2nd BOSNIAN TURKISH CYTOPATHOLOGY SCHOOL

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ORGANIZED BY:

Turkish Division of the International Academy of Pathology (TDIAP)
Turkish Society of Cytopathology
Department of Pathology, Clinical Centre of the University of Sarajevo, Bosnia and Herzegovina

LUNG CYTOLOGY
Predictive markers and molecular tests

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ipatimup

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

No financial disclosures

Pathologists' role

Historically	2016
• Guiding the surgeons' hands	• Oncologists' best mate
• Pattern recognition	• Understanding disease
• Tumour burden	• Disease biology
• Basements (hiding behind a microscope)	• Integral part of the multidisciplinary team

Ancillary Studies in Cytology Challenges

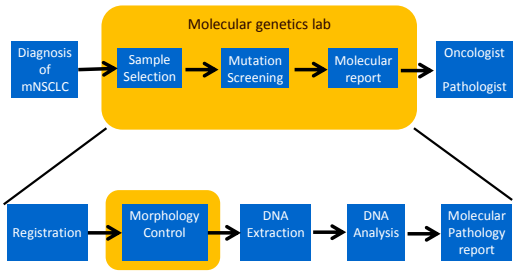
- To select the correct test for a limited sample quantity.
- Avoid jumping from a histological adapted technique directly to cytological material.
- Use appropriate controls for cytological material.

Pre-analytical issues

- The variability in material and fixatives is a major factor preventing standardization of some procedures using cytology.
- Spray and ethanol fixation results in better DNA quality than air drying (but both give reliable results in clinical specimens).
- Due the similarities with histological material, cell-blocks are more easy to handle on the molecular lab.

Dejmek A. Cancer Cytopathology 2013
Schmitt F. Cytopathology 2011

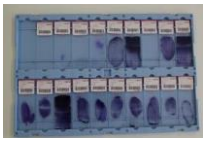
MOLECULAR TESTING WORKFLOW

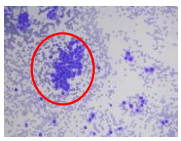


Simple protocol for DNA extraction from archival stained FNA smears

1. *Slide Selection and Assessment*
   . For cases with a high tumor content (>20%) the marking of areas of tumors is unnecessary. (1 cell = 6 pg DNA)

2. *Removing the Coverslip*
   . 48-72hs in xylene or substitute



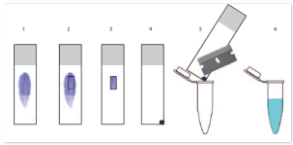


Enrichment by macrodissection if necessary.

Acta Cytologica

Simple protocol for DNA extraction from archival stained FNA smears

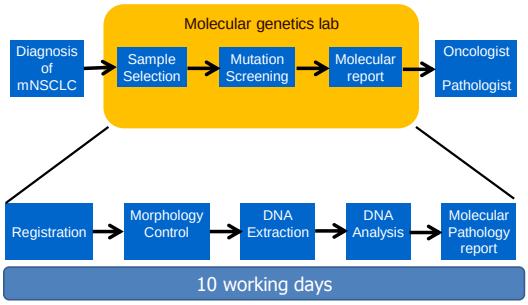
3. *Collecting the Tissue*



4. *Tissue Lysis and DNA Extraction*

Acta Cytologica 2012;56:632–635

EGFR mutation status testing workflow



Minimizing Delays in DNA Retrieval: The "Freezer Method" for Glass Coverslip Removal.

. The slide is placed flat in the freezer (at a temperature of -20ºC) for 1-3 minutes.

Santos GC et al. Cancer Cytopathology 2013

KEY POINTS TO USE MOLECULAR TECHNIQUES IN CYTOLOGY

- Collect good and well-preserved material.
- Validate molecular studies on cytological material.
- Control the cases morphologically.

Schmitt FC. Cytopathology 2011

Rapid On-Site Evaluation

- The increasing need to assess specific targetable mutations and/or genetic aberrations implicates in a detailed evaluation of the collected material with formulation of a preliminary diagnosis followed by extra passes or adequate triage.
- Only an experienced cytopathologist can consistently and correctly decide on handling of the specimens and failure to do so, may result in direct negative consequences to patient care.

Cancer Cytopathology 2012

THE PETHALS AND THORNS OF ROSE Disadvantages

- Need of an experienced on-site cytopathologist because relies only on morphology.
- Equivocal on-site diagnosis may prematurely end a procedure.
- Need of extra-time from the cytopathologist with financial under compensation of pathologist´s time.

Cancer Cytopathology 2012

International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

Journal of Thoracic Oncology • Volume 6, Number 2, February 2011

PATHOLOGY CONSIDERATIONS FOR GOOD PRACTICE

- Small biopsy and cytology samples should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

LUNG CYTOLOGY and TUMOR TYPING

TABLE 3. Sensitivity and Specificity of Cytologic Tumor Subtyping

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV,[a] % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
SCLC vs. non-SCLC	100 (29.2–100)	100 (97.9–100)	100 (29.2–100)	100 (97.9–100)	100 (98.1–100)
Squamous vs. nonsquamous	87 (66.4–97.2)	98 (94.6–99.6)	87 (66.4–97.2)	98 (94.6–99.6)	97 (93.2–98.6)
Adenocarcinoma vs. nonadenocarcinoma	98 (93.9–99.6)	79 (63.2–89.7)	94 (88.7–97.2)	92 (77.5–98.3)	93 (89.1–96.3)

- Cytology is a powerful tool in the diagnosis of lung cancer, in particular in the distinction of adenocarcinoma from squamous cell carcinoma.

J Thorac Oncol. 2011;6: 451–458

LUNG CYTOLOGY and TUMOR TYPING

TABLE 5. Frequency and Accuracy of Morphologic vs. Immunohistochemistry-Aided Diagnoses of Adenocarcinoma and Squamous Cell Carcinoma in Cytology

	Frequency	Accuracy
Diagnosis based on morphology	146 (88%)	96%
Diagnosis based on IHC	14 (9%)	100%
Diagnosis that needs IHC but cellularity insufficient (diagnosis: NSCLC-NOS)	5 (3%)	na

J Thorac Oncol. 2011;6: 451–458

Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

Fernando Schmitt, MD, PhD[1,2,*], José Carlos Machado, md[3]

Table 2
Common immunocytochemistry markers used in lung cytology

	Markers					
Lung Cancer Subtype	TTF-1	Napsin A	P63	P40	CK5/6	Neuroendocrine Markers
ADC	+/–[a]	+	–/+	–	–	–
SqCC	–	–	+	+	+	–
SCLC	+	–	–	–	–	+
NSCLC-NOS	+/–	–	–	–	–	–
Metastatic carcinoma	–/+	–	–/+	–/+	–/+	–/+

[a] Especially mucinous ADC.

Review

Acta Cytologica 2015;59:5–6
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p40: A p63 Isoform Useful for Lung Cancer Diagnosis – A Review of the Physiological and Pathological Role of p63

Ana Rita Nobre[a, b] André Albergaria[a, c] Fernando Schmitt[a, c]

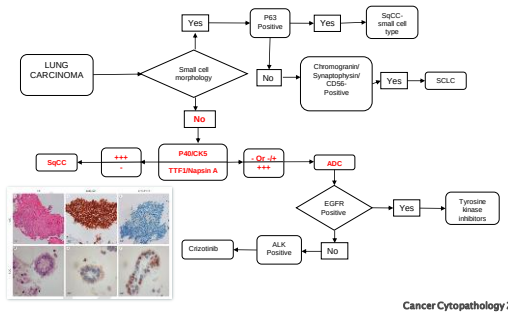
Table 1. Distribution of TTF-1, p63, and p40 in lung cancer

	Histological diagnosis					SE	SP	PPV	NPV	Ref.
	ADC	SCC	LCC/SC	ADSC	LCL					
TTF-1	110/130 (77)	0/50 (0)	n.a.	n.a.	n.a.	0.77	1.00	1.00	0.59	65
p63	27/110 (18)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.82	0.65	1.00	
p40	0/130 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1+/p63+	26/180 (14)	0/50 (0)	n.a.	n.a.	n.a.	0.14	1.00	1.00	0.25	
TTF-1+/p63–	133/180 (84)	0/50 (0)	n.a.	n.a.	n.a.	0.84	1.00	1.00	0.83	
TTF-1–/p63+	4/180 (2)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.98	0.93	1.00	
TTF-1–/p63–	35/180 (19)	0/50 (100)	n.a.	n.a.	n.a.	0.19	1.00	1.00	0.26	
TTF-1+/p40+	0/180 (0)	0/50 (0)	n.a.	n.a.	n.a.	0.00	1.00	0.00	0.22	
TTF-1+/p40–	141/180 (78)	0/50 (0)	n.a.	n.a.	n.a.	0.78	1.00	1.00	0.56	
TTF-1–/p40+	0/180 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1–/p40–	39/180 (22)	0/50 (0)	n.a.	n.a.	n.a.	0.00	0.78	0.00	0.78	
TTF-1	28/30 (93)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.93	1.00	1.00	0.83	66
p63	0/30 (16)	10/10 (100)	1/1 (100)	5/5 (100)	n.a.	1.00	0.76	0.33	1.00	
p40	5/30 (17)	10/10 (100)	0/1 (0)	5/5 (100)	n.a.	1.00	0.83	0.67	1.00	
TTF-1+/p63+	8/30 (27)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.27	1.00	1.00	0.31	
TTF-1+/p63–	20/30 (67)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.67	1.00	1.00	0.50	
TTF-1–/p63+	1/30 (3)	10/10 (100)	1/1 (100)	2/5 (40)	n.a.	1.00	0.97	0.91	1.00	
TTF-1–/p63–	1/30 (3)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
TTF-1+/p40+	0/30 (0)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.00	1.00	0.00	0.74	
TTF-1+/p40–	28/30 (96)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.93	1.00	1.00	0.83	
TTF-1–/p40+	1/30 (3)	10/10 (100)	0/1 (0)	2/5 (40)	n.a.	0.33	0.97	0.91	0.76	
TTF-1–/p40–	1/30 (3)	0/10 (0)	1/1 (100)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
p63	79/237 (33)	81/81 (100)	n.a.	n.a.	82/152 (54)	1.00	0.67	0.52	1.00	67
p40	7/205 (3)	81/81 (100)	n.a.	n.a.	0/152 (0)	1.00	0.97	0.92	1.00	
TTF-1	114/44 (77)	0/24	3/12	0/1	n.a.	0.77	1.00	1.00	0.62	69
p63	13/44 (28)	24/24 (100)	7/12	1/1 (100)	n.a.	1.00	0.80	0.65	1.00	
p40	1/29 (3)	23/23 (100)	3/12 (25)	1/1 (100)	n.a.	1.00	0.97	0.96	1.00	
Napsin A	11/29 (38)	0/13 (0)	1/12 (8)	0/1 (0)	n.a.	0.38	1.00	1.00	0.45	

Integrative data of the expression of TTF-1, p63, and p40 in subtypes of lung cancer and comparison of the sensibility and specificity of these markers for SCC. Values in parentheses are percentages. ADSC = Adenosquamous carcinoma; LCC = large cell carcinoma; LCL = large cell lymphoma; n.a. = not assessed; NPV = negative predictive value; PPV = positive predictive value; SC = sarcomatoid carcinoma; SE = sensitivity; SP = specificity; Ref = reference.

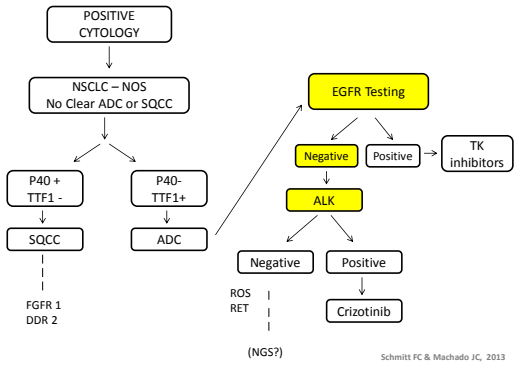
Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD[1,2] and Helena Barroca, MD[3]

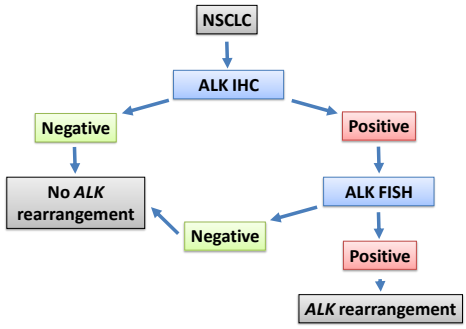


Cancer Cytopathology 2011

ALGORITHM FOR ANCILLARY TESTING IN NSCLC



Diagnostic algorithm for ALK testing

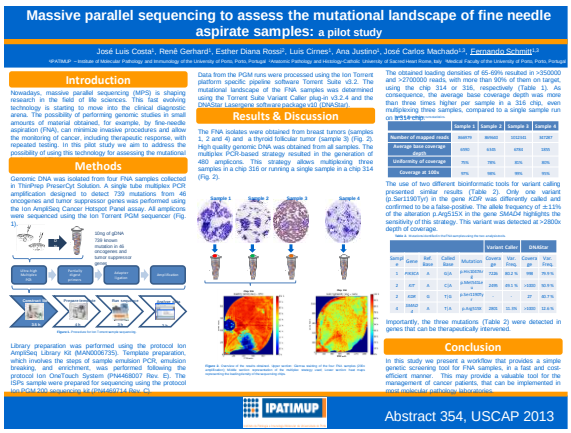


3 tier approach to NGS in oncology

- Clinical
  - Focused Panels detecting defined clinically relevant alterations (SNVs, CNVs, InDels, Fusion Transcripts)
- Translational
  - Broad Panels detecting a large number of defined alterations covering the drug development (oncology pipeline) spectrum (recruitment for clinical trials)
- Exploratory
  - Exome Sequencing (T/N)
  - RNAseq
  - WGS (T/N)

Genomic Analysis in Cytology

- Next generation sequence (NGS) has recently entered in the routine clinical molecular diagnostics by providing a highly multiplexed platform for simultaneous screening of multiple genes at a high analytical sensitivity with minimal amounts of DNA.
- Cytologic specimens offer a number of advantages in terms of molecular testing and minimally invasive procedures such as FNA are often used to establish a diagnosis.



Genomic Analysis in Cytology

- However, cytology specimens are still underutilized for molecular testing because most molecular testing requests are directed reflexively to the histology and cytology is used only when there is no concurrent core biopsy (CB) or the CB is insufficient for testing.
- The other issue is that molecular laboratories are not always validated all the different types of cytologic preparations (smears, cytospins, LBC, cell-blocks) and prefer to treat all specimens as they do with the FFPE histology blocks.

Pre-analytical challenges for genomic analysis in cytology

- Pre-analytical factors are determinant for the success of NGS sequencing and recognizing these factors can enable better processing or triaging of specimens to further improve NGS success.
- In cytology specimens, these factors may be especially important because of the wide variations in sample types, preparations and adequacy criteria.

Chen H. et al. Cancer 2015

Pre-analytical factors that affects NGS analysis in cytology

- Specimen cellularity.
- Type of preparation.
- Type of fixative and stains.
- Type of glass slides.
- Tumour fraction.
- DNA yield
- Input DNA

Chen H. et al. Cancer 2015

Role of the Cytopathologists

- There is a wide interobserver variation among cytopathologists in estimating tumor fraction, even with predefined adequacy criteria.
- Some cytopathologists demonstrate high cancellation rates, resulting in low NGS failure rates, whereas others have low cancellation rates with high NGS failure rates.
- The variation among cytopathologists in tumor fraction estimation and selecting cases for NGS is also seen in the selection of substrate (cell blocks versus smears) and the number an type of glass slides sent for testing.

Roy-Chowdhuri et al. Cancer Cytopathology 2015

Consistency and Reproducibility of Next-Generation Sequencing and Other Multigene Mutational Assays: A Worldwide Ring Trial Study on Quantitative Cytological Molecular Reference Specimens

Umberto Malapelle, Clara Mayo-de Las Casas, Miguel A. Molina, Rafael Rnell, Spasenija Savic, Michel Bihl, Lukas Bubendorf, Manuel Salto-Tellez, Dario de Biase, Giovanni Tallini, David H. Hwang, Lynette M. Sholl, Rajyalakshmi Luthra, Sinchita Roy-Chowdhuri, Birgit Weynand, Sara Vander Borght, Edoardo Missiaglia, Massimo Bongiovanni, Daniel Stieber, Philippe Vielh, Fernando Schmitt, Alexandra Rappa, Massimo Barberis, Francesco Pepe, Pasquale Pisapia, Nicola Serra, Elena Vigliar, Claudio Bellevicine, Matteo Fassan, Massimo Rugge, Carlos E. de Andrea, Maria D. Lozano, Fulvio Basolo, Gabriella Fontanini, Marina N. Nikiforova, Yuri E. Nikiforov, Suzanne Kamel-Reid, Gilda da Cunha Santos, Giancarlo Troncone for the Molecular Cytopathology Meeting Group

This interlaboratory ring trial study shows that next-generation sequencing and other multigene mutational assays are robust and accurate with cytological samples. In particular, the performance of laboratories using next-generation sequencing is excellent, regardless of the platform or gene panel type.

Cancer Cytopathology 2017

EDITORIAL DOI:10.1111/cyt.12015

Molecular diagnostics and the training of future tissue- and cell-based pathologists

STAGE A 0–12 months	STAGE B 12–18 months	STAGE C 24–30 months	STAGE D 12 months
Introductory lectures on molecular diagnostics	Compulsory 2–3 month attachment in molecular diagnostics	Compulsory 2–3 month attachment in molecular diagnostics	Option 1: One year full-time tissue molecular diagnostics (see Table 2) Option 2: One year 'superspeciality' attachment with part-time practice in a subspecialty and part-time reporting the related molecular tests Option 3: Mixture of diagnostics and research
Year 1 assessment at end-of-year to exit stage	Exit stage with FRCPath Part 1 examination	Exit stage with FRCPath Part 2 examination with/without modules in autopsy and gynaecological cytology (additional 3 months each)	End of training

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