

Performance Attributes of Microdissected Slide-Format and Dedicated Needle Aspiration Biopsy Analysis of Indeterminate Thyroid Nodules

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INTRODUCTION:

- Molecular analysis of cytology indeterminate thyroid nodules can help predict biological aggressiveness (benign versus low grade malignancy versus high grade malignancy)
- Two general testing formats are available to choose from consisting of:
 - 1) Separate dedicated needle biopsy/rinse
 - 2) Material from cytology/surgical pathology microscopic slides
- We previously reported that two-stage molecular testing (mutation panel and microRNA classifier) diagnostic yield and performance with needle aspirate/rinse specimens.
- Here we extend the review to the different slide formats that are available, incorporating microdissection to enrich for cells of interest

METHODS:

- Microdissection under stereoscopic guidance was used to select material from archived slides that underwent various preservative treatment
- Microdissected material was tested for mutations using NGS and for miRNAs using qPCR
- Molecular results of microdissected material were compared to those from needle/aspirate rinse specimens

Table 1. Markers in Mutation panel and microRNA classifier panel

Next Generation Sequencing Mutation Panel	
BRAF	BRAF_V600E, BRAF_K601E, BRAF_A598V
HRAS	HRAS_G12V, HRAS_G13R, HRAS_Q61K, HRAS_Q61L, HRAS_Q61R
NRAS	NRAS_G12D, NRAS_G13R, NRAS_Q61R, NRAS_Q61K, NRAS_Q61P
KRAS	KRAS_G12D, KRAS_G12V, KRAS_G13D, KRAS_Q61R
PIK3CA	PIK3CA_E542K, PIK3CA_H1047L, PIK3CA_H1047R
RNA Fusion Transcripts	RET-PTC1
	RET-PTC3
	PAX8-PPAR γ
10 MicroRNA (MiR) Classifier Panel	
Down-regulated	miR-204-5p, miR-139-5p, miR-29b-1-5p, miR-155-5p, miR-138-1-3p
Up-regulated	miR-375, miR-551-b-3p, miR-146b-5p, miR-31-5p, miR-222-3p

Table 2. Slide formats tested for mutation panel and miRNA classifier diagnostic yield

Slide Format	Total (n=206)
Pap stained smear (PS)	64
Diff Quik smear (DS)	53
Unstained FFPE cell block section (CB)	38
Pap stained monolayer prep (MP)	35
Unstained FFPE core biopsy section (Core)	16

Table 3. Diagnostic yield from various slide formats compared to needle aspirate specimens

Format	Insufficiency Rate	
	Mutation Analysis	miRNA Classifier
PS	1.6%	4.7%
DS	1.9%	3.7%
CB	2.6%	2.6%
MP	0.0%	5.7%
Core	0.0%	6.3%
Asp	1.4%	4.7%

- The five slide formats all performed well with low insufficiency rates equivalent to or lower than that seen for dedicated needle aspirates/rinses (Asp) previously reported (n=4503)
- Recourse to slide format testing for insufficient needle aspirate samples enabled full molecular combination testing to be achieved in virtually all cases

CONCLUSIONS:

- **Ancillary two-stage molecular testing combining mutational analysis and miRNA classifier can be accomplished with equivalent effectiveness on dedicated needle aspirates/rinses or by microdissection of all cytology/histopathology slide formats**
- **The ability to evaluate both aspirates and slides enables a definitive result in nearly all patients with indeterminate thyroid nodules**