

Thyroid Cytology Smear Slides: An Untapped Resource for ThyroSeq Testing

Marina N. Nikiforova¹, Marcos Lepe², Lindsey Tolino¹, Abigail Wald¹, Zubair Baloch², Yuri E. Nikiforov¹

¹University of Pittsburgh Medical Center, Pittsburgh, PA ²University of Pennsylvania, Philadelphia, PA

INTRODUCTION

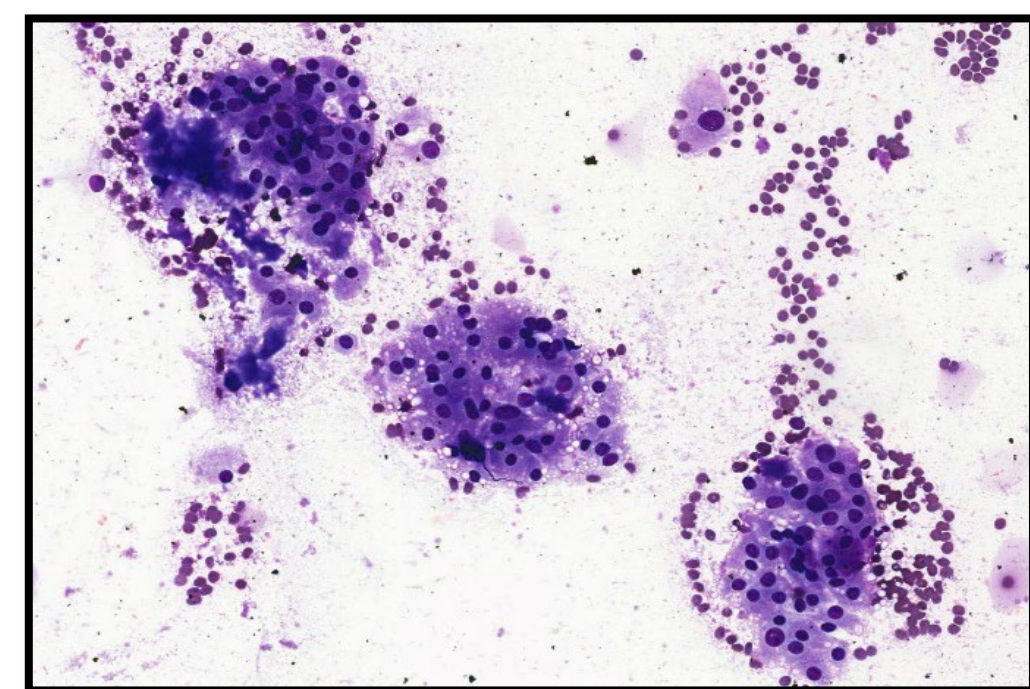
ThyroSeq® v3 Genomic Classifier (GC) is a molecular test used to improve diagnosis in thyroid nodules classified as indeterminate by fine-needle aspiration (FNA) cytology and streamline patient management. FNA samples collected into a preservative solution are an ideal sample type for *ThyroSeq®* testing; however, in some cases FNA smear slides are the only available specimen.

METHODS

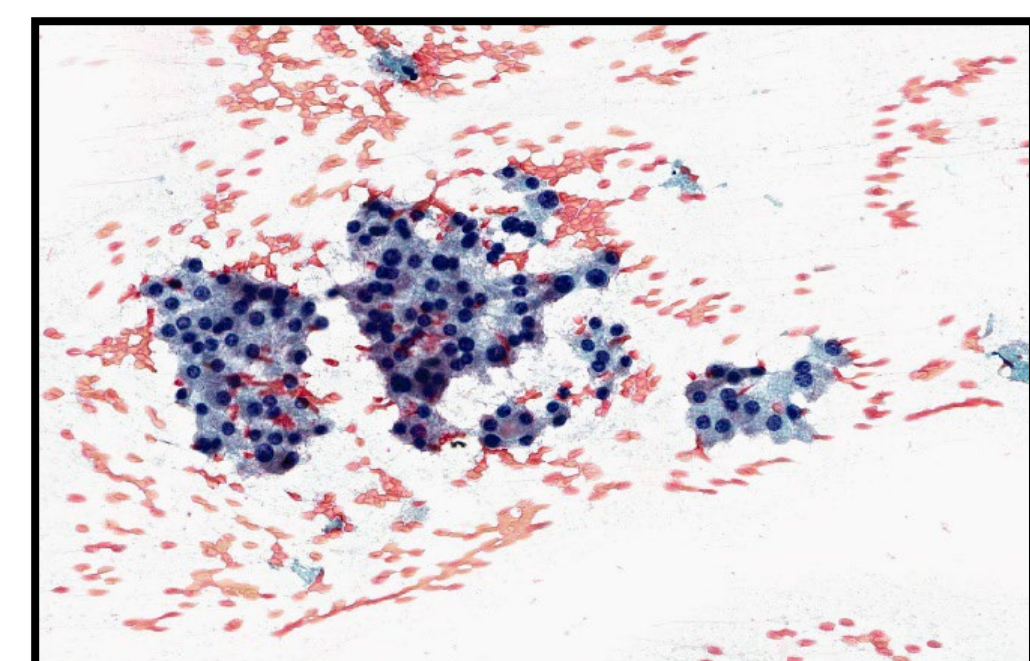
Performance of *ThyroSeq v3 GC* in cytology smears was evaluated by blindly testing 31 routinely prepared FNA cytology smears (17 Diff-Quik® and 14 Papanicolaou stained) from 16 patients with prior *ThyroSeq* results on FNA samples collected directly into preservative solution.

After un-blinding, *ThyroSeq* results of cytology smears were compared with initial FNA results and correlated with surgical outcome.

Routinely Prepared Cytology Smears (n=31, from 16 patients)



Diff-Quik® stained, n=17



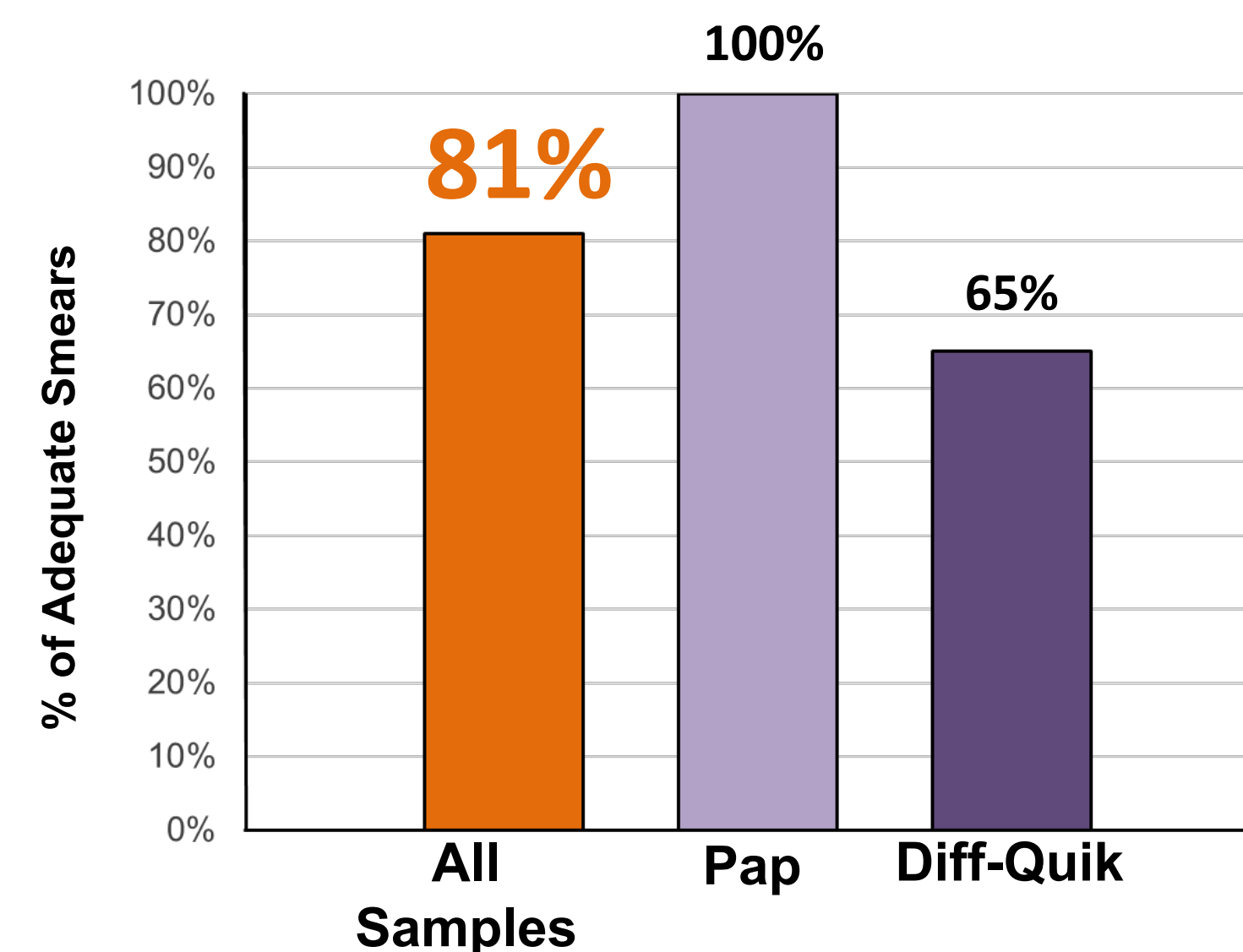
Papanicolaou- stained, n=14

ThyroSeq®
 Thyroid Genomic Classifier

- Blindly tested
- Each FNA smear contained at least 10 cell groups with 10-20 cells per group

RESULTS

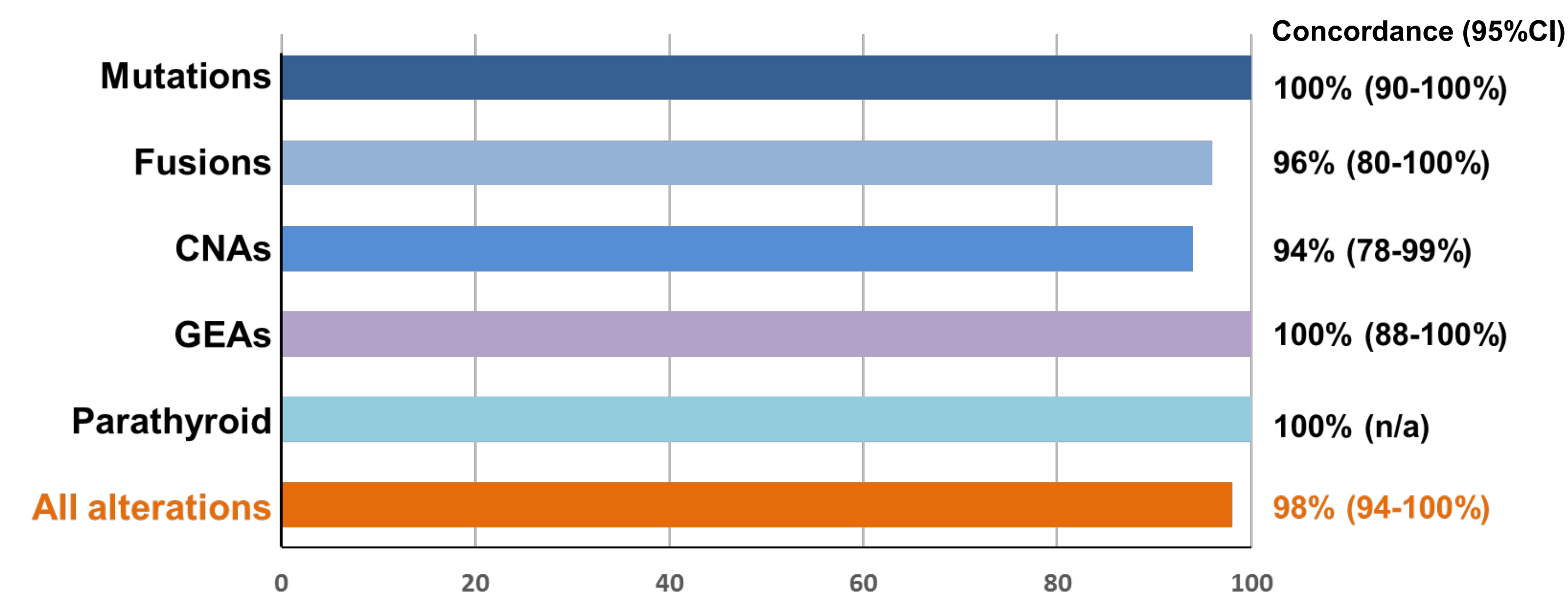
❖ Adequacy of Cytology Smears for ThyroSeq Testing



- 25/31 (81%) smears yielded adequate quantity and quality of nucleic acids for *ThyroSeq* testing
- 14/14 (100%) Papanicolaou stained smears were acceptable for testing
- 11/17 (65%) Diff-Quik were acceptable for testing, 6 failed due to poor RNA quality from cell fixation procedure

❖ ThyroSeq Performance in Adequate Cytology Smears

After un-blinding, results from cytology smears were compared with original FNA results for concordance. All classes of alterations (mutations, fusions, copy number alterations [CNAs], and gene expression alterations [GEAs]) were represented in these samples.



Concordance for detection of all molecular alterations was 98%
 Concordance for Genomic Classifier calls was 100%

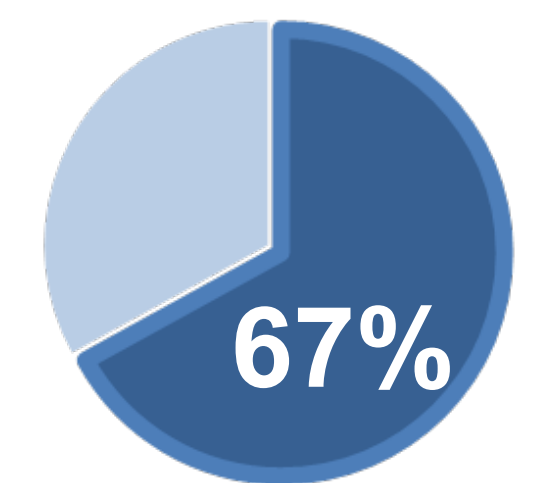
❖ FNA Diagnosis – ThyroSeq Results – Surgical Follow-up

Surgical pathology diagnosis correlation revealed that 67% of thyroid nodules were cancers/NIFTP and 33% benign follicular adenomas. One sample positive for parathyroid was diagnosed as parathyroid adenoma.

Patient ID	Bethesda Category	ThyroSeq Result: FNA	ThyroSeq Result: Smears	Surgical Outcome
1	III	POS	POS	FA
2	IV	POS	POS	FA
3	IV	POS	POS	HCC
4	IV	POS	POS	FVPTC
5	IV	POS	POS	HCA
6	III	POS	POS	PTC
7	IV	POS	POS	FA
8	III	POS	POS	FVPTC
9	IV	POS	POS	FA
10	IV	POS	POS	PTC
11	V	POS	POS	PTC & FVPTC
12	IV	POS	POS	PA
13	IV	POS	POS	PTC
14	III	POS	POS	FVPTC
15	V	POS	POS	NIFTP
16	IV	POS	POS	NIFTP

Positive Predictive Value

Thyroid Nodules



Parathyroid Nodule



FA, follicular adenoma; HCA, Hürthle cell adenoma; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; PTC, papillary thyroid cancer; FVPTC, follicular-variant papillary thyroid cancer; HCC, Hürthle cell carcinoma; PA, parathyroid adenoma

CONCLUSIONS

- ✓ Routinely prepared thyroid FNA cytology smears with adequate cellularity can be successfully employed for *ThyroSeq GC* testing in ~80% of cases
- ✓ A higher adequacy for testing was seen in Papanicolaou stained smears as compared to Diff-Quik® stained smears
- ✓ All types of genetic alterations and Genomic Classifier were accurately (98-100%) classified by *ThyroSeq* in thyroid cytology smears
- ✓ *ThyroSeq* testing of routine cytology smears may eliminate the need to repeat FNA biopsies for patients with indeterminate cytology