Molecular Cytopathology
Part 2

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Key Objectives

- Cytology provides the versatility of specimen preparations that offer a variety of options for molecular testing
- A multitude of pre-analytical factors impact tissue quality and the success of molecular testing
- **The pathologist plays a key role in triage and specimen handling that can improve the success of molecular testing**

Key Element of Specimen Selection

Role of the pathologist in **specimen selection** is finding the best fit (molecular assay) for the sample

- **Modulate the specimen** to fit the assay
- **Modulate the assay** to fit the specimen

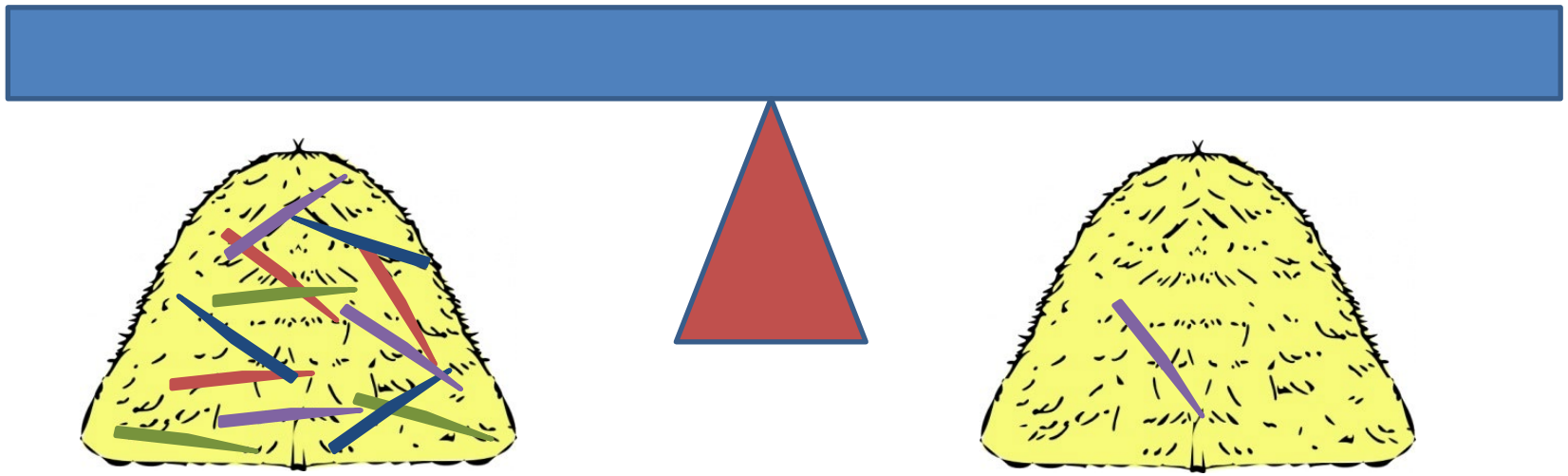
Mutation Analysis Assay Design is a Balancing Act

Most mutational assay design is a balancing act between **Clinical** and **Analytical** Sensitivity

Mutation Analysis Assay Design is a Balancing Act

Clinical Sensitivity:
How many of the possible changes does the test detect?

Analytical Sensitivity:
How sensitively can the test detect a rare change in a background of normal?

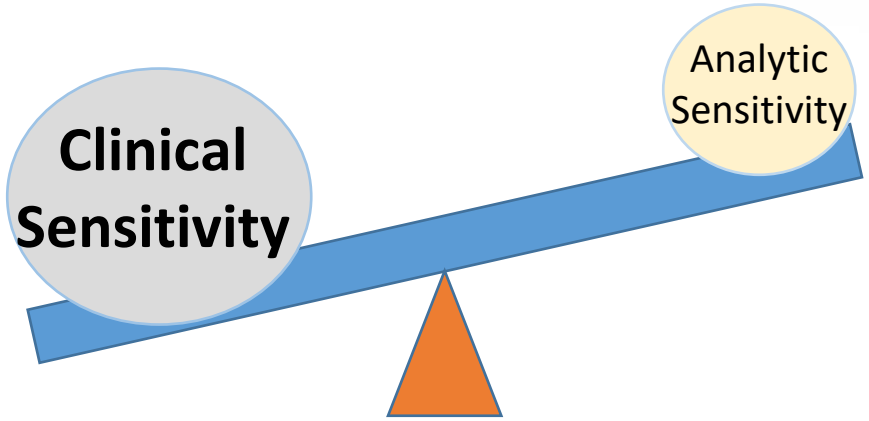


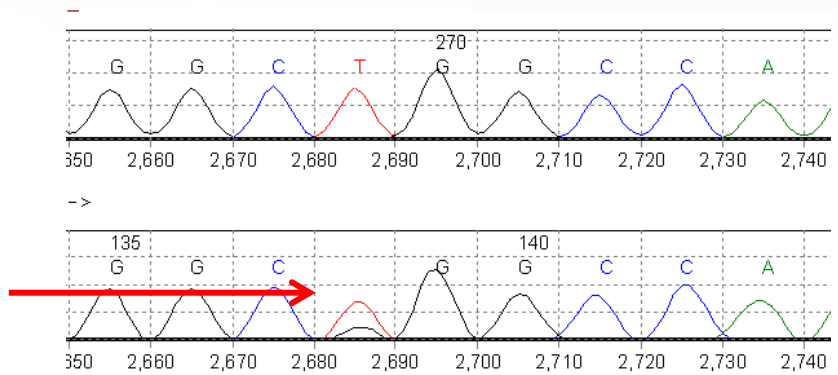
- The test can identify needles of many different colors, but need to exist at a relatively high level

- The test can identify only a few colors of needles, but can pick them out even when they are very rare

Pic courtesy: Dara L Aisner MD PhD, University of Colorado

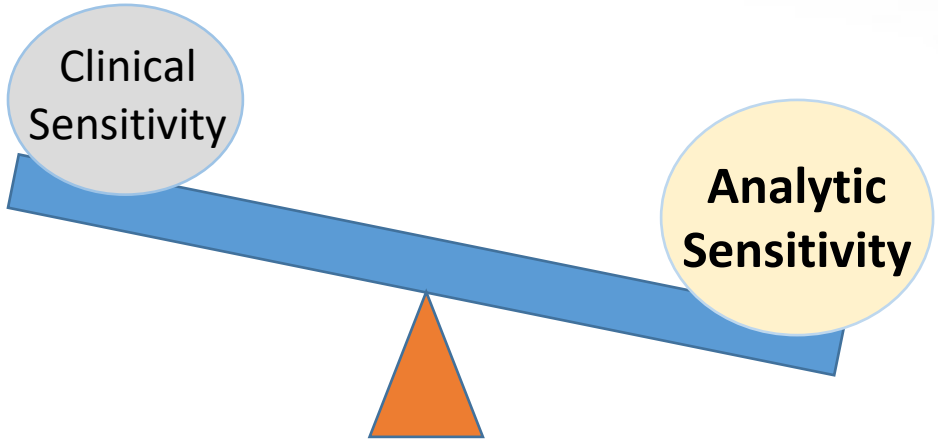
Sanger Sequencing
Low Analytic/High Clinical Sensitivity

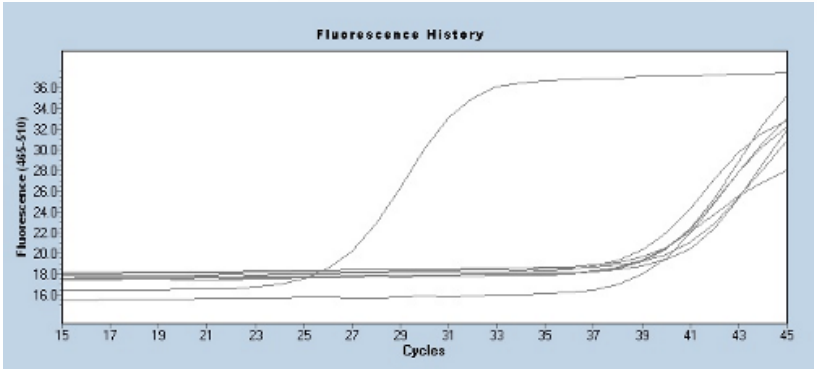




- High **clinical sensitivity**: Sanger sequencing should detect ALL potential changes
- **Analytic sensitivity** is among the lowest of all testing methods: ~15-20% of alleles need to be mutant to be detected reliably

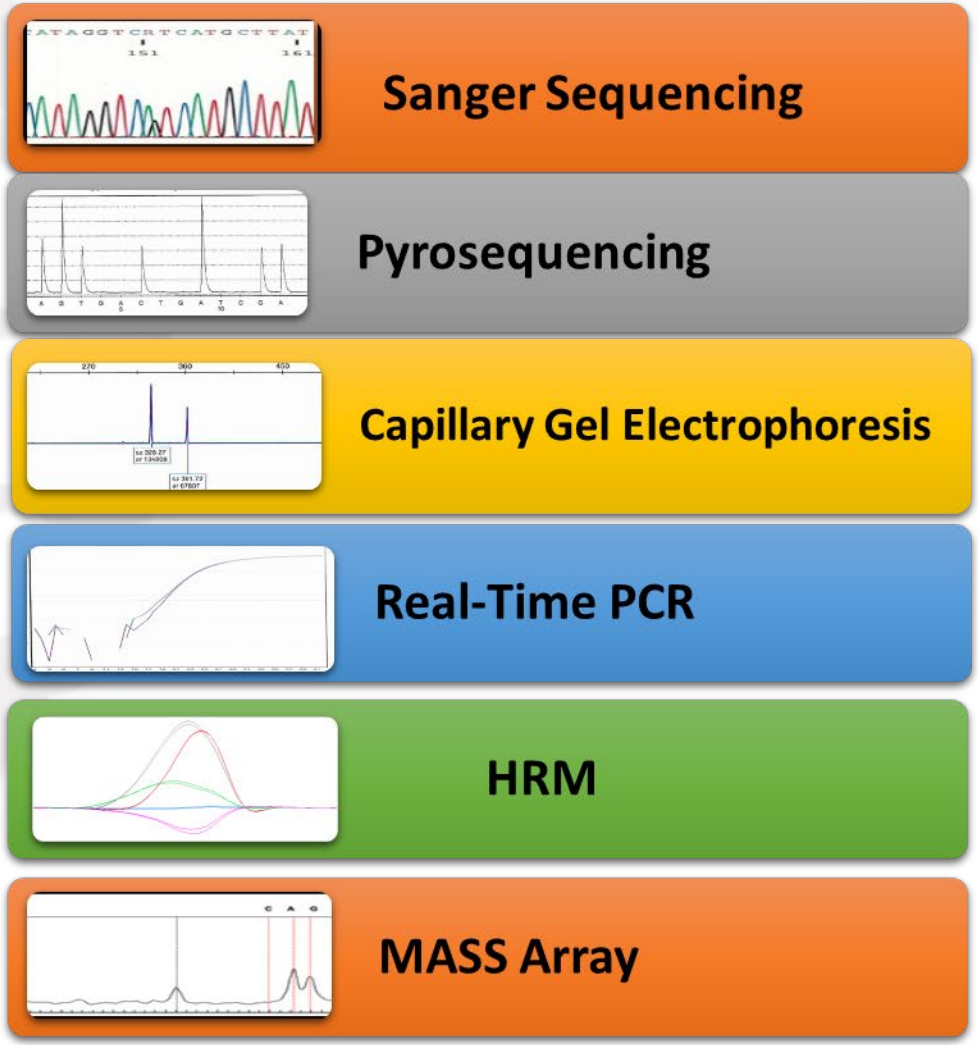
Real-time PCR
High Analytic/Low Clinical Sensitivity

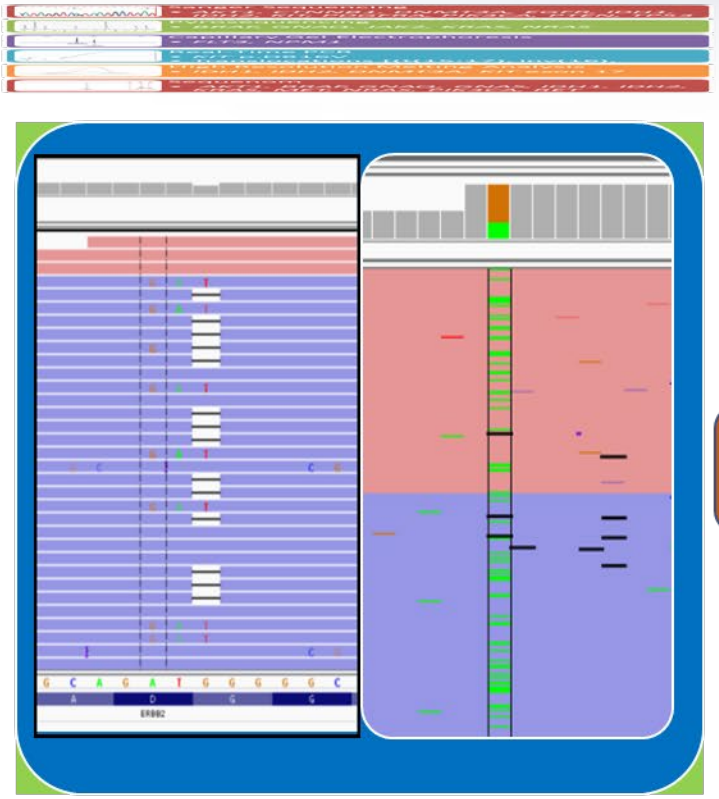




- Can have a very high **analytic sensitivity** (~1-5% allelic)
- The mutations identified are strictly those which are part of the assay design (low **clinical sensitivity**)

Next Generation Sequencing: A Multiplexed Assay for Clinical Testing

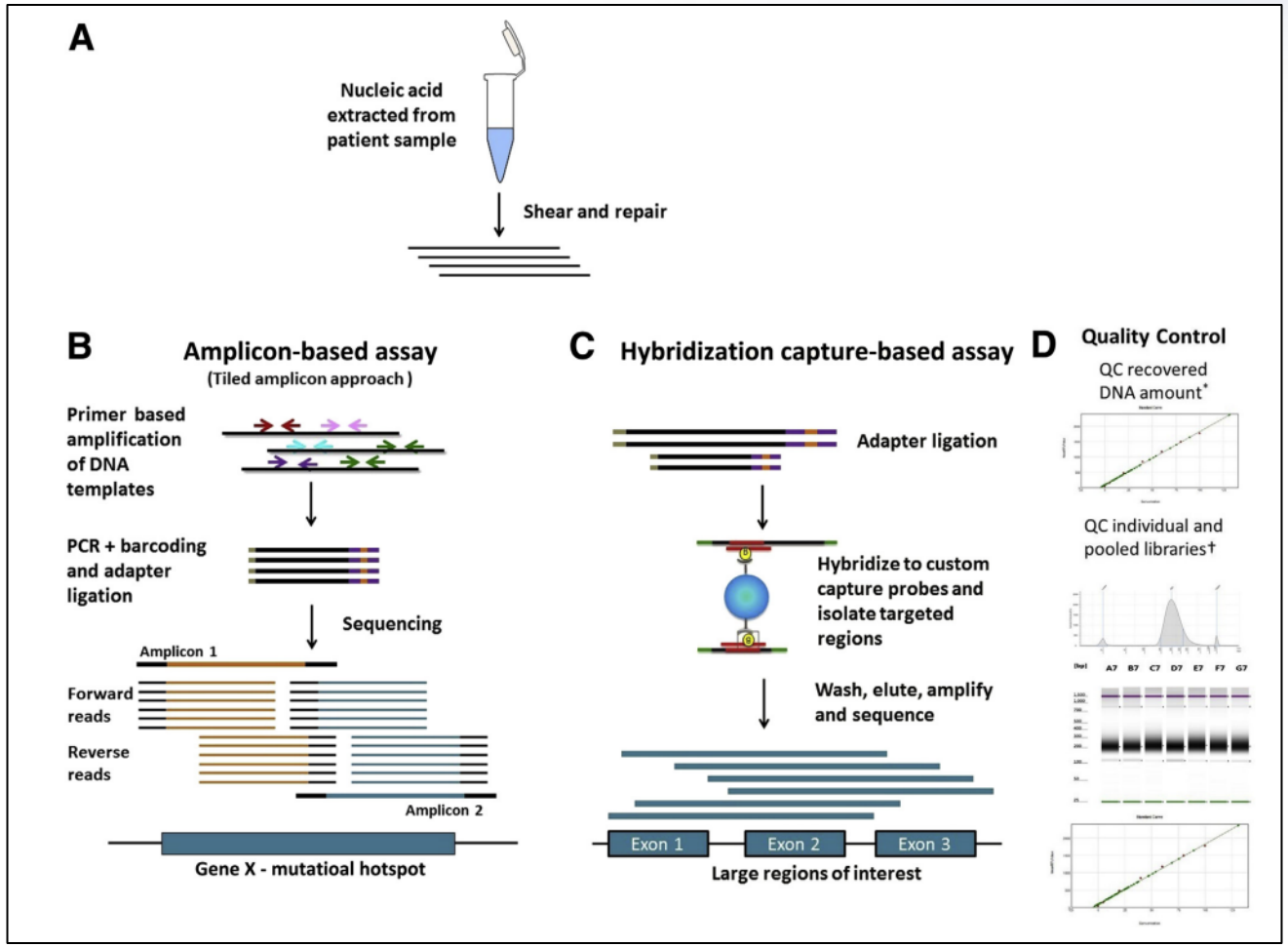




Next Generation Sequencing

- Simultaneous screening of multiple genes
- High analytic sensitivity ~5-10%
- Single platform for multiple genomic alterations (SNVs, insertions and deletions, CNVs, gene rearrangements, TMB, MSI

Next Generation Sequencing



Molecular Testing: The Analytics

Molecular Diagnostics in an Era of Targeted Therapy

Challenges:

Doing more with less

Limited sample size

Targeted therapy and evaluating multiple markers in tumor specimens

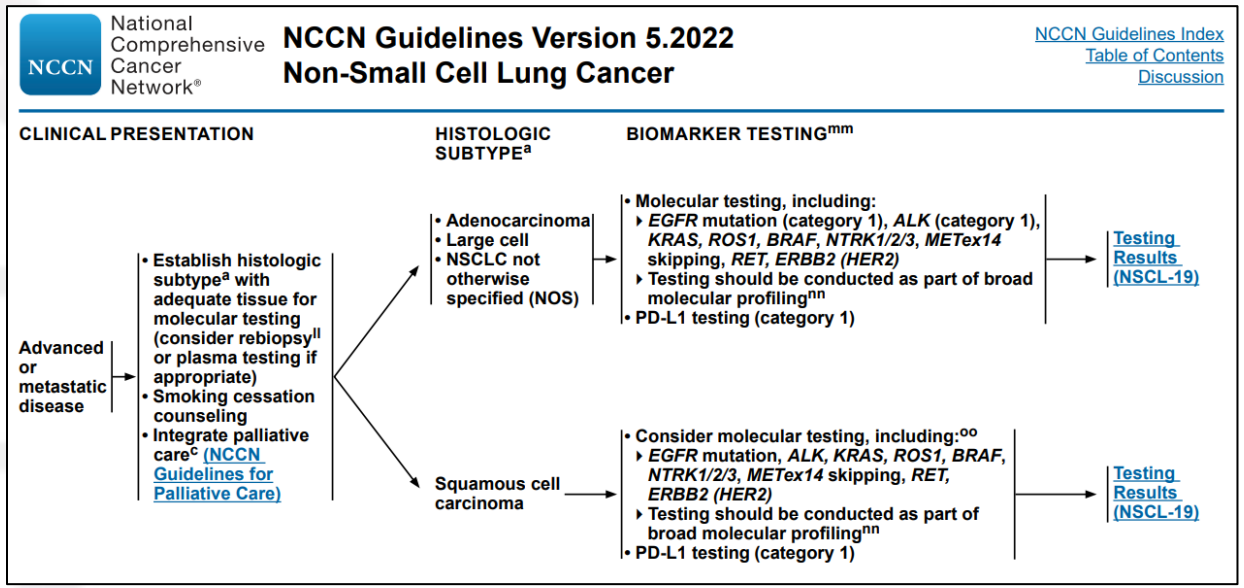
Turn around time

Timely and accurate reporting

How Often are Small Specimens Inadequate for Biomarker Testing?

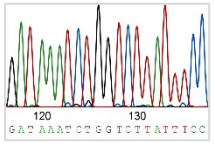
- Depends on the number of different tests (genes, assays) that are required
- Depends on the adequacy of the tissue obtained (viability, cellularity, tumor fraction etc.)
- Depends on how the tissue is handled

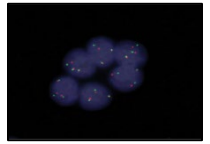
How Often are Small Specimens Inadequate for Biomarker Testing?

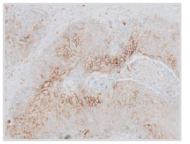


EGFR exon 19 **deletion or** ***L858R*** **mutation positive**	NSCL-20
EGFR S768I, L861Q, **and/or** ***G719X*** **mutation positive**	NSCL-23
EGFR exon 20 **insertion mutation positive**	NSCL-24
KRAS G12C **mutation positive**	NSCL-25
ALK **rearrangement positive**	NSCL-26
ROS1 **rearrangement positive**	NSCL-29
BRAF **V600E mutation positive**	NSCL-31
NTRK1/2/3 **gene fusion positive**	NSCL-32
METex14 **skipping mutation positive**	NSCL-33
RET **rearrangement positive**	NSCL-34
ERBB2 (HER2) **mutation positive**	NSCL-35
PD-L1 ≥50% and negative for actionable molecular biomarkers above	NSCL-36
PD-L1 ≥1%–49% and negative for actionable molecular biomarkers above	NSCL-37
PD-L1 <1% and negative for actionable molecular biomarkers above	NSCL-38

10 targets + TMB+ MSI







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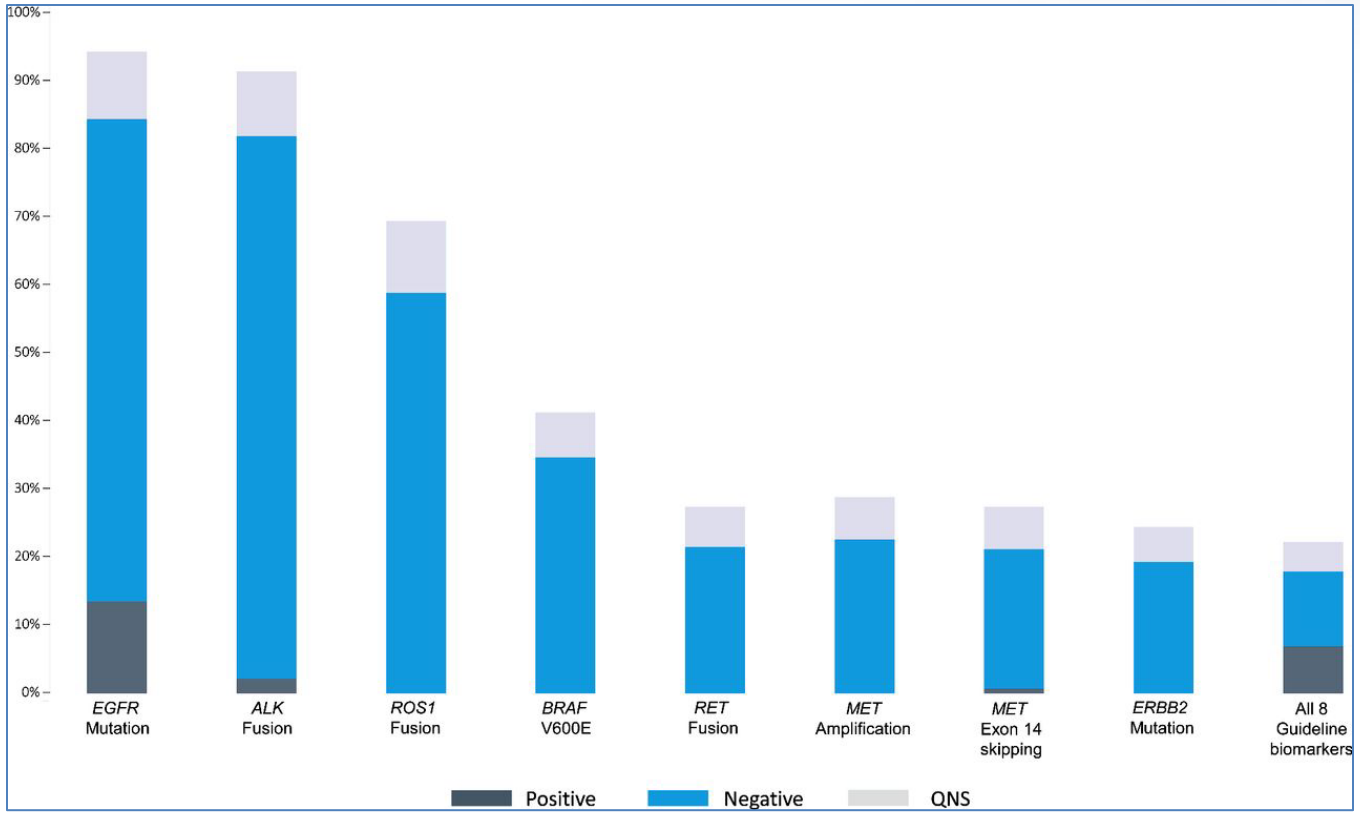
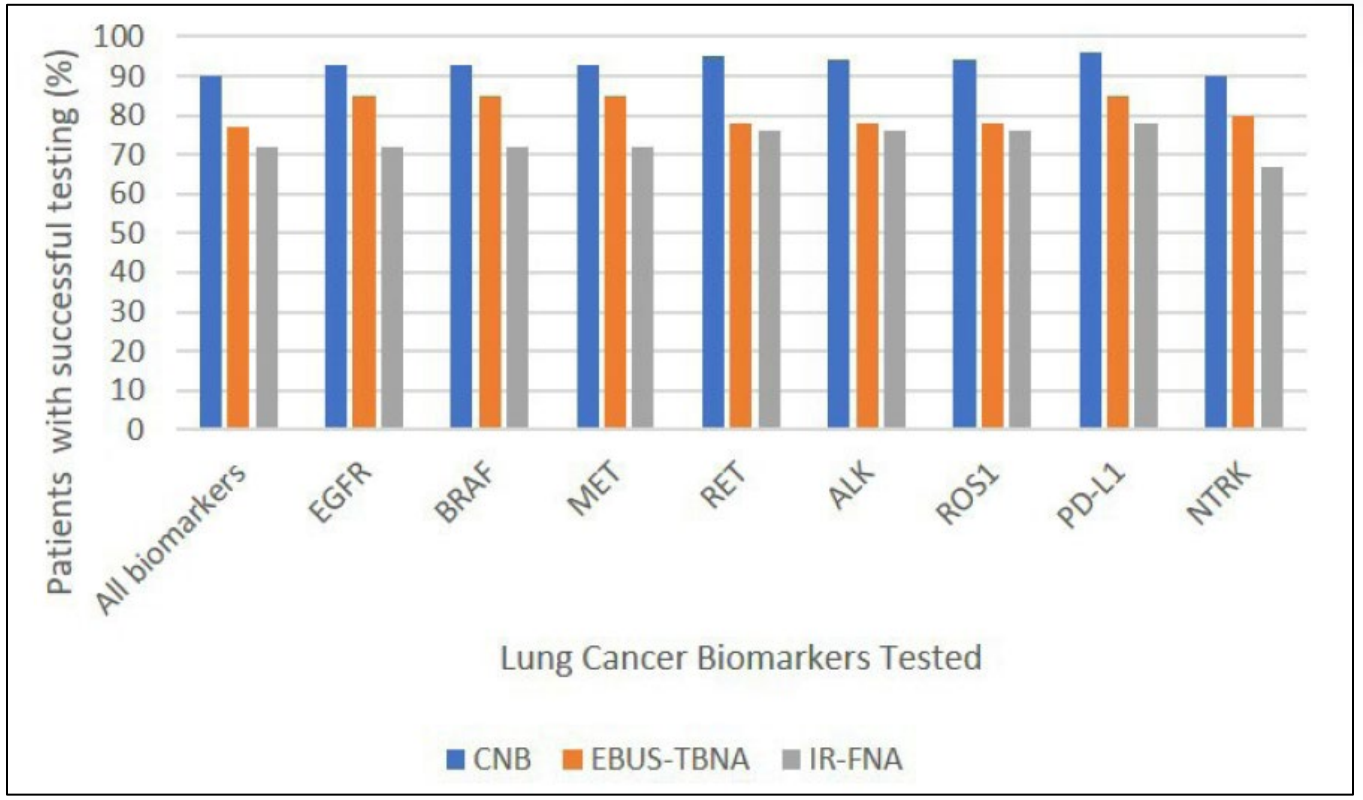


Reference: Leighl et al. Clin Cancer Res 2019;25:4691-4700

How Often are Small Specimens Inadequate for Biomarker Testing?



Reference: Faber et al. J Clin Pathol. 2021 May 5:jclinpath-2021-207597. doi: 10.1136/jclinpath-2021-207597.

How Often are Small Specimens Inadequate for Biomarker Testing?

- Depends on the number of different tests (genes, assays) that are required
- Depends on the adequacy of the tissue obtained (viability, cellularity, tumor fraction etc.)
- Depends on how the tissue is handled

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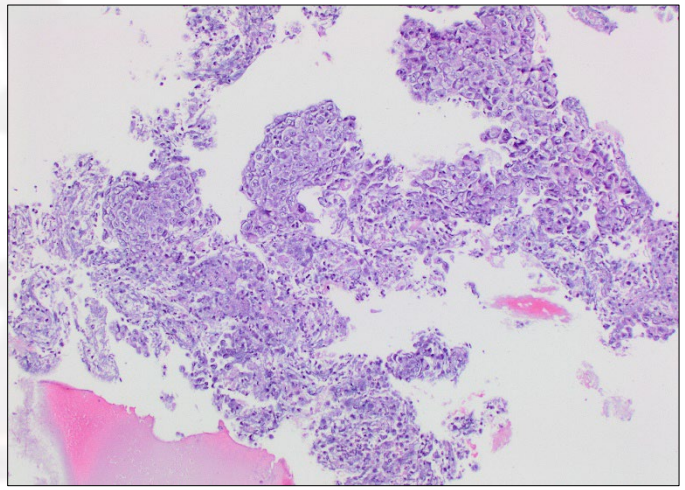

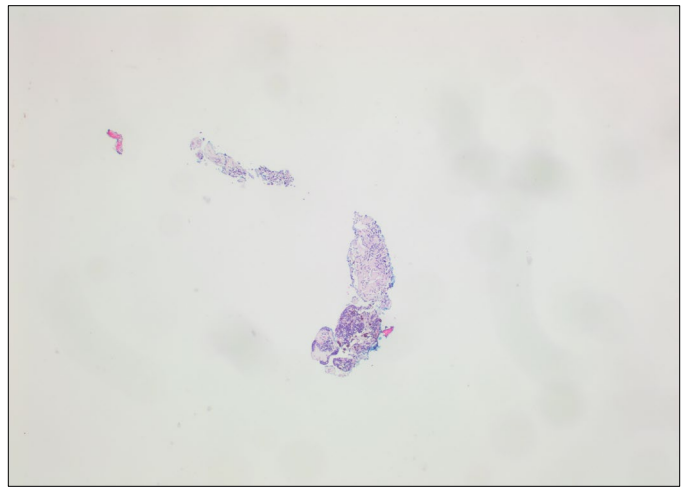

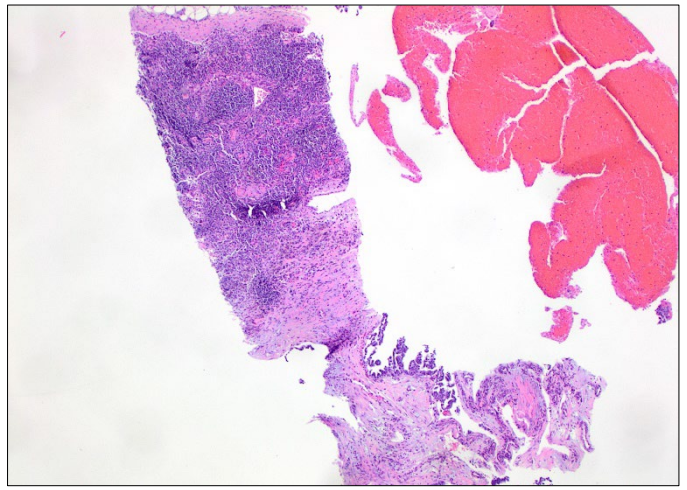

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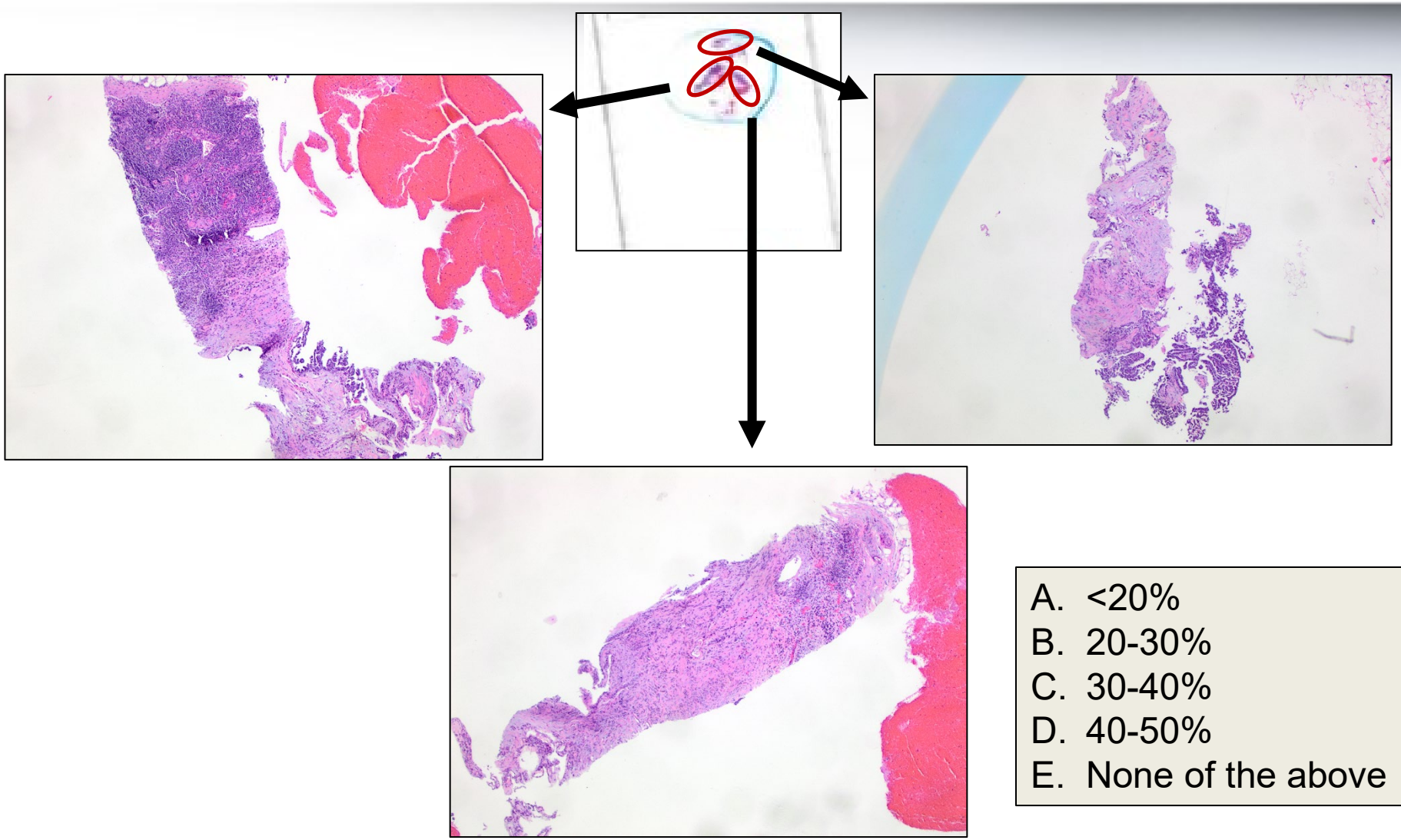
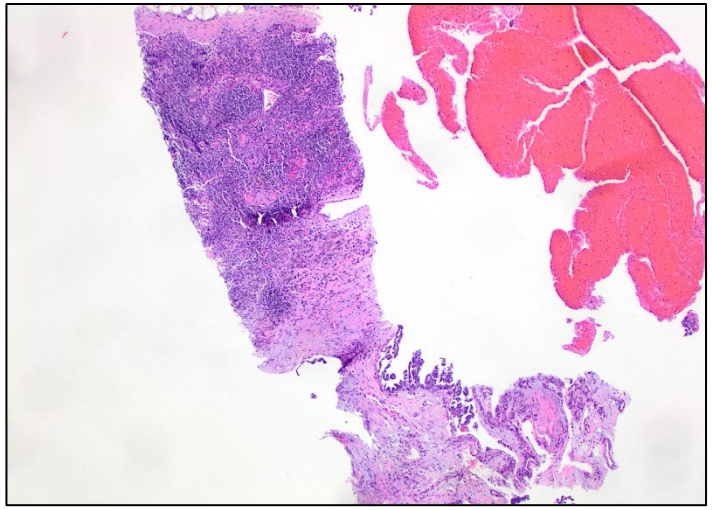
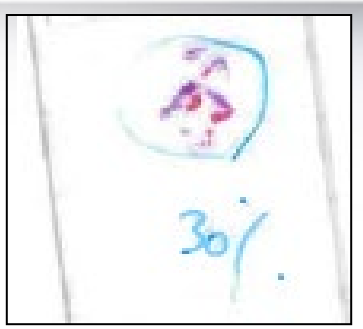
Case Example 1

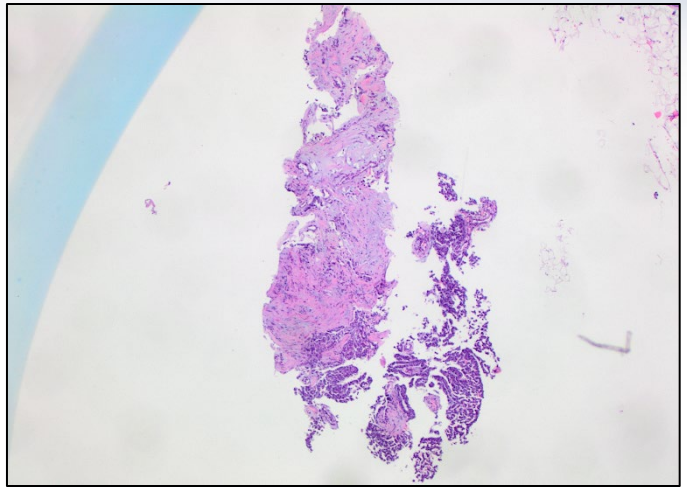


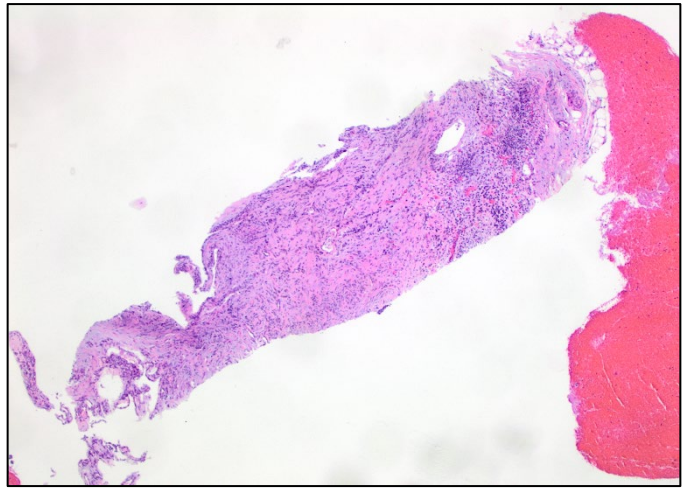
A. <20%
B. 20-30%
C. 30-40%
D. 40-50%
E. None of the above

Case Example 1









A. <20%
B. 20-30%
C. 30-40%
D. 40-50%
E. None of the above

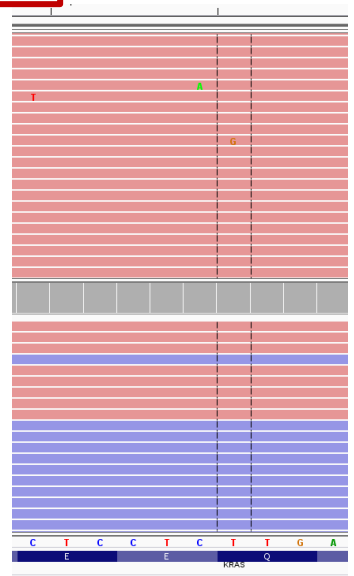
Issues with Low Tumor Fraction Samples

Filtered variant calls - 9 (373 total rows)

Gene	HGVS	dbSNP	COSMIC	dbCG	Strand	Loc	Type	Freq	Coverage	Variant(+)	varcov
KRAS	NM_004985.3(KRAS):c.183A>C p.Q61H	rs17851045	COSM554	***2***	-	Exon 3	SNV	2.1	2207	A->C	46
TP53	NM_000546.5(TP53):c.733G>A p.G245S	rs28934575	COSM6932		-	Exon 7	SNV	2	1631	G->A	33
JAK1	NM_002227.2(JAK1):c.2100T>G p.S700R				-	Exon 15	SNV	2.6	1290	T->G	33
JAK1	NM_002227.2(JAK1):c.2096C>A p.A699D				-	Exon 15	SNV	2.6	1290	C->A	34
CSF1R	NM_005211.3(CSF1R):c.1596_1598del p.L537del				-	Exon 11	Indel	2.4	1392	GCTC->C	33
POLE	NM_006231.2(POLE):c.4330dupG p.V1444fs*6				-	Exon 34	Indel	2.1	1353	T->GT	29
PIK3CA	NM_006218.2(PIK3CA):c.2179dupA p.T727fs*11				+	Exon 14	Indel	2.9	966	G->GA	28
FGFR1	NM_015850.3(FGFR1):c.376G>A p.E126K				-	Exon 4	SNV	5	600	G->A	30
NOTCH1	NM_017617.3(NOTCH1):c.7067C>G p.A2356G				-	Exon 34	SNV	2.5	1229	C->G	31

Detecting a clinically relevant mutation at a low VAF in a low tumor sample:

- Call? (confidence of real call vs artifact/noise)
- Confirm? (High sensitivity orthogonal platform)
- Ignore? (potential patient care problem)
- Cancel? (patient needs rebiopsy)



← Tumor sample

← Normal control

← Reference seq

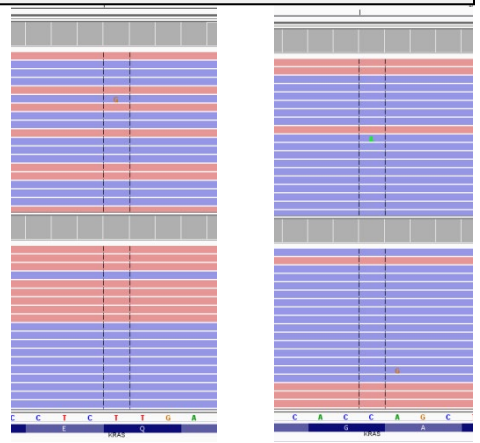
Issues with Low Tumor Fraction Samples

Filtered variant calls - 18 (345 total rows)

Gene	HGVS	dbSNP	COSMIC	dbCG	Strand	Loc	Type	Freq	Coverage	Variant(+)	varcov
KRAS	NM_004985.3(KRAS):c.183A>C p.Q61H	rs17851045	COSM554	***2***	-	Exon 3	SNV	2.5	2000	A->C	50
KRAS	NM_004985.3(KRAS):c.34G>T p.G12C	rs121913530	COSM516	***3***	-	Exon 2	SNV	1.1	2211	G->T	25
RNF43	NM_017763.4(RNF43):c.1932del p.K644fs*56				-	Exon 9	Indel	51.3	665	AT->T	341
FBXW7	NM_033632.3(FBXW7):c.2001_2005delinsAGT p.S668fs*2				-	Exon 12	Indel	2.9	1470	GAGTG->AGT	42
FBXW7	NM_033632.3(FBXW7):c.2009del p.G670fs*37				-	Exon 12	Indel	4	1470	GA->A	59
FBXW7	NM_033632.3(FBXW7):c.2009dupG p.V671fs*23				-	Exon 12	Indel	4.3	1470	A->GA	63
FBXW7	NM_033632.3(FBXW7):c.2002_2004delinsGAGTG p.S668				-	Exon 12	Indel	2.2	1470	AGT->GAGTG	33
FBXW7	NM_033632.3(FBXW7):c.2001del p.S668fs*39		COSM34018		-	Exon 12	Indel	6.9	1470	GA->A	102
FBXW7	NM_033632.3(FBXW7):c.2001dupG p.S668fs*26				-	Exon 12	Indel	3.8	1470	A->GA	56
EGFR	NM_005228.3(EGFR):c.2214dupT p.K739*				+	Exon 19	Indel	2.6	1441	G->GT	37
PTCH1	NM_000264.3(PTCH1):c.3913_3915delinsA p.D1305fs*19				-	Exon 23	Indel	11.2	430	GAC->A	48
PTCH1	NM_000264.3(PTCH1):c.3913del p.D1305fs*67				-	Exon 23	Indel	28.4	430	GA->A	122
PTCH1	NM_000264.3(PTCH1):c.3921del p.R1308fs*64				-	Exon 23	Indel	97.6	420	CA->A	410
TSC1	NM_000368.4(TSC1):c.3127_3129del p.S1043del				-	Exon 23	Indel	2.8	1041	GCAG->G	29
PIK3CB	NM_006219.2(PIK3CB):c.1658del p.N553fs*55				-	Exon 11	Indel	4	1250	AT->T	50
FGFR1	NM_015850.3(FGFR1):c.376G>A p.E126K				-	Exon 4	SNV	8.8	556	G->A	49
RB1	NM_000321.2(RB1):c.13del p.T5fs*60				+	Exon 1	Indel	90	590	CA->C	531
RB1	NM_000321.2(RB1):c.13A>C p.T5P				+	Exon 1	SNV	15.4	593	A->C	91

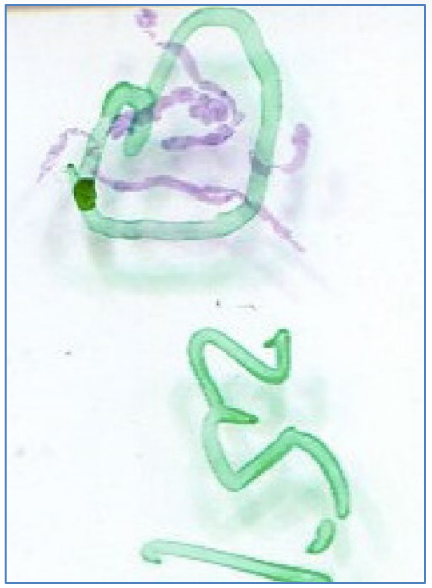
Detecting a clinically relevant mutation at a low VAF in a low tumor sample that is reproducible vs not:

- Call? (confidence of real call vs artifact/noise)
- Ignore? (potential patient care problem)
- Cancel? (patient needs rebiopsy)



Case Example 2

- Patient with lung adenocarcinoma
- Known *EGFR* mutation
- Progression on TKI therapy
- Suspecting *EGFR* resistance

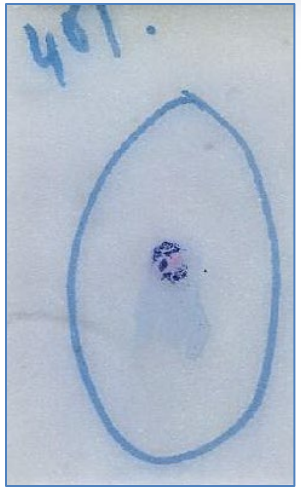


Somatic Mutations

Gene	Standardized Nomenclature (HGVS)	Location	DNA change	Protein change	dbSNP ID	COSMIC ID
APC	NM_000038.5(APC):c.4348C>T p.R1450*	Exon 16	SNV	Nonsense	rs121913332	COSM13127
ATM	NM_000051.3(ATM):c.6951G>C p.K2317N	Exon 47	SNV	Missense		
EGFR	NM_005228.3(EGFR):c.2155G>T p.G719C	Exon 18	SNV	Missense	rs28929495	COSM6253
EGFR	NM_005228.3(EGFR):c.2303G>T p.S768I	Exon 20	SNV	Missense	rs121913465	COSM6241

Allelic Frequency <10%

Case Example 2



I. Mutations in ordered genes

Gene	Standardized Nomenclature (HGVS)	Location	DNA change	Protein change	dbSNP ID	COSMIC ID
EGFR	NM_005228.3(EGFR):c.2155G>T p.G719C	Exon 18	SNV	Missense	rs28929495	COSM6253
EGFR	NM_005228.3(EGFR):c.2369C>T p.T790M	Exon 20	SNV	Missense	rs121434569	COSM6240
EGFR	NM_005228.3(EGFR):c.2303G>T p.S768I	Exon 20	SNV	Missense	rs121913465	COSM6241

II. Mutations in non-ordered genes

Gene	Standardized Nomenclature (HGVS)	Location	DNA change	Protein change	dbSNP ID	COSMIC ID
APC	NM_000038.5(APC):c.4348C>T p.R1450*	Exon 16	SNV	Nonsense	rs121913332	COSM13127
TP53	NM_000546.5(TP53):c.517del p.V173*	Exon 5	Deletion	Nonsense		COSM43732

Allelic Frequency

- Sensitizing mutation 25%
- Resistance mutation 10%

Tumor Fraction: FNA versus CNB

FNA samples are inherently enriched in tumor cells

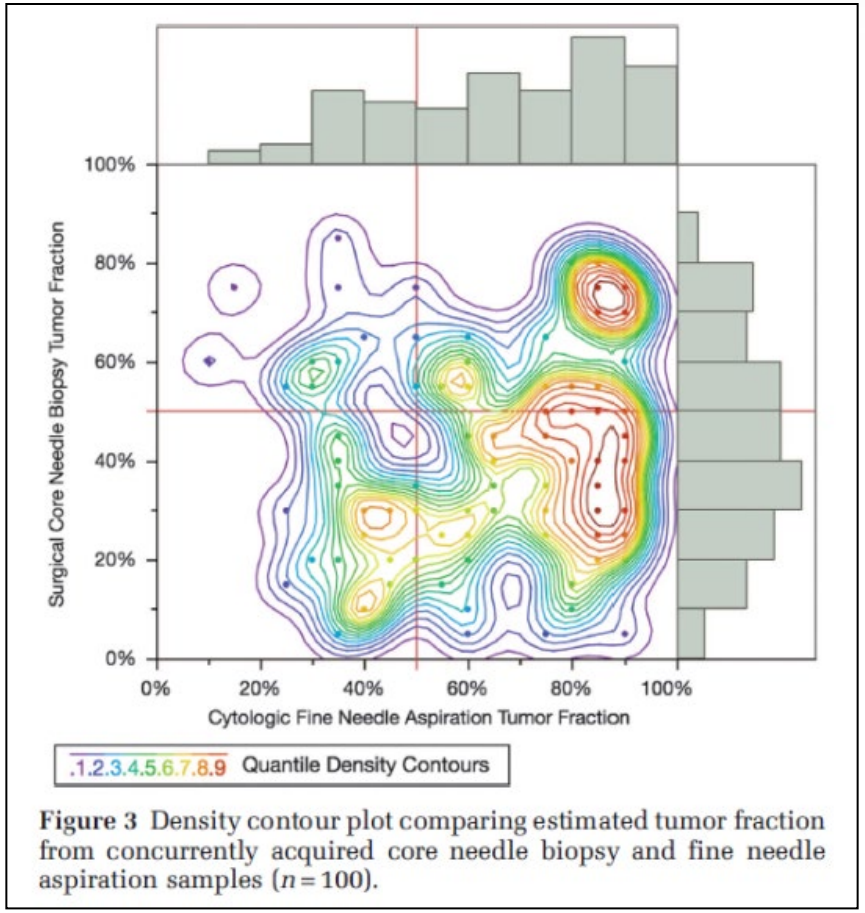


Figure 3 Density contour plot comparing estimated tumor fraction from concurrently acquired core needle biopsy and fine needle aspiration samples ($n = 100$).

Reference: Roy-Chowdhuri S, Chen H, et al. Mod Pathol. 2017 Apr;30(4):499-508.

Cytology Samples Provide Excellent Substrates for NGS

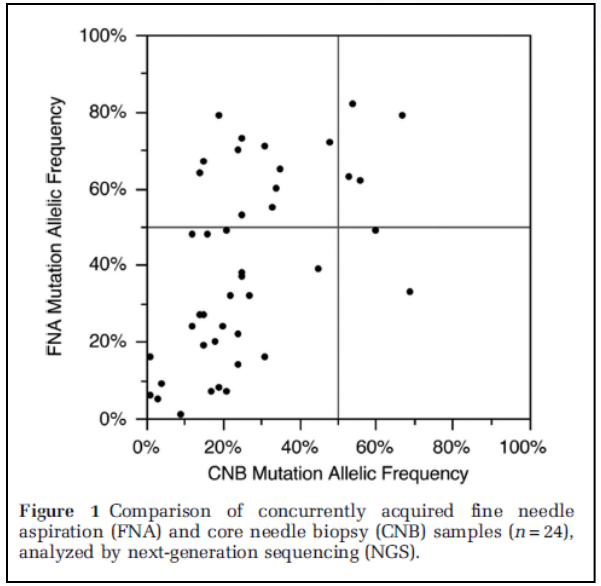


Figure 1 Comparison of concurrently acquired fine needle aspiration (FNA) and core needle biopsy (CNB) samples ($n = 24$), analyzed by next-generation sequencing (NGS).

Mod Pathol. 2017 Apr;30(4):499-508.

- FNA samples are inherently enriched in tumor cells
- FNA samples had lower numbers of underperforming amplicons
- Normalized average amplicon coverage is significantly higher in FNA samples

Cytology Samples Provide Excellent Substrates for NGS

Next-Generation Sequencing of Cytologic Preparations: An Analysis of Quality Metrics

David H. Hwang, MD [1]; Elizabeth P. Garcia, PhD[2]; Matthew D. Ducar, MS[3]; Edmund S. Cibas, MD [1]; and Lynette M. Sholl, MD[1,2]

TABLE 3. Comparison of Quality Metrics

Quality Metric	Smears/LBPs	Core Biopsies	Cell Blocks	*P*
Adequacy rate, n/N (%)	23/26 (88)	77/87 (89)	29/30 (97)	.41
Initial DNA concentration, ng/μL	6.84	7.70	10.45	.70
Postshearing fragment size, bp	317.2	411.7	385.8	<.001
Post–library preparation fragment size, bp	356.3	336.3	355.6	.21
Fragment size difference, bp	52.5	−72.3	−47.6	<.001
Insert size, bp	191	177	179	<.001
Total reads[a]	2.79×10^7 [1.085]	2.48×10^7 [0.983]	2.50×10^7 [1.002]	.29
Passing-filter reads aligned[a]	2.59×10^7 [1.085]	2.30×10^7 [0.982]	2.29×10^7 [1.003]	.33
Percent passing-filter unique reads aligned[a]	96.3% [1.001]	94.3% [1.001]	94.1% [1.000]	.70
Mean target coverage[a]	400.3% [1.181]	156.0% [0.989]	147.8% [1.006]	.04
Percentage of loci with >100× coverage[a]	97.2% [1.013]	76.2% [0.988]	77.0% [1.003]	.24
Percent duplication[a]	32.0% [0.929]	70.5% [1.001]	70.5% [0.996]	<.001
Percent selected bases[a]	49.5% [1.019]	49.0% [1.010]	48.7% [1.003]	.14
Percent usable bases on bait[a]	26.7% [1.049]	11.1% [1.002]	10.7% [0.999]	.03

Abbreviations: bp, base pair; LBP, liquid-based preparation.
Median values are presented.
[a] Values within square brackets are values normalized by the flow cell average; *P* values are based on the normalized values.

Smears/LBP show better quality metrics than FFPE

- higher mean insert size
- higher mean target coverage
- higher percent usable bases
- lower duplication rate
- lower post-shearing fragment size

What about Fluorescence In-situ Hybridization (FISH) in Cytology?

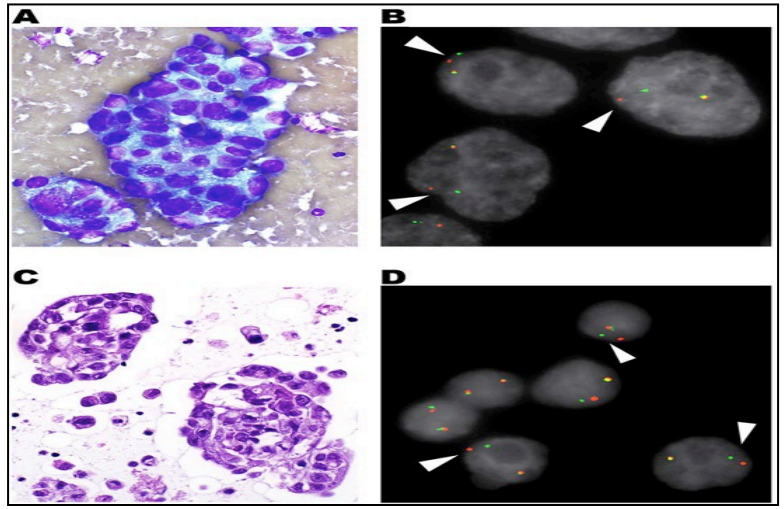
FISH in Cytology

- Can be performed on a variety of cytology specimen preparations
- Minimum requirements (number of cells) are assay dependent
- Tumor fraction less of a concern
- Tumor nuclei have to be easily distinguished from background cells by DAPI stain
- Cytology smears, cytospins, LBC have whole nuclei and therefore no truncation artifact as seen with FFPE sections

FISH in Cytology

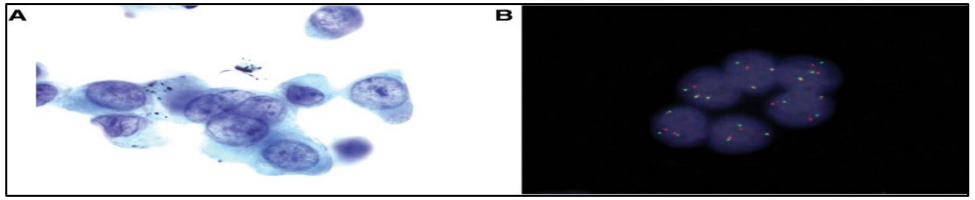
The Use of Stained Cytologic Direct Smears for *ALK* Gene Rearrangement Analysis of Lung Adenocarcinoma

Bryan L. Betz, PhD[1]; Catherine A. Dixon, MLS (ASCP)[1]; Helmut C. Weigelin, MLS (ASCP)[1]; Stewart M. Knoepp, MD, PhD[2]; and Michael H. Roh, MD, PhD[1]



ALK Status Testing in Non–Small-Cell Lung Carcinoma by FISH on ThinPrep Slides with Cytology Material

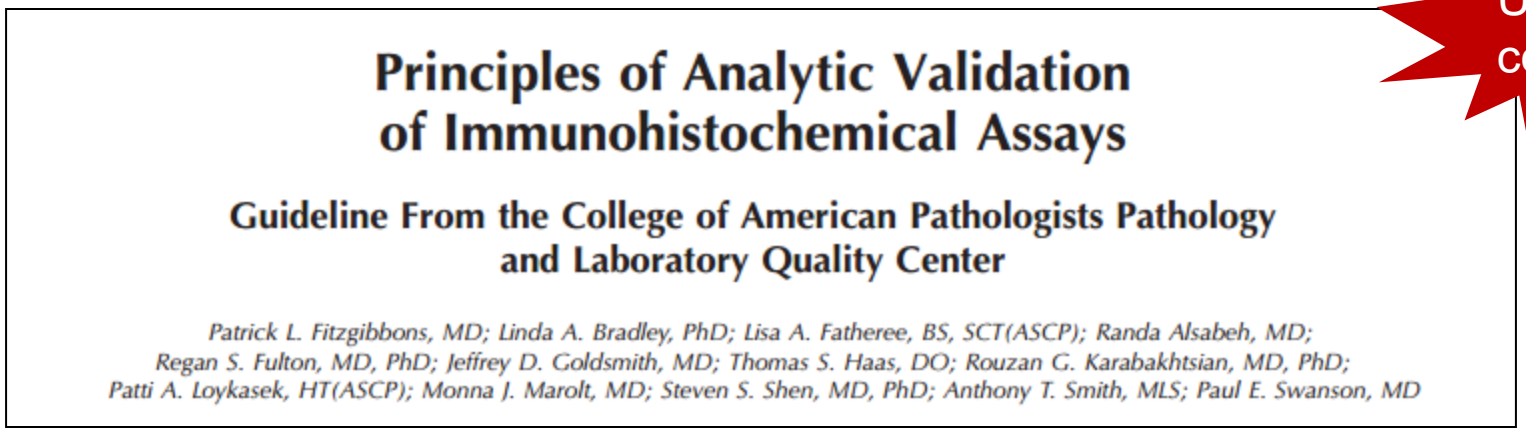
Eugen C. Minca, MD, PhD, Christopher P. Lanigan, MS,* Jordan P. Reynolds, MD,* Zhen Wang, MD, PhD,* Patrick C. Ma, MD,† Joseph Cicenia, MD,‡ Francisco A. Almeida, MD,‡ Nathan A. Pennell, MD,† and Raymond R. Tubbs, DO**



Biomarker Testing by IHC in Cytology

Actionable Biomarker Testing by IHC

- It is becoming increasingly common for actionable biomarker testing by IHC (e.g. ALK, ROS1, BRAF V600E, PD-L1, MSI) emphasizing the need for appropriate validation



Principles of Analytic Validation of Immunohistochemical Assays

Guideline From the College of American Pathologists Pathology and Laboratory Quality Center

Patrick L. Fitzgibbons, MD; Linda A. Bradley, PhD; Lisa A. Fatheree, BS, SCT(ASCP); Randa Alsabeh, MD; Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karabakhtsian, MD, PhD; Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, MLS; Paul E. Swanson, MD

Update coming

Arch Pathol Lab Med. 2014 Nov;138(11):1432-43. doi: 10.5858/arpa.2013-0610-CP.

Actionable Biomarker Testing by IHC

REVIEW ARTICLE

Principles of Analytic Validation of Clinical Immunohistochemistry Assays

Jeffrey D. Goldsmith, MD, Patrick L. Fitzgibbons, MD,† and Paul E. Swanson, MD‡*

Similarly, if IHC is run on cytologic preparations, including smears, cytospins, cell blocks, and ThinPrep preparations (or core samples submitted with aspirate fluid or other preparation to the cytology laboratory in CytoLyt or other nonformalin solutions), reasonable efforts should be made to assure that these assays perform adequately before they are used on patient samples. The selection of markers tested and number of cases included in these separate validation studies must be determined by the laboratory medical director.

Update coming

Reference: Goldsmith JD et al., Adv Anat Pathol. 2015 Nov;22(6):384-7.

Biomarker Testing by IHC in Cytology May Require Optimization

- Methanol-based fixation can decrease IHC accuracy by loss or decrease of immunogenicity when formalin-optimized protocols are used
- Some studies report post-fixation of alcohol-fixed specimens in formalin may restore immunogenicity
- Therefore, rigorous validation and protocol optimization are critical for immunostaining cytologic specimens

Reference:
Sauter JL et al., Cancer Cytopathol. 2016 Feb;124(2):89-100.
Kinsella MD et al. Diagn Cytopathol. 2013 Mar;41(3):192-8.
Jain D et al. Cancer Cytopathol. 2019 May;127(5):325-339.

Which Cytology Specimen Requires Additional Validation?

- Cytology direct smear
- FFPE cell blocks of effusion specimens sitting in fridge O/N
- Cellient cell block
- FFPE cell block of FNA collected in RPMI/ CytoLyt/ saline/ ethanol

Molecular Cytopathology: Integrating Cytology and Molecular

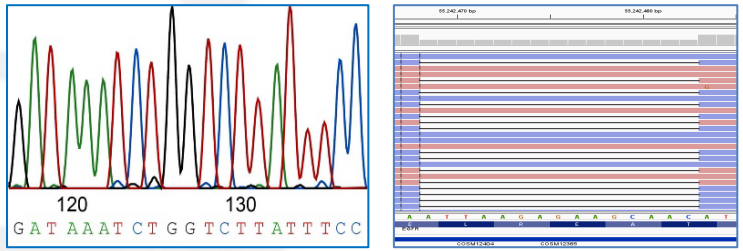
Three Cardinal Rules of Molecular Cytopathology

Sinchita's Rule of Three

- Know your test
- Know your specimen
- Know the clinical relevance

Three Cardinal Rules of Molecular Cytopathology

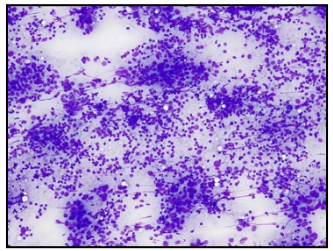
Know your test



Molecular assay

- Assay design, capabilities, limitations
- Analytical versus clinical sensitivity

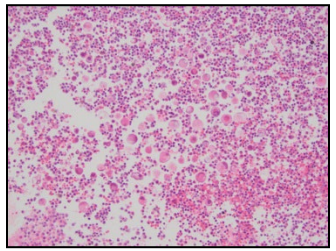
Know your specimen



Specimen adequacy

- Selecting the best specimen for testing
- Modulating the specimen for the assay

Know the clinical relevance



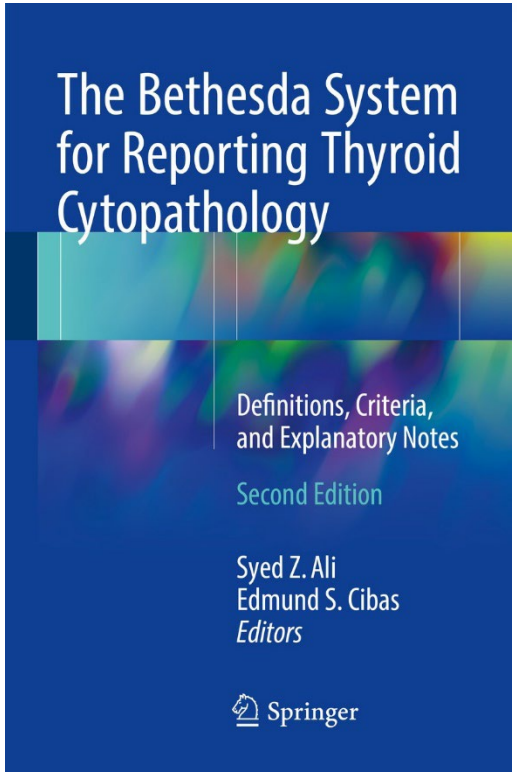
Correlation

- Recognizing false negative/positive
- Clinical significance of the findings

Harnessing the Power of Molecular Cytopathology

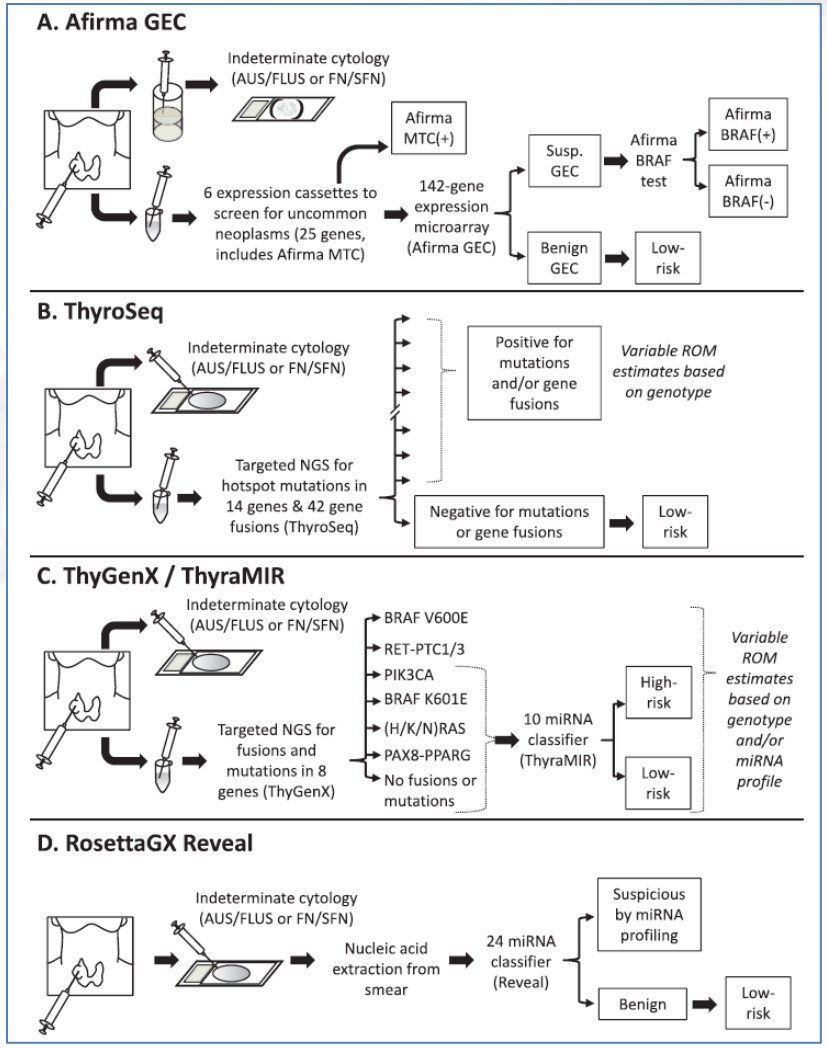
Molecular Testing in Thyroid Cytology

- FNA preferred sampling modality
- Non-diagnostic/indeterminate rates are relatively high



	Diagnostic Category	ROM [1] (NIFTP [2] Considered as Cancer (%))	ROM [1] (NIFTP [2] NOT Considered as Cancer (%))	Management Options
I.	**Nondiagnostic/unsatisfactory** (Cyst fluid, acellular specimen, other (e.g., obscuring blood, artefacts))	5–10	5–10	Correlate with clinical/radiological findings Consider repeat FNA [3]
II.	**Benign** (Benign follicular nodule, Chronic lymphocytic (Hashimoto) thyroiditis, Granulomatous (subacute) thyroiditis)	0–3	0–3	Surveillance and US [4] follow up
III.	**Atypia of undetermined significance, *or* follicular lesion of undetermined significance**	6–18	10–30	Correlate with clinical/radiological findings Consider repeat FNA [3] Consider molecular testing
IV.	**Follicular neoplasm *OR* Follicular carcinoma** (specify if Hürtle cell features)	10–40	25–40	Consider molecular testing, Lobectomy
V.	**Suspicious for malignancy**	45–60	50–75	Total thyroidectomy or lobectomy
VI.	**Malignant** (Papillary thyroid carcinoma, Poorly differentiated thyroid carcinoma, Medullary thyroid carcinoma, Anaplastic thyroid carcinoma, Squamous cell carcinoma, Carcinoma with mixed features, Metastatic malignancy, Non-Hodgkin lymphoma, Other)	94–96	97–99	Total thyroidectomy or lobectomy

Molecular Testing in Thyroid Cytology



Nishino M, Nikiforova M. Arch Pathol Lab Med. 2018 Apr;142(4):446-457. doi: 10.5858/arpa.2017-0174-RA.

Molecular Analysis of Residual ThinPrep Material From Thyroid FNAs Increases Diagnostic Sensitivity

Jeffrey F. Krane, MD, PhD[1] Edmund S. Cibas, MD[1] Erik K. Alexander, MD[2] Ralf Paschke, MD[3] and Markus Eszlinger, PhD[3]

Molecular Diagnosis Using Residual Liquid-Based Cytology Materials for Patients with Nondiagnostic or Indeterminate Thyroid Nodules

Hyemi Kwon[1], Won Gu Kim[1], Markus Eszlinger[2], Ralf Paschke[2], Dong Eun Song[3], Mijin Kim[1], Suyeon Park[1], Min Ji Jeon[1], Tae Yong Kim[1], Young Kee Shong[1], Won Bae Kim[1]

Next-Generation Sequencing Identifies Gene Mutations That Are Predictive of Malignancy in Residual Needle Rinses Collected From Fine-Needle Aspirations of Thyroid Nodules

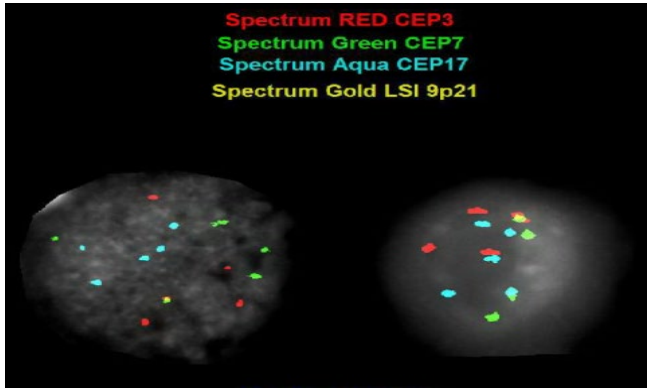
Maren Y. Fuller, MD; Dina Mody, MD; April Hull, CT(ASCP); Kristi Pepper, MT(ASCP); Heather Hendrickson, MT(ASCP); Randall Olsen, MD, PhD

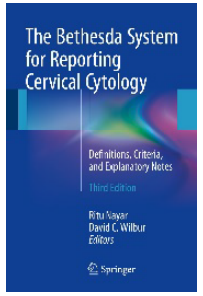
Not Malignant

The Power of Molecular Cytopathology

Genomic alterations without a malignant diagnosis

- Atypia: e.g. Gyn cytology, urine cytology





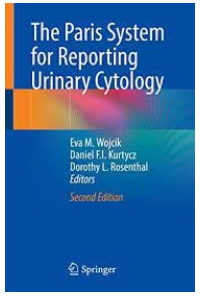


Table 4. Relative risk of the diagnostic categories outlined in The Paris System, based on studies to date.

Category	Risk of malignancy, %	Management
Unsatisfactory/nondiagnostic	<5–10	repeat cytology, cystoscopy in 3 months if increased clinical suspicion
Negative for high-grade urothelial carcinoma	0–10	clinical follow-up as needed
Atypical urothelial cells	8–35	clinical follow-up as needed, potential use of ancillary testing
Suspicious for high-grade urothelial carcinoma	50–90	more aggressive follow-up, cystoscopy, biopsy
Low-grade urothelial neoplasm	~10	need cystoscopy and biopsy to further evaluate grade and stage
High-grade urothelial carcinoma	>90	more aggressive follow-up, cystoscopy, biopsy, staging
Other malignancy	>90	more aggressive follow-up, cystoscopy, biopsy, staging

The Power of Molecular Cytopathology

Genomic alterations without a malignant diagnosis

- Negative for malignancy: pancreatic cyst fluid

ORIGINAL ARTICLE

Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia

Aatur D Singhi,[1] Kevin McGrath,[2] Randall E Brand,[2] Asif Khalid,[2] Herbert J Zeh,[3] Jennifer S Chennat,[2] Kenneth E Fasanella,[2] Georgios I Papachristou,[2] Adam Slivka,[2] David L Bartlett,[3] Anil K Dasyam,[4] Melissa Hogg,[3] Kenneth K Lee,[3] James Wallis Marsh,[3] Sara E Monaco,[1] N Paul Ohori,[1] James F Pingpank,[3] Allan Tsung,[3] Amer H Zureikat,[3] Abigail I Wald,[1] Marina N Nikiforova[1]

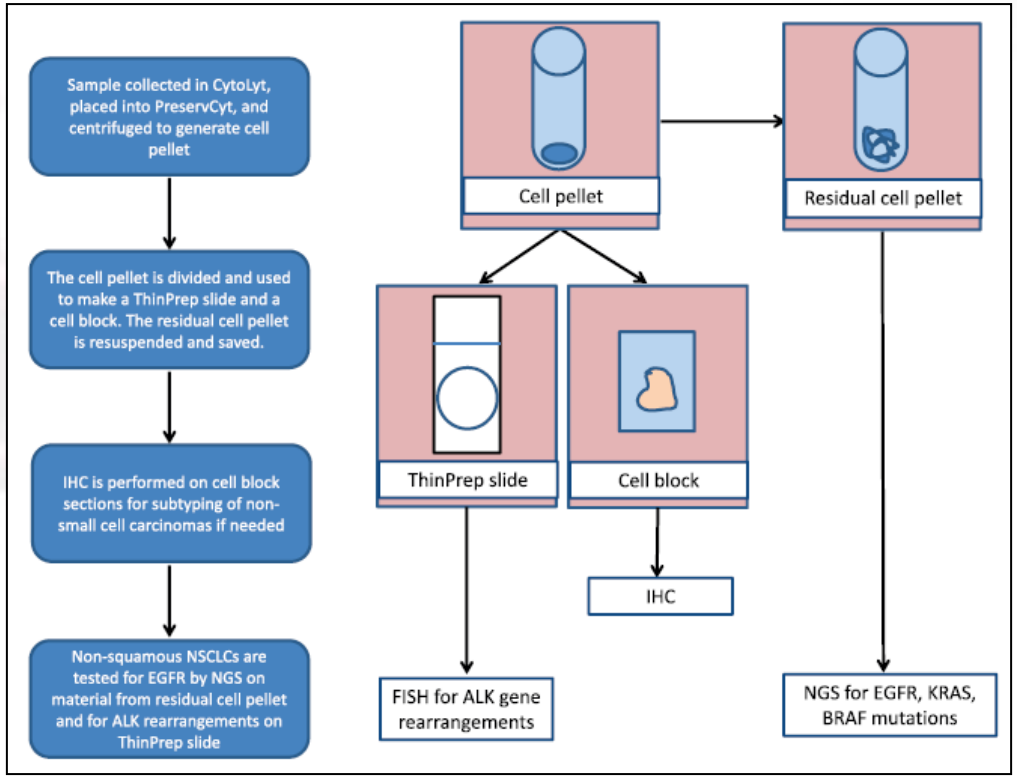
Table 1
Key genetic mutations and/or deletions in pancreatic cysts

Pancreatic Cyst Type	*KRAS*	*GNAS*	*RNF43*	*VHL*	*CTNNB1*	*TP53*	*PIK3CA*	*PTEN*	*CDKN2A*	*SMAD4*
Intraductal papillary mucinous neoplasm	+	+	+	–	–	+[a]	+[a]	+[a]	+[a]	+[a]
Mucinous cystic neoplasm	+	–	–	–	–	+[a]	+[a]	+[a]	+[a]	+[a]
Serous cystadenoma	–	–	–	+	–	–	–	–	–	–
Solid-pseudopapillary neoplasm	–	–	–	–	+	+[b]	+[b]	–	–	–
Non-neoplastic cysts	–	–	–	–	–	–	–	–	–	–

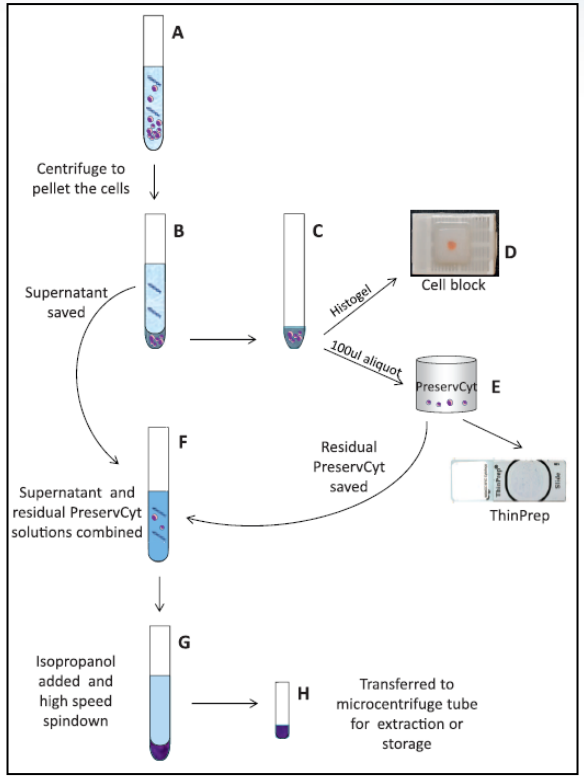
[a] Alterations in these genes are associated with advanced neoplasia.
[b] Although mutations in these genes have been described, they are rare findings.
+, presence; –, absence.

References:
Singhi AD, McGrath K et al. Gut. 2018 Dec;67(12):2131-2141.
Theisen BK et al. Surg Pathol Clin. 2016 Sep;9(3):441-56. doi: 10.1016/j.path.2016.04.008

Utilizing Residual Liquid-Based Samples

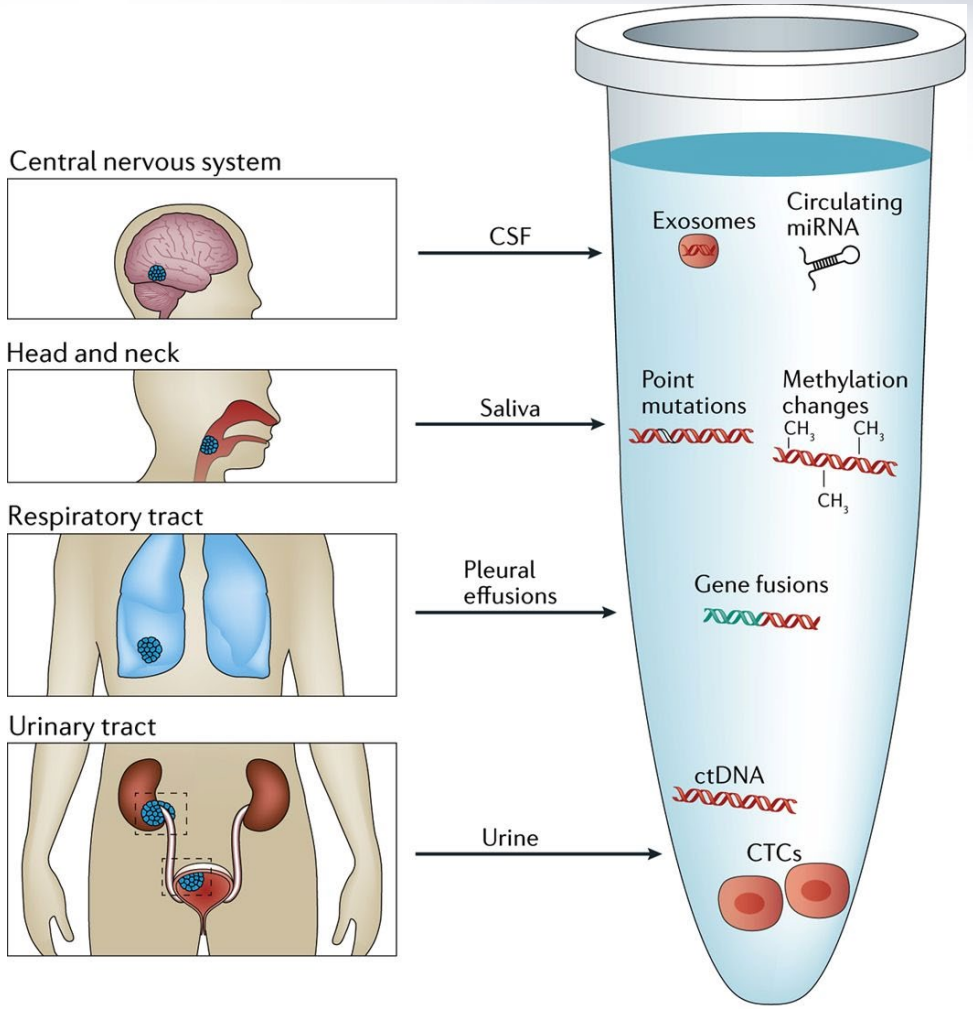


Doxtader EE et al. Arch Pathol Lab Med. 2019 Jun;143(6):670-676.



Tian SK et al. Arch Pathol Lab Med. 2016 Nov;140(11):1200-1205.

Cytology Specimens May Serve as a Liquid Biopsy Testing Option



Nature Reviews | Clinical Oncology

Siravegna, G. *et al. Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2017.14

Next Generation Molecular Cytopathology

Modern Pathology
https://doi.org/10.1038/s41379-018-0006-x

USCAP

ARTICLE

Salvaging the supernatant: next generation cytopathology for solid tumor mutation profiling

Sinchita Roy-Chowdhuri[1] · Meenakshi Mehrotra[2] · Ana Maria Bolivar[3] · Rashmi Kanagal-Shamanna[2] · Bedia A. Barkoh[2] · Brette Hannigan[3] · Stephanie Zalles[3] · Wenrui Ye[3] · Dzifa Duose[4] · Russell Broaddus[1] · Gregg Staerkel[1] · Ignacio Wistuba[4] · L. Jeffrey Medeiros[2] · Rajyalakshmi Luthra[2,4]

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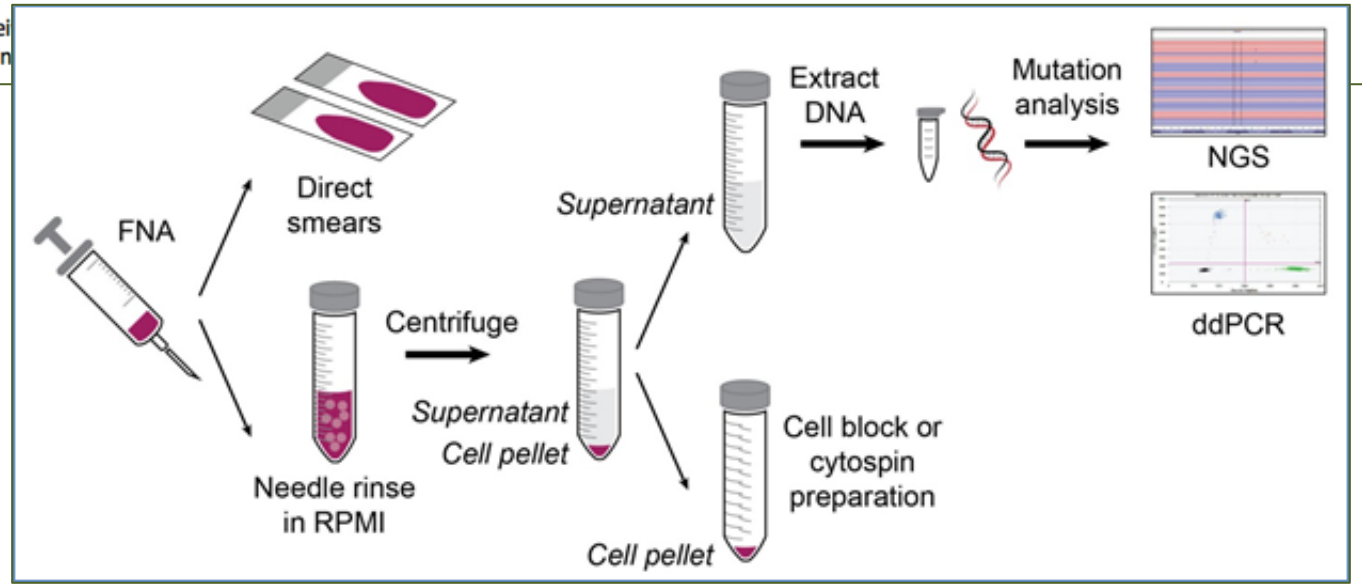


Next Generation Molecular Cytopathology

Liquid biopsy assay for lung carcinoma using centrifuged supernatants from fine-needle aspiration specimens

B. Hannigan[1†], W. Ye[1†], M. Mehrotra[2], V. Lam[3], A. Bolivar[1], S. Zalles[1], B. A. Barkoh[2], D. Duose[4], P. C. Hu[1], R. Broaddus[5], J. Stewart[5], J. Heymach[3], L. J. Medeiros[2], I. Wistuba[4], R. Luthra[2] & S. Roy-Chowdhuri[5*]

Centrifuged Supernatants from FNA Provide a Liquid Biopsy Option for Clinical Next-Generation Sequencing of Thyroid Nodules

Wenrui Ye, PhD[1]; Brette Hannigan, MS[1]; Stephanie Zalles, MS[1]; Meenakshi Mehrotra, PhD[2]; Bedia A. Barkoh[2]; Michelle D. Williams, MD[3]; Maria E. Cabanillas, MD[4]; Beth Edeiken-Monroe, MD[5]; Peter Hu, PhD[1]; Dzifa Duose, PhD[6]; Ignacio I. Wistuba, MD[6]; L. Jeffrey Medeiros, MD[2]; John Stewart, MD, PhD[3]; Rajyalakshmi Luthra, PhD[2]; and Sinchita Roy-Chowdhuri, MD, PhD [3]

Liquid biopsy of fine-needle aspiration supernatant for lung cancer genotyping

Nicolas Guibert[a,b], Hisashi Tsukada[c], David H. Hwang[d], Emily Chambers[b], Edmund S. Cibas[d], Tejus Bale[d], Julianna Supplee[a], Bryan Ulrich[a], Lynette M. Sholl[d], Cloud P. Paweletz[a], Geoffrey R. Oxnard[b,*]

Comparison of Cytocentrifugation Supernatant Fluid and Formalin-Fixed Paraffin-Embedded Tissue for Targeted Next-Generation Sequencing

Nafiseh Janaki, MD [1,2]; Aparna Harbhajanka, MD[1,2]; Claire W. Michael, MD[1,2]; Phillip Bomeisl, DO[1,2]; Jay Wasman, MD[1,2]; Maureen Atchley, BS[1]; Kristina Miskiewicz, BS[1]; David Alouani, PhD[1,2]; and Navid Sadri, MD, PhD [1,2]

Rapid point-of-care testing for epidermal growth factor receptor gene mutations in patients with lung cancer using cell-free DNA from cytology specimen supernatants

SHIHO ASAKA[1,2], AKIHIKO YOSHIZAWA[1,3], KAZUSA SAITO[4], YUKIHIRO KOBAYASHI[1,2], HIROSHI YAMAMOTO[5], TATSUYA NEGISHI[1], RIE NAKATA[1,2], KAZUYUKI MATSUDA[1], AKEMI YAMAGUCHI[6] and TAKAYUKI HONDA[1]

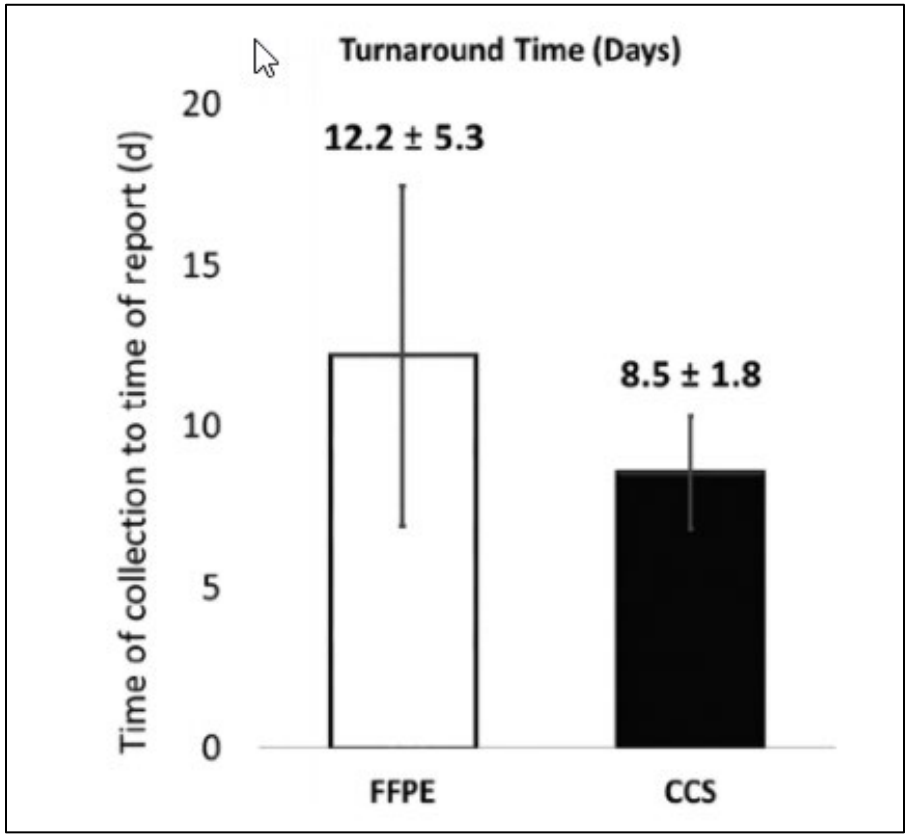
Less is More. Less is Faster and Cheaper

Use of cytology centrifuged supernatants improves cost and turnaround time for targeted next generation sequencing

HamzaN. Gokozan MD[1,2] | Aparna Harbhajanka MD[1,2] | Philip Bomeisl DO[1,2] | Claire W. Michael MD[1,2] | Navid Sadri MD, PhD[1,2]

TABLE 2 Cost savings associated with CCS workflow

FFPE		CCS
Specimen Received		Specimen Received
CB for cytology diagnosis		CB for cytology diagnosis and sample adequacy
Discard CCS		Save CCS
Order molecular testing		Order molecular testing
Retrieve CB	$2 ($24/hr)	
Cut unstained slides off of CB	$2 ($24/hr) $1 (15 unstained slides)	
First and last recut H&E for sample adequacy	$23 fixed	
Slides delivered to second pathologist	$1 ($24/hr)	
Review-adequacy assessment	$15 ($150/hr)	
Unstained Slides delivered to molecular lab		CCS delivered to molecular lab
Macro/microdissection of unstained slides	$4 ($24/hr)	
Deparaffinization	$2 ($24/hr)	
Nucleic acid extraction		Nucleic acid extraction
NGS		NGS
	$50 per case Total Savings	



Molecular Cytopathology Summary

Cytology is an Underutilized Goldmine of Genomic Data

- Lack of awareness regarding utility of cytology specimens for molecular testing
- Lack of standardization across cytology laboratories for specimen processing
- Reluctance of molecular labs to validate a variety of cytologic specimen preparations
- Overall reluctance of cytopathologists to sacrifice irreplaceable cytologic smears from the diagnostic archives

The Pathologist Plays a Key Role in the Success of Molecular Testing

- The pathologist plays a key role in specimen handling that can improve the success of molecular diagnostics

- Be the bridge between the clinical team and the molecular lab

  1. Know your test

  - *Modulate the platform for the specimen* (high analytic sensitivity)

  2. Know your specimen

  - *Modulate the specimen for the platform* (tumor enrichment)

  3. Know your clinical team

  - *Prioritize testing to answer clinically relevant questions*

  4. Know the limitations

  - *Be prepared to make molecular cytopathologic correlations*

The Pathologist Plays a Key Role in the Success of Molecular Testing

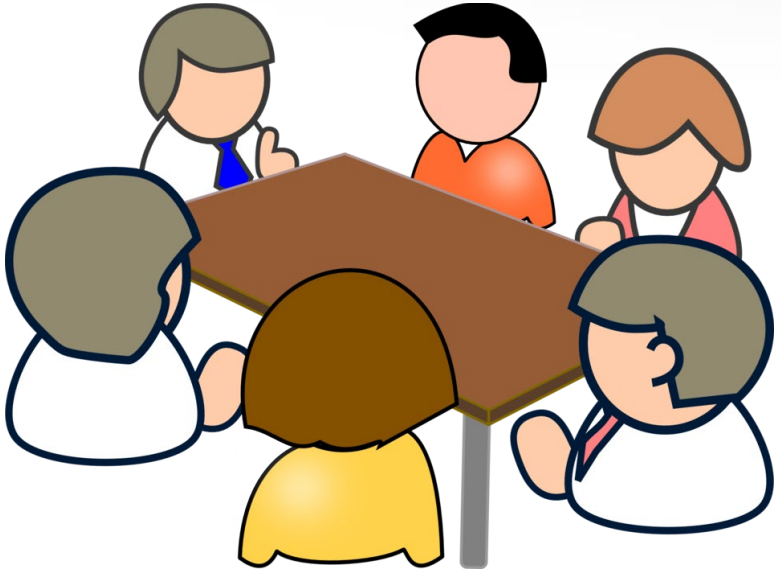
Why does this matter?

- Rendering an **accurate diagnosis**: sampling, accessioning, processing, interpretation

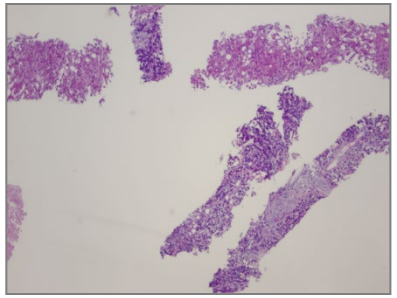
Team work is critical for patient care

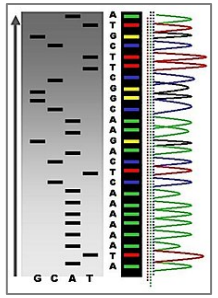
- Molecular testing and reporting also relies on teamwork
- Molecular test results are key determinants for therapeutic decisions and patient outcome

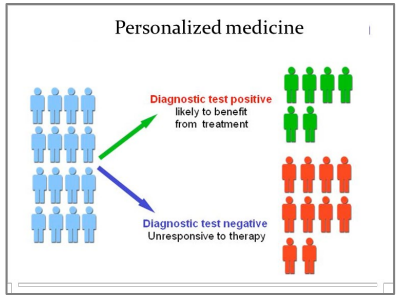
Pathologists Need to be "*Integrative Diagnosticians*"



Greg Fuller, MD PhD







Pathologists are the gatekeepers for ensuring the patient is matched to the appropriate treatment

Thank You

Questions?

@Sinchita_Roy

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MD Anderson Cancer Center
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