

Molecular testing from stained slides



Diagnostic challenges in lung cancer cytology 17.6.18

Hanoch Goldshmidt. PhD, MBA

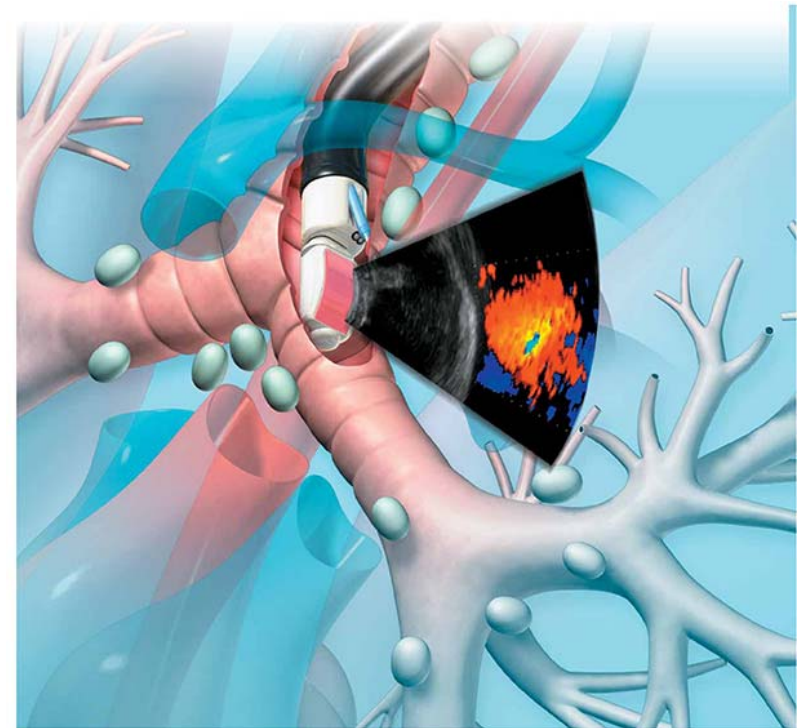
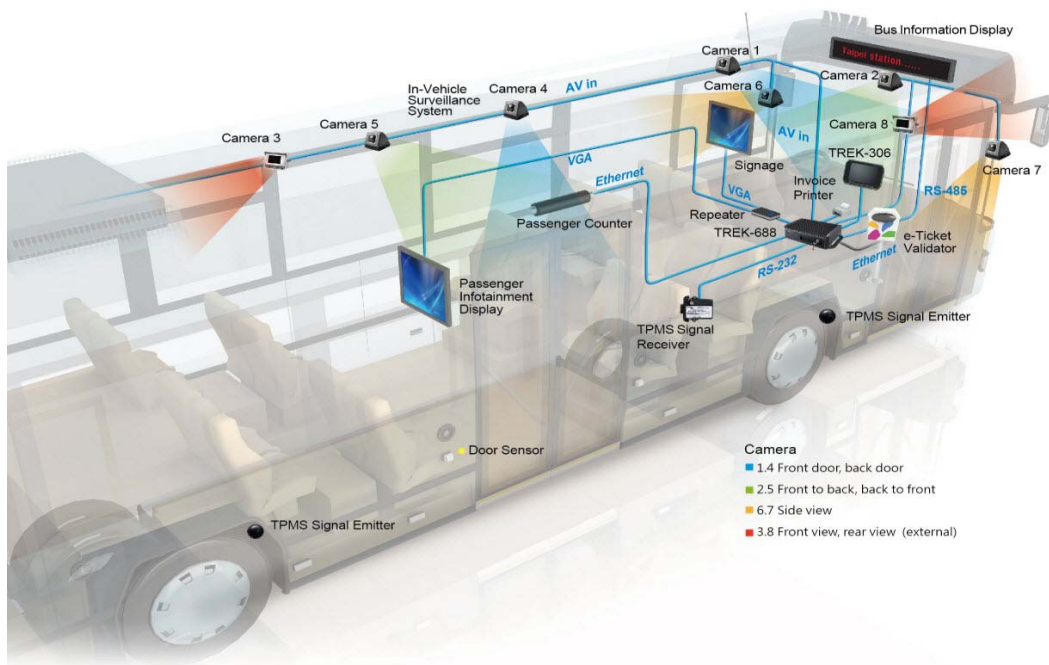
TASMC

WHAT IS ROSE?

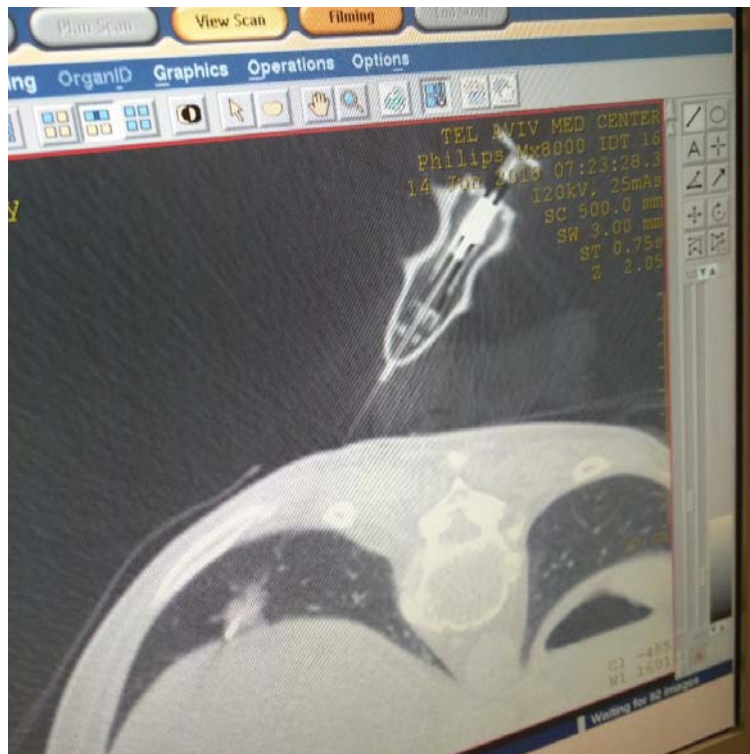
- **Rapid on-site evaluation, or ROSE, is a service that pathologists and cytotechnologists commonly perform to check the cellular adequacy of fine-needle aspiration smears and cell block preparations.**
 - What for?
 - inform the operator to avoid having to repeat the procedure
 - Preliminary diagnosis
- Additional samples and material can be requested



EBUS - endobronchial US



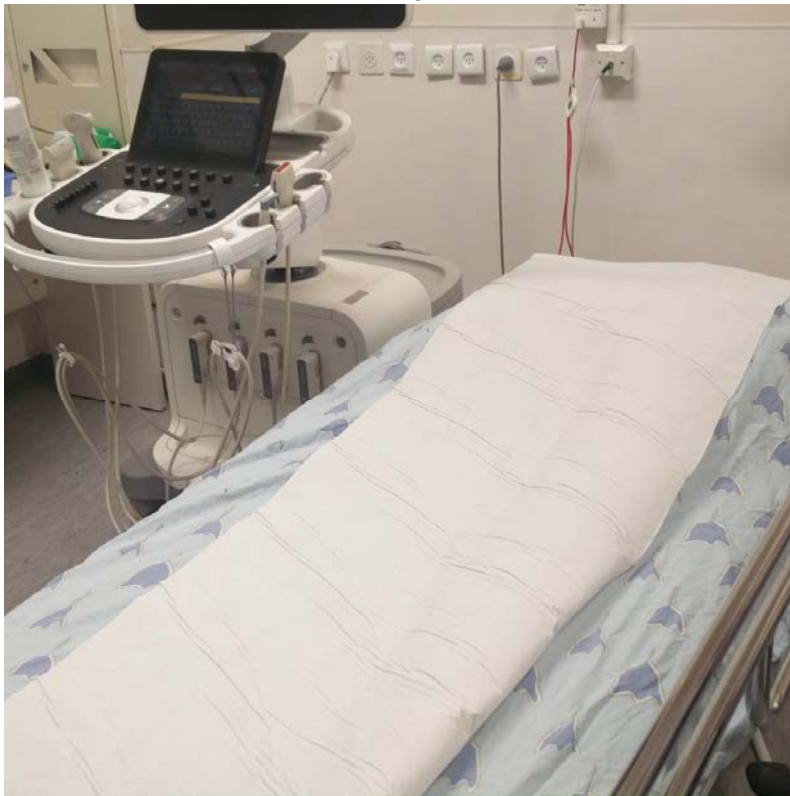
CT-guided



TASMC ROSE PROCEDURE:

Prepare the instruments

US



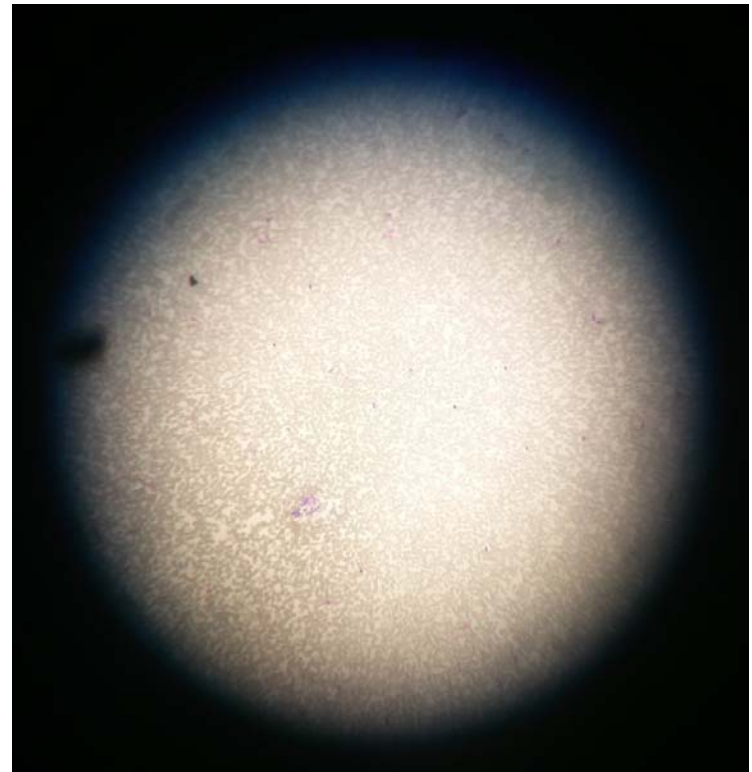
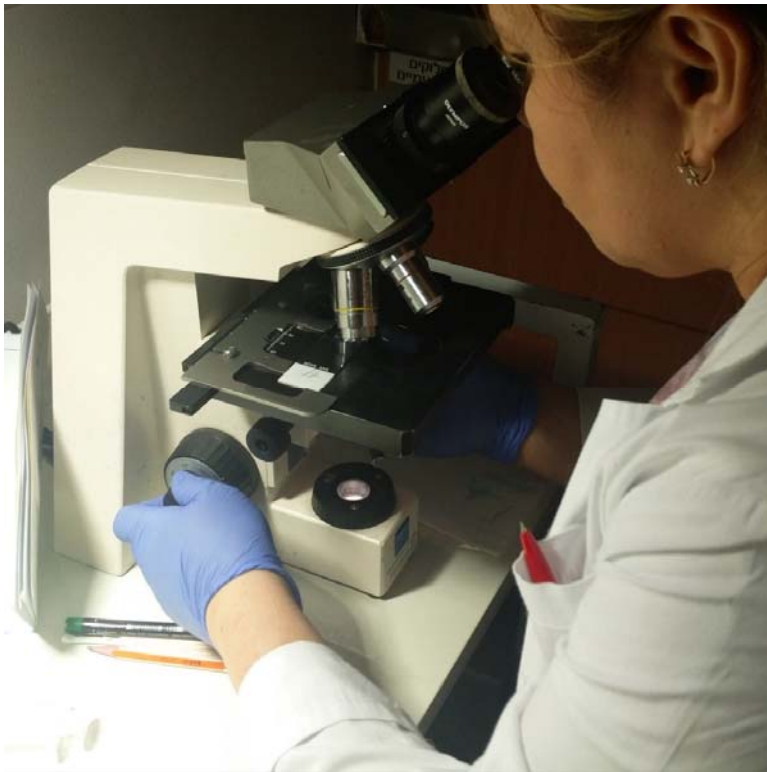
Prepare the slides



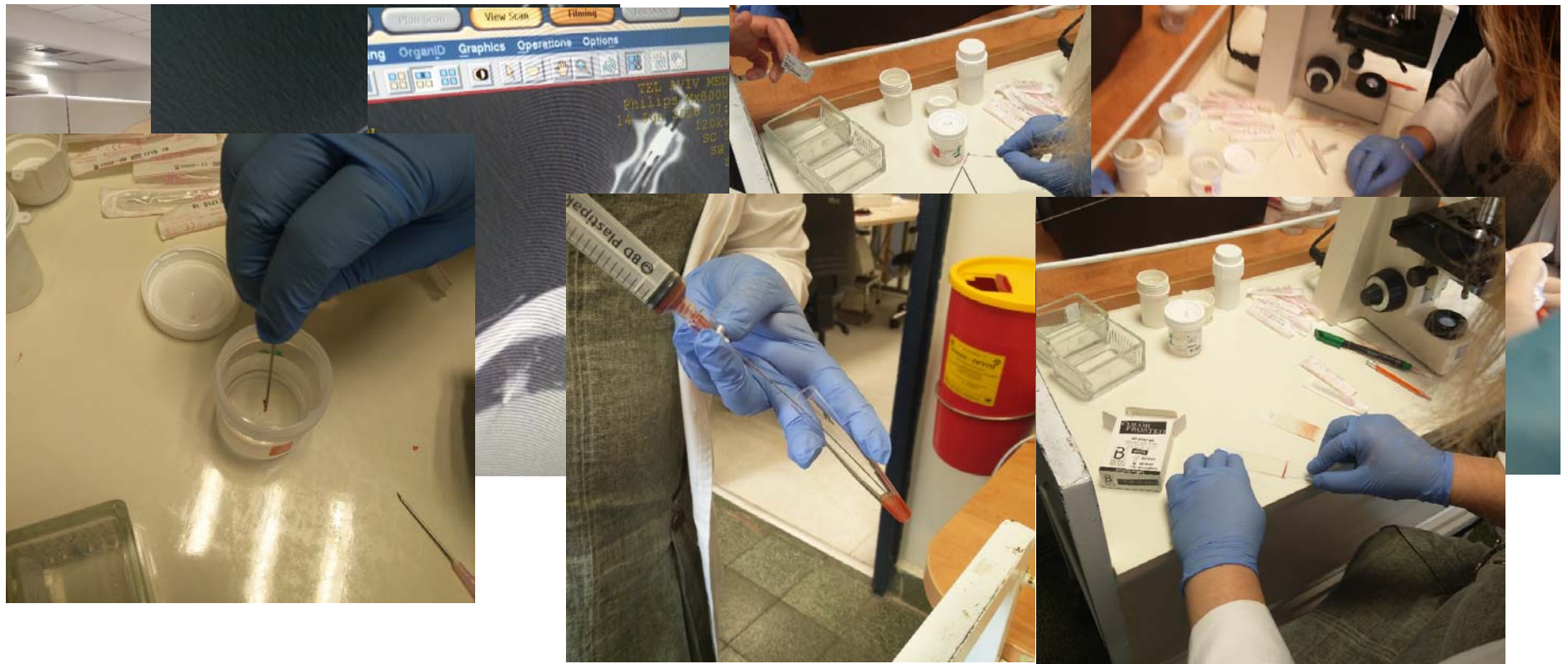
DRY and stain using Romanowsky Quik Diff protocol



Diagnose and discuss with physician if another biopsy is needed. Save slides for further molecular tests



ROSE during CT-guided CNB / FNA



Evolution of a rapid onsite evaluation (ROSE) service for endobronchial ultrasound guided (EBUS) fine needle aspiration (FNA) cytology in a UK Hospital: A 7 year audit



Tracey Stevenson¹  | Manish Powari² | Christopher Bowles³

- “good purity of tumor DNA in cytology FNA samples when compared with biopsy samples”
- “DNA extracted from EBUS sourced cases (n =50) gave on average twice the amount of DNA as that extracted from FFPE (n =325) (16 vs.8 ug total DNA, measured using Nanodrop)”
- “DNA from FFPE required 1.6 amplicon repeats per patient to obtain a result whereas DNA from EBUS cytofluid material required 0.46 amplicon repeats per patient”



TABLE 7 Average concentrations of DNA extracted from EBUS, effusions, and FFPE material

Sample type	Number	Average concentration (ng/ μ L)	Max concentration (ng/ μ L)	Min concentration (ng/ μ L)	Average reflex test rate (1 in)	Average amplicon retest (amplicons per patient)
FFPE	325	161.5077846	983.5	0	10.8	1.6
EBUS cytofluid	50	320.86	926.3	18.2	25	0.46
Effusion fluid	51	573.46	977.5	41.85	12.75	1.1
FNA other	10	272.73	852.3	16.11	10	0.8

Adequacy of Core Needle Biopsy Specimens and Fine-Needle Aspirates for Molecular Testing of Lung Adenocarcinomas



Frank Schneider, MD, Matthew A. Smith, MD, Molly C. Lane, Liron Pantanowitz, MD, Sanja Dacic, MD, PhD, and N. Paul Ohori, MD

From the Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.

Key Words: Fine-needle aspiration; Core needle biopsy; Lung cancer; Adenocarcinoma; Adequacy; Molecular testing

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- “When paraffin-embedded tissue is used for molecular testing of lung cancer, CNB specimens are more likely than FNA specimens to provide adequate tissue for molecular testing.
- Obtaining a sufficient FNA specimen depends on the tumor size and the individual performing the biopsy”

Improving Adequacy of Small Biopsy and Fine-Needle Aspiration Specimens for Molecular Testing by Next-Generation Sequencing in Patients With Lung Cancer

A Quality Improvement Study at Dartmouth-Hitchcock Medical Center

Vijayalakshmi Padmanabhan, MD; Heather B. Steinmetz, BS; Elizabeth J. Rizzo, BS; Amber J. Erskine, BS; Tamara L. Fairbank, BS; Francine B. de Abreu, PhD; Gregory J. Tsongalis, PhD; Laura J. Tafe, MD

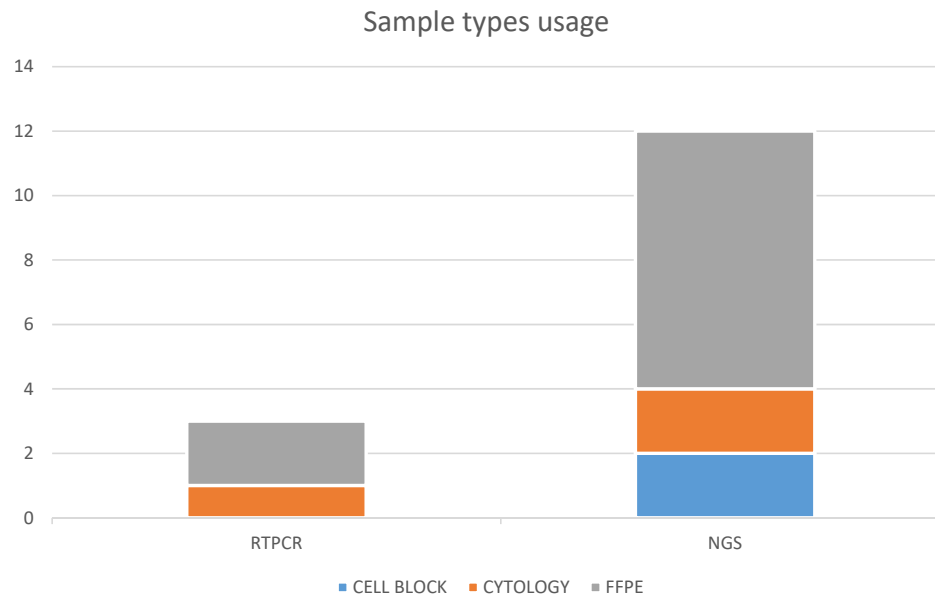
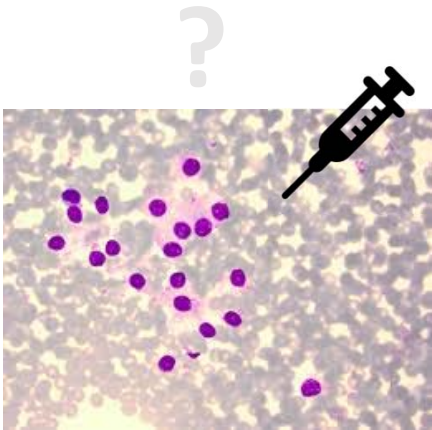
“During the study period, FNA samples were consistently more adequate for molecular testing than CT-guided NCB samples, as seen in Tables 2 and 3, unlike the study by Schneider et al.”

“Many variables are likely to play a role, including type of lesion; operator experience and the material itself”



Using cytology samples for molecular diagnosis TASMC

2018 - Cases that we extracted cytology and FFPE DNA samples



DNA concentration and Quality

Quantity

	Cytology concentration (ng/ul)	FFPE concentration(ng/ul)
median	7.5	35.5

Quality

dsDNA/total DNA rate	ng/ul-qubit	nanodrop-ng/ul	sample	extraction date
40%	17.20	43	c17-03699	5.12.17
4%	0.08	2	c18-01233	25.3.18
5%	0.48	9	c18-09185	7.5.18
17%	8.35	49	18-02045-1	12.3.18
43%	9.92	23.3	18-06799	20.3.18
14%	4.03	29	18-00468	26.2.18

- Data from cases with FFPE tissue + cytology



conclusion

- Rapid onsite evaluation is a service that prevents repeating procedures
- Rapid onsite evaluation provides suitable material for molecular testing including NGS and RT-PCR.
- When lung cancer material becomes so crucial for so many tests, we should have good procedures to utilize all the available samples, including cytology smears
- Factors to consider:
 - amount of material
 - Percentage of tumor cells
 - quality of DNA from these samples



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