# Optimal preparation of the cytological specimens for future molecular testing – our experience at the Lady Davis Carmel Medical Center

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In the era of personalized medicine, an accurate detection of the molecular aberrations that are associated with cancer is crucial for patients management.

This molecular information is being increasingly exploited to treat advanced stage lung cancer patients with tailored, targeted therapy.

During the management of these patients, minimally invasive procedures to obtain samples for tissue diagnoses are desirable.

Technological advances in imaging modalities and instruments such as computed tomography and endobronchial ultrasound (EBUS) have facilitated accurate and precise sampling of the latter.

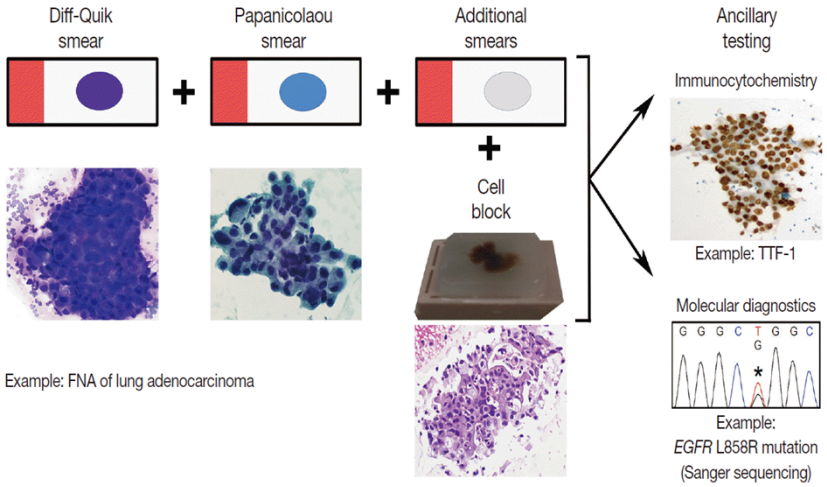
Cytological fine-needle aspirates are often utilized for this purpose and are important not only for rendering diagnoses to subtype patients' lung cancers, but also for ascertaining molecular diagnostic information for treatment purposes.

One commonly held misconception is that FNAs are generally insufficient for the performance of molecular assays.

However, it has been demonstrated that cellularity on the order of 100–500 cells are sufficient for DNA sequencing-based assays .

For fluorescence *in-situ* hybridization (FISH) assays, 100 analyzable tumor cell nuclei are generally sufficient.

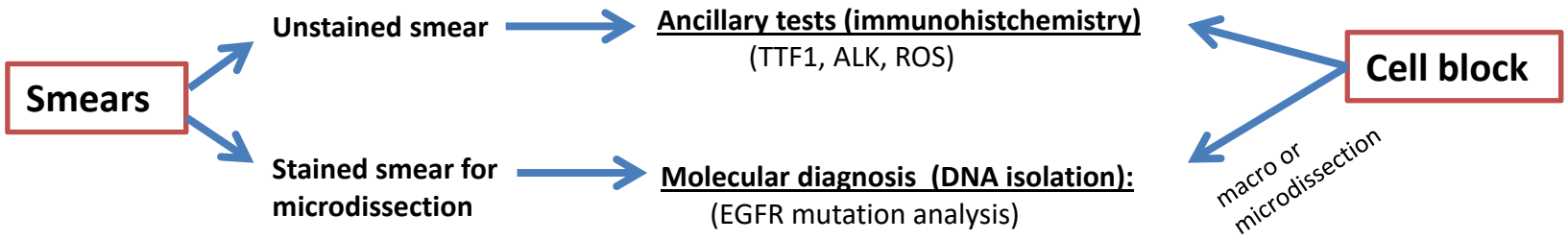
This is an example of fine-needle aspiration (FNA) processing workflow (Michael H. Roh, Ann Arbor, Michigan University, USA):



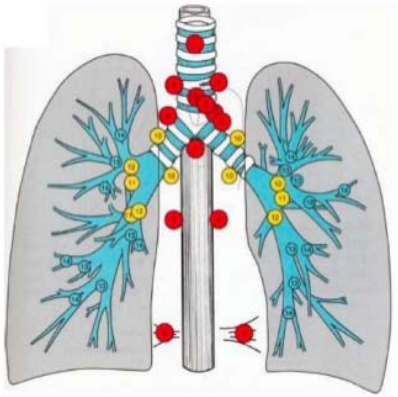

- The contents of a needle pass, obtained during an FNA procedure, are expelled onto a slide to prepare smears. Typically, a pair of smears (one Diff-Quik stained and one Papanicolaou stained) is prepared per needle pass.

- Additional smears can be prepared from a single needle pass by distributing the cellular material across more than two slides

- A cell block can also be prepared



**Our experience at the Carmel Medical Center, with FNA hilar or mediastinal lymph node biopsies**



Eight years ago, Dr. Abramowitz Amir (Pulmonary Medicine, Carmel Hospital) started to sample hilar or mediastinal lymph nodes, using *Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration* (*EBUS-TBNA*).

The tight collaboration between Dr. Abramowitz and our cytology lab technicians was established, building a learning curve in order to finally obtain high quality cytological material for an accurate diagnosis.

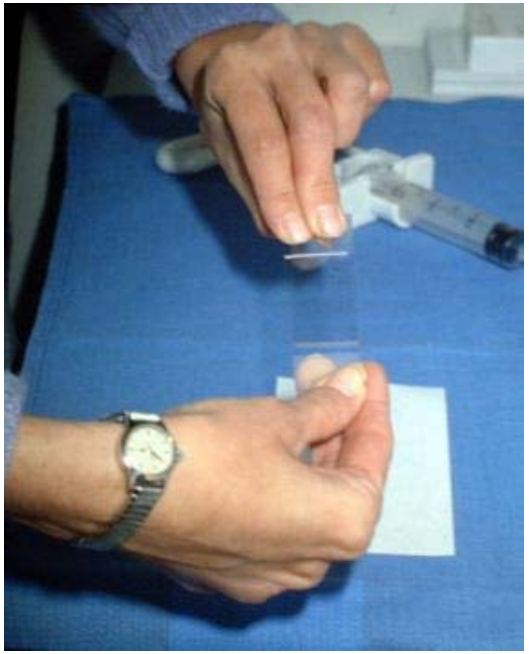
During this collaboration, we have learned that best results were obtained from concentrating the FNA content in a cell block, rather than using smears on the slides.

Cell block cytology was successfully used for ancillary tests (immunohistochemistry) and also provide sufficient material for molecular analyses.

**Our recommendations:**

**The clinician who performs the FNA**

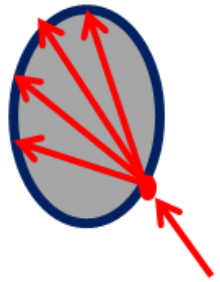
**The cytotechnican who processes the biopsy**



## Our recommendations to the clinician who performs the FNA:

1. It is important to focus on lymph nodes that are not vascular rich or necrotic.
   - Necrosis is not suitable for immunohistochemical or molecular analysis.
   - Smaller lymph nodes may be better (less necrotic).
   - U.S helps to detect more suitable lymph nodes)

2. Improve the aspiration technique:
   - the needle should reach the tumor inside the lymph node. In order to achieve this, the needle should cross the lymph node, from one capsular site to the opposite capsular site
   - In order to reduce sampling error and bloody samples, use a "fanning technique"
     Fanning, consist in modifying angle of needle insertion into the lesion, by changing either elevator position, or great wheel rotation, in order to sample tissue from different sector of the lesion. The fanning method assures directing the needle to different parts of the lesion with each pass may increase the quality of the sample. Advancing the needle repetitively through the same tract may result in bloodier samples with decreased quality.



3. General recommendation for number of passes (ERCP and EUS, A case based aproach. Linda S. Lee):
   - Lymph node: 3-4 passes (if molecular analysis is needed: 4 passes)
   - Other: Mass: 4-6 passes, Subepithelial lesions: 5 passes, Liver lesions: 2-3 passes

4. Concentrate all FNA samples in a formalin container and send to the cytology lab.

5. Attach a detailed clinical report including biopsy site and presumed diagnosis are mandatory (including the need for molecular analysis).

## Our recommendations to the cytotechnicians who process the FNA:

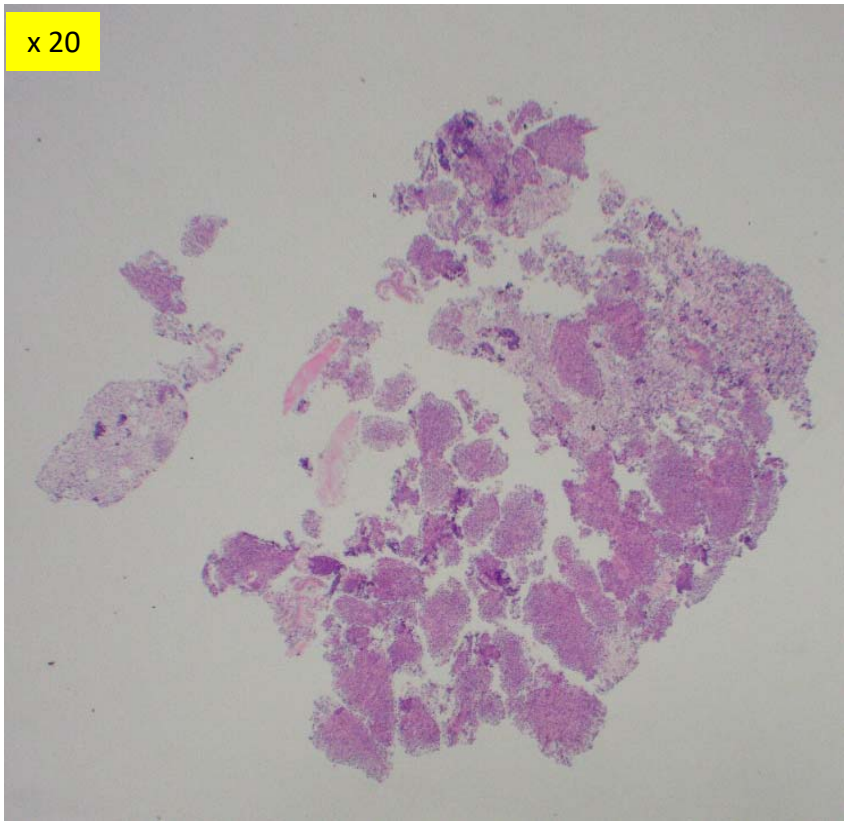
1. After identification and registration of the sample, the content is collected by a pipette into the formalin contained and submitted centrifuged for 10 minutes.

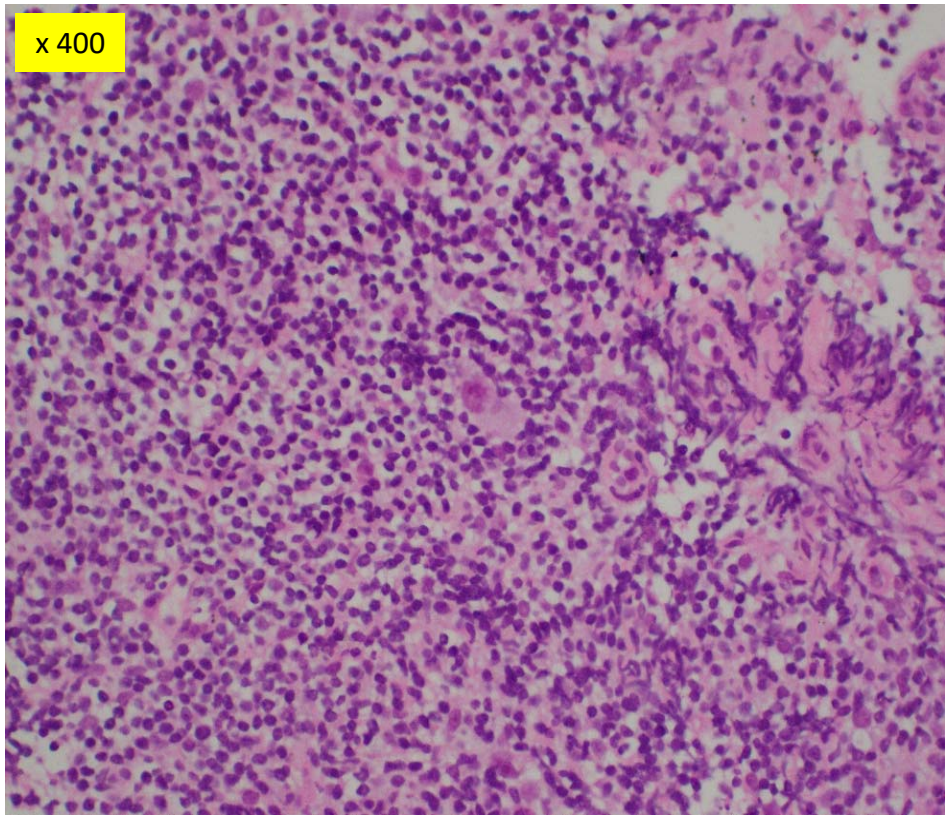
2. The sediment is introduced into a cassette .

3. A paraffin block is made and an HE stained slide is performed.

4. Based on the clinical information, immunohistochemistry is performed if needed, saving as much material as possible for the molecular analysis if required. In this respect, the clinical information is crucial.

High quality cell blocks:




(Dr. Abramowitz and Meira)

Currently at the Carmel Medical Center

When suspicious for lung neoplasia and FNA was performed, the cell blocks provide sufficient diagnostic material in almost 100% of the cases and in most cases material was also sufficient for a molecular analysis.

When suspicious for sarcoidosis , in over 90% of the biopsies, the cell blocks provide sufficient diagnostic material allowing to see well preserved granulomas.

Thank You !