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ThyroSeq® Thyroid Genomic Classifier

Performance of ThyroSeq v3 Genomic Classifier in Fixed Cytology Smears

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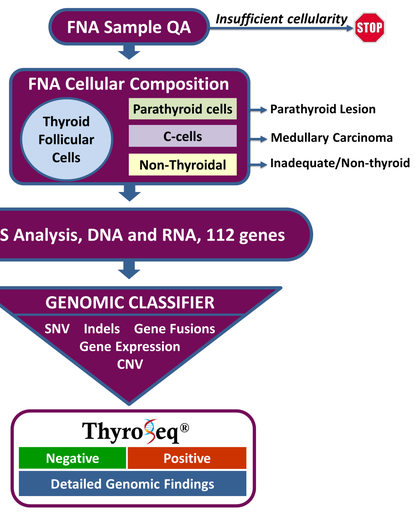
INTRODUCTION

ThyroSeq v3 Genomic Classifier (GC) is a molecular test used to improve diagnosis in thyroid nodules with indeterminate fine-needle aspiration (FNA) cytology and inform patient management.

Scheme of ThyroSeq Test Flow



FNA samples collected into a dedicated preservative solution vial is a standard sample type for ThyroSeq testing. However, in some cases, routine FNA smears are the only specimen type available.

AIMS

To evaluate ThyroSeq v3 GC analytical performance in:

1. FNA smears prepared for cytopathologic interpretation
2. Reference cytology slides with gene mutations at varying allelic frequencies

METHODS

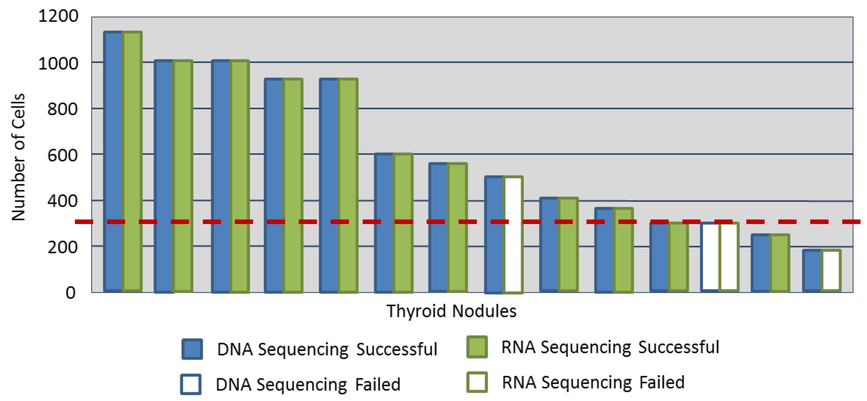
ThyroSeq v3 GC analysis was performed on:

- 33 clinical routinely prepared Diff-Quik® or Papanicolaou (PAP) stained slides, with ~200 to 1000 cells per slide, from 14 nodules
- Four reference cytology slides (Horizon Diagnostics) with various mutation frequency

Results were compared to FNAs collected into preservative solution (ThyroSeq*Preserve*)

RESULTS

ThyroSeq Performance in Cytology Smears (n=33, 14 nodules)



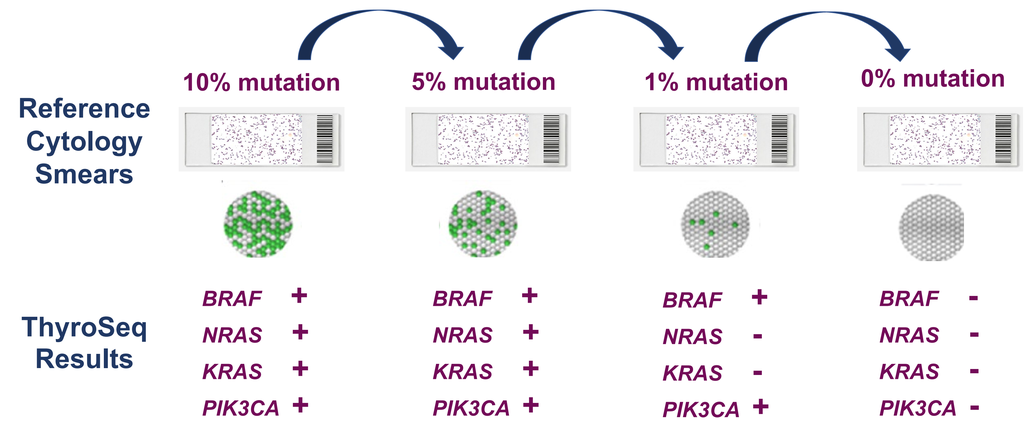
- In cytology smears, adequate DNA results for mutations and copy number alterations were obtained in 93% (13/14) of samples and adequate RNA results for gene fusions and gene expression in 79% (11/14) of samples.
- Two smears with <200-300 cells showed failure of RNA analysis.
- One old cellular smear (6 yrs) failed RNA due degraded nucleic acids.

Comparison of Test Informative Rate Using Cytology Smears vs. Dedicated Collection Tube

FNA Preparation	Informative Result Rate	Required Cellularity	Turnaround Time
 Fixed cytology smears (Diff-Quik, Pap)	79-93%	200-300 cells	8-9 days
 FNA in ThyroSeq*Preserve*	96%	200-300 cells	5-7 days

- The rate of informative results was higher in FNAs collected into preservative solution as compared to cytology smears (79-93% vs. 96%).
- The turnaround time was 1-3 days longer due to additional preparatory steps for cytology smears as compared to FNA samples collected into preservative solution.

Accuracy of Detection of Mutations in Reference Cytology Smears from World-Wide Ring Trial Study[1]



- Unstained reference cytology slides (Horizon Diagnostics) used in a Worldwide Ring Trial Study[1] with *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations at various frequency (10%, 5%, 1%, and 0%) were used to evaluate the accuracy of mutation detection in cytology smears.
- ThyroSeq detected all mutations down to 5% and *BRAF* and *PIK3CA* mutations down to 1% allele frequency. No mutations were detected in the negative control slide.

CONCLUSIONS

- The results of this study provide evidence that ThyroSeq v3 GC testing can be effectively performed using routinely prepared cytology smears
- Overall, the test informative results were obtained in 79-93% of cytology smears
- The minimum required cellularity of cytology smears is 200-300 cells
- Both PAP-stained and Diff-Quik-stained smears can be used
- Gene mutations could be reliably detected down to 5% allele frequency
- Additional studies are underway to provide further analysis of cytology smear adequacy for ThyroSeq testing

REFERENCES

1. Malapelle U, et al. Consistency and reproducibility of next-generation sequencing and other multigene mutational assays: A worldwide ring trial study on quantitative cytological molecular reference specimens. *Cancer Cytopathol.* 2017 Aug;125(8):615-626.
2. Nikiforova MN, et al. Analytical Performance of the ThyroSeq v3 Genomic Classifier for Cancer Diagnosis in Thyroid Nodules. *Cancer.* 2018 Apr;124(8):1682-1690.