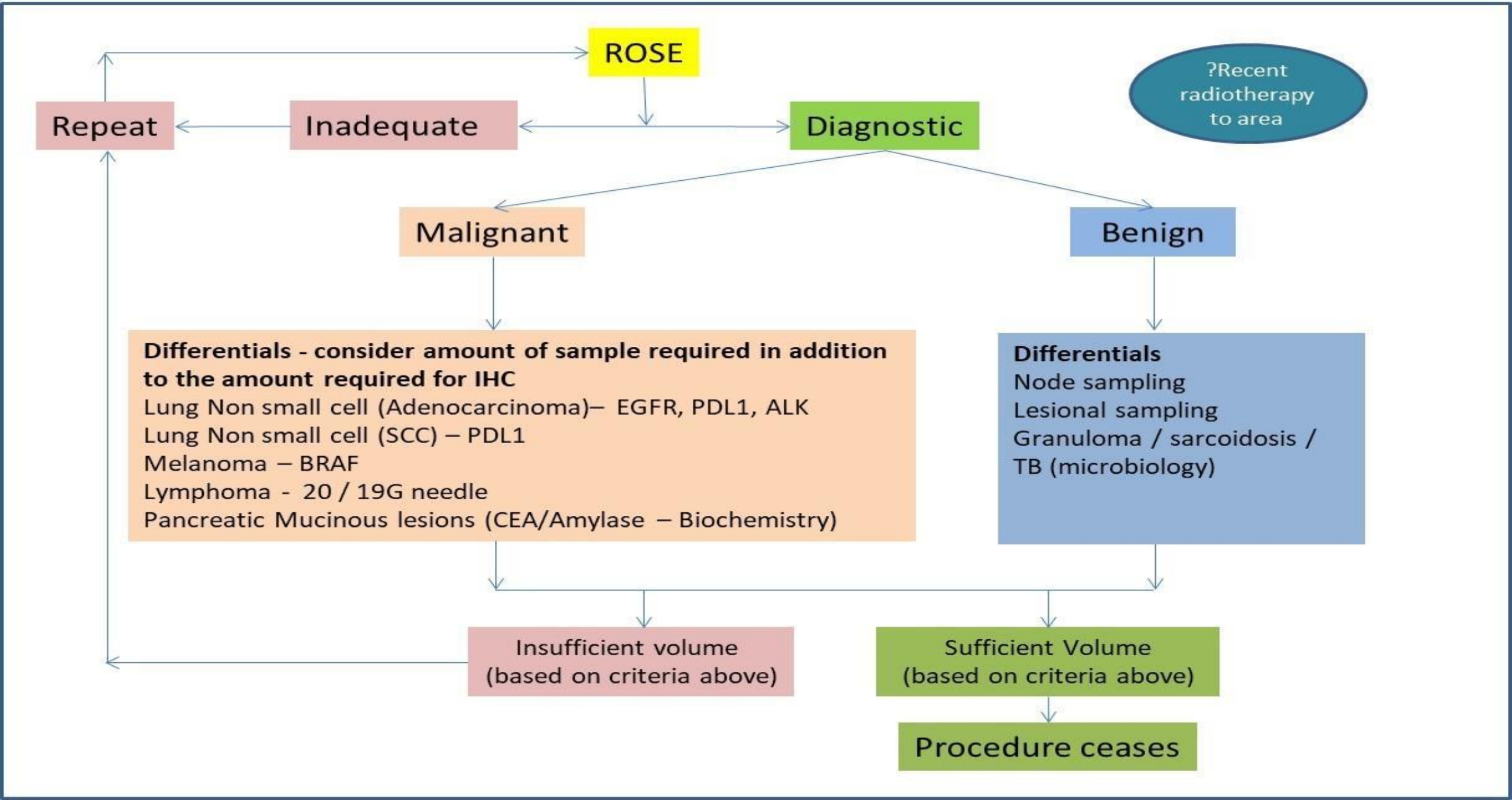


(A) Smears stained via giemsa stain on slides. **(B)** Background colloid. FNAC thyroid shows abundance of colloid with hemosiderin-laden macrophages. **(C)** Excessive dwell time during a fine-needle aspiration can lead to clotted blood entrapping cellular material of interest which obscures cytomorphologic features.

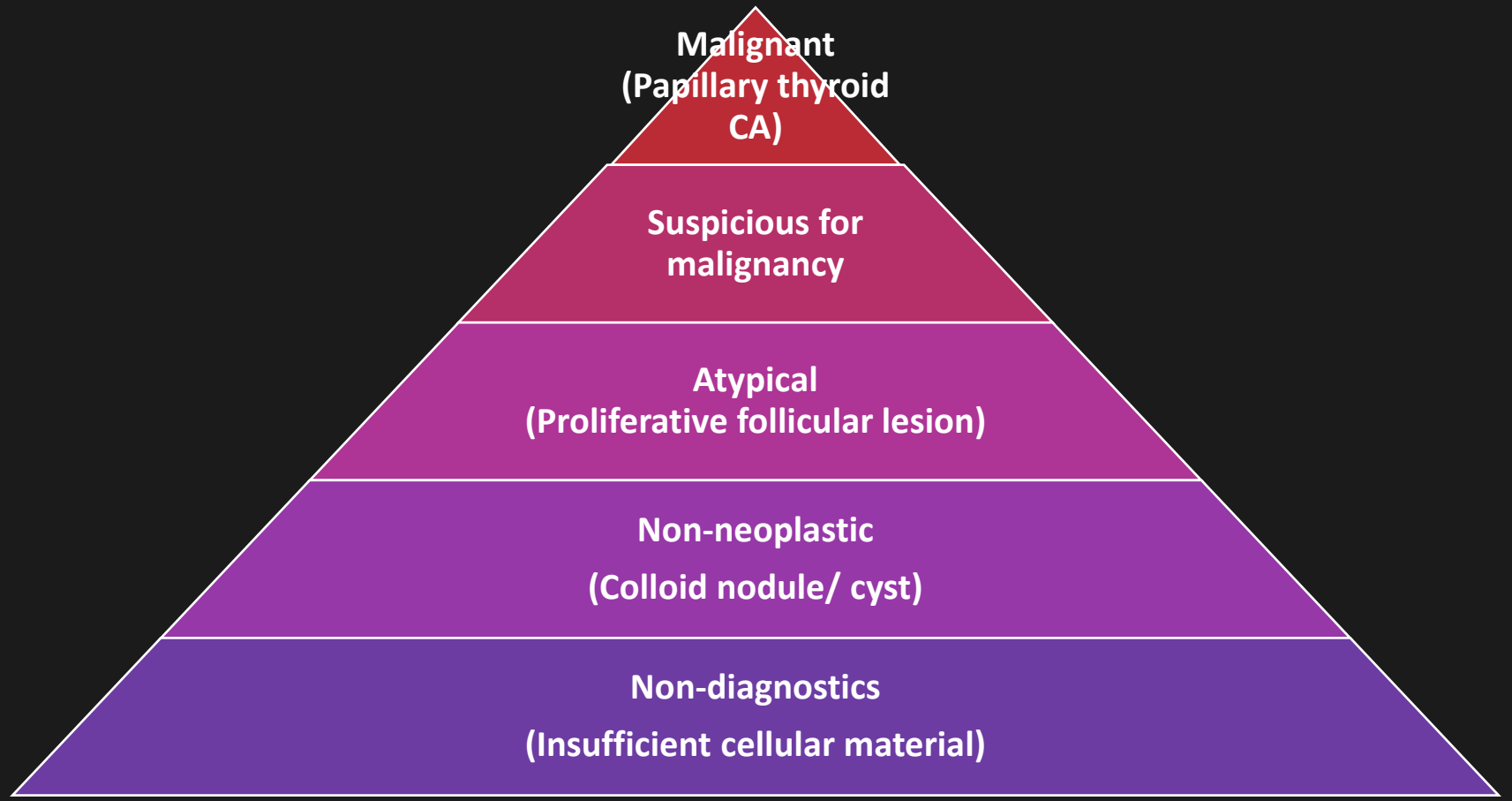


Schematic diagram to show purpose and algorithmic flow of rapid on-site evaluation (ROSE) for endobronchial ultrasound–guided transbronchial needle aspirate (EBUS-TBNA) specimens. Abbreviation: Pap, Papanicolaou.

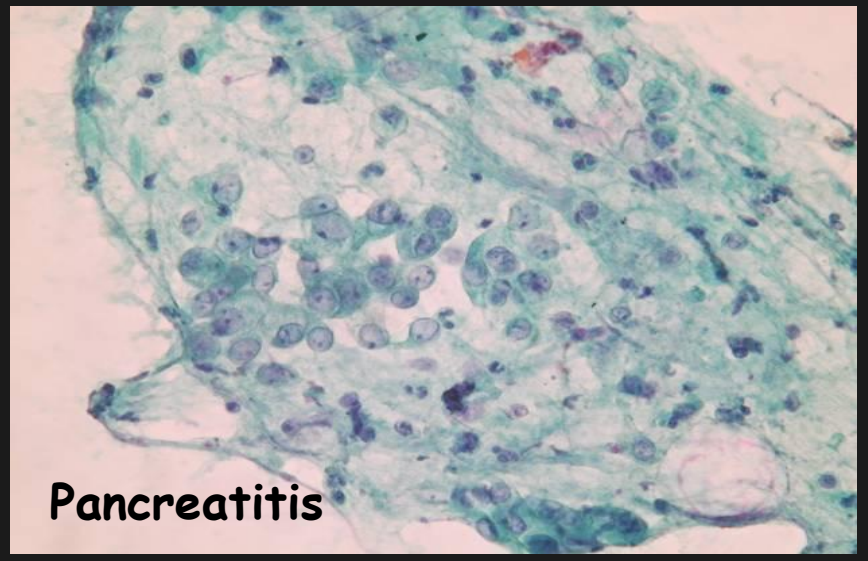
ROSE assessment criteria for amount of sample required in addition to immunocytochemistry.

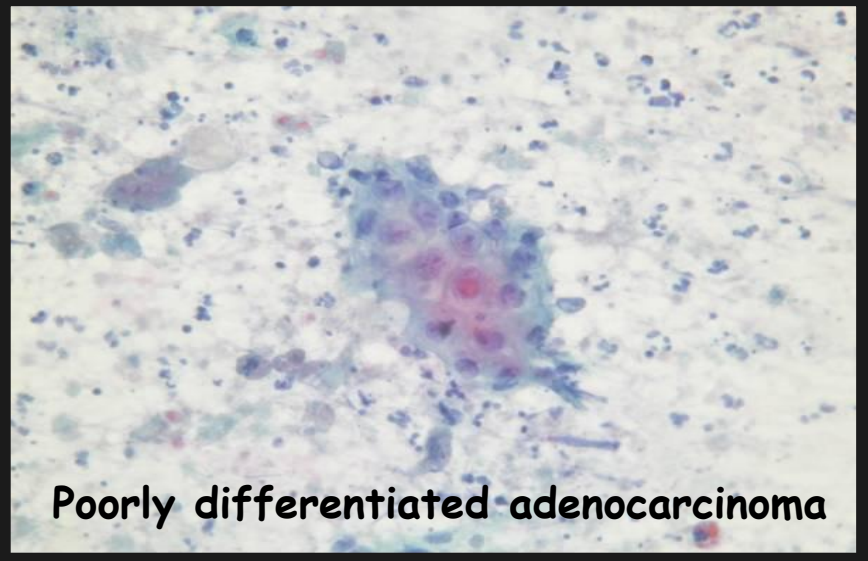


ROSE Interpretation algorithm



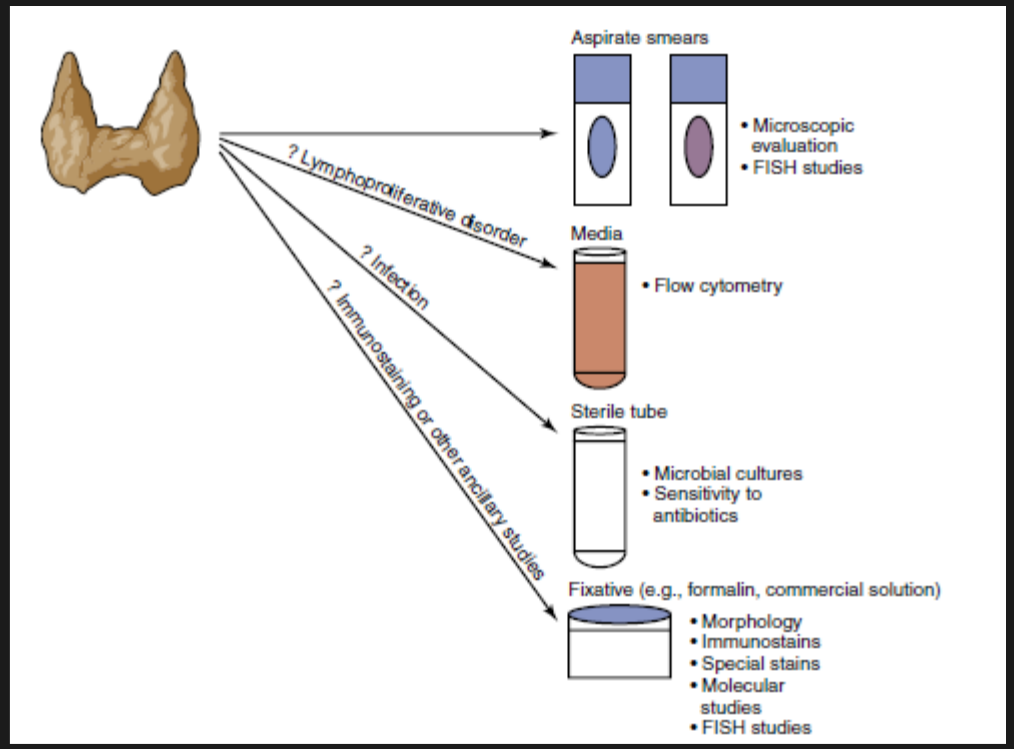
Differential diagnosis





Ancillary studies

- Immunocytochemistry
- Molecular studies (mutations, translocations)
- Flow cytometry
- Microbiological (cultures)



Basis of ROSE Interpretation

- The interpretation and comprehensive analyzing of ROSE should not be carried out until the specimen is obtained precisely from target lesions.
- Otherwise, the ROSE interpretation is worthless or even misleading clinical decision.
- If target lesion is not obtained, interventional modes and modalities should be modified to attempt repeatedly with the help of ROSE.

Microscopic interpretation. Cytologists need to be meticulous in their scrutiny of the slide and objective in its interpretation. Shown here is a weekly discussion around the multi-head microscope, which contributes to cytology training

Specific terminology during ROSE

- Inadequate sampling → repeat sampling (if the clinician deems it's safe)
- Adequate sampling → stop procedure
- Diagnostic sampling but more material required → repeat sampling (if the clinicians deems it's safe)
- Diagnostic sampling and sufficient cell yield → Stop procedure
- For patient safety, cytotechnologists are not permitted to give a diagnostic report on the ROSE sample. The role of ROSE is to provide enough information for sample triage not patient management (for example, a clinician may ask if they can refer to oncology or not based on the ROSE. The clinician must wait for the authorized report).

Criteria for ROSE recommended by WHO

- Discuss the case with the clinician about the area of interest and sampling and route to sampling (contaminant information).
- Be ready with relevant imaging, clinical history and clinical suspicion of malignancy.
- Record this information obtained.
- Do not allow this information to bias your assessment of the sample.
- Be aware of the diagnostic pitfalls and risks such as recent radiotherapy, contamination due to route of needle, inflammatory changes, pancreatitis.

- Key communication points between cytotechnologist and surgeon include:
  - The presence of blood in the syringe in aspiration
  - The feedback regarding the volume of sample obtained
  - The feedback regarding the description of sample obtained
  - The feedback regarding more or less suction being required for the next pass
  - The ROSE assessment - inadequate, diagnostic and or enough material
  - Ensure the clinician gives you sufficient time to assess the sample
- Good communication helps to ensure that the sample is collected in the best way for ease of preparation and secures sufficient time to assess the sample.

Health & Safety considerations

- Ensure that you are not standing too close to the clinician on removal of the EUS/EBUS guide wire. This lead to risk of injury to eyes.
- When walking around the endoscopy clinic be aware of the trip hazards, wires from equipment and suction tubes.
- Ensure you wear the appropriate personal protective equipment.
- Be aware of sharps and local needle stick injury policy.
- Be aware of local infection control policy.

Training & competency assessment

Laboratory training

- Diagnostic interpretation.
- Diagnostic pitfalls
- How to review the slide efficiently
- Speed review sessions
- Review of the cases from previous week

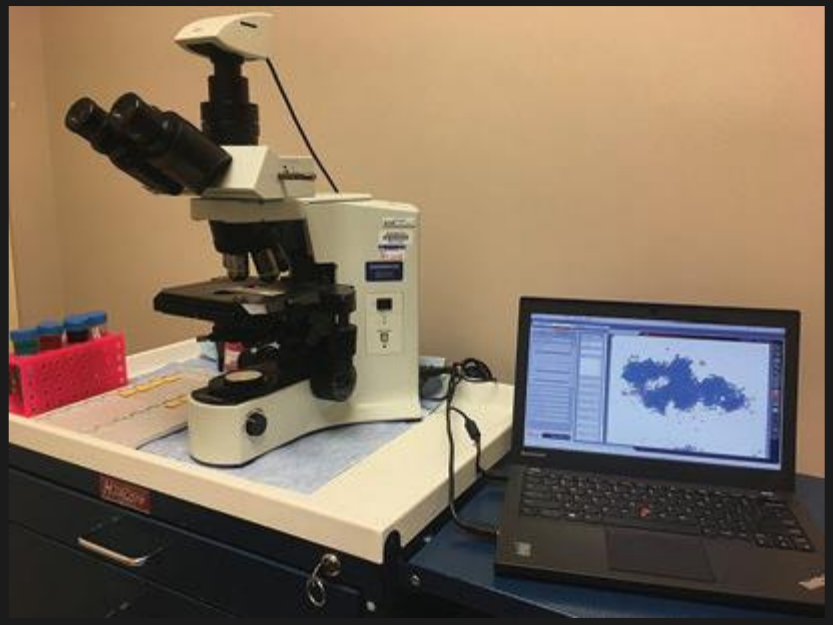
Competency assessment

- Terminology
- Awareness
- Communications with clinical team
- Limitations of practice
- Building relationships, and trust and confidence from the clinical team

An effective training plan is vital in ROSE. The individual needs to obtain the skills to feel confident in their decision making and remain calm in a pressured environment. Selection of staff with the right aptitude is crucial.

Pitfalls & challenges in ROSE

- Some hospitals or institutions may not have ROSE readily accessible.
- Amount of time ROSE can potentially take can be varied. The average amount of time per site sampled can range from 12 to 22 min, and often, more than one site is sampled.
- Tele-cytopathology can be employed as a potential time-efficient technique in busy cytopathology laboratories. Setup requires a microscope connected to a high-resolution digital camera, video software, secure intranet, access, and appropriate validation.



Setup for tele-cytopathology illustrating a microscope connected to a high-resolution digital camera equipped with appropriate video software and secure intranet access.

Advantages and disadvantages of ROSE.

Advantages

- Reduces the need for additional sampling (core-needle biopsies) with a lower risk of procedure
- Cost-effective (fewer ancillary techniques)
- Improves the adequacy rate and overall diagnostic yield
- Assists further diagnostic triage
- Decreases the number of passes
- needed for an adequate sample

Disadvantages

- Need for an experienced on-site cytopathologist
- Equivocal on-site diagnosis may prematurely end a procedure
- Relies solely on morphology
- Need for optimal clinical-pathologist communication
- Need for optimal staining quality

References

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Thank You!

- Results of studies vary depending on clinical setting and investigators (proceduralists, pathologists).
- **Adequacy**: if a sample provides sufficient material for a diagnosis.
- **Diagnostic yield**: rate at which a diagnosis is made.
- **Accuracy**: concordance between cases in which a diagnosis is rendered and a gold standard.

Once the needle is in the nodule, vacuum is generated in the syringe and sampling begins.