SAT-630

Analytical Validation of a Telomerase Reverse Transcriptase (*TERT*) Promoter Mutation Assay

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INTRODUCTION

- Telomeres are condensed DNA-protein structures on the ends of chromosomes. Over the life of a cell, telomeres shorten with cell division, leading to DNA damage and decreased cellular proliferation. Telomerase activation, which can occur with *TERT* promoter mutations, is a phenomenon cancer cells use to maintain telomere length and induce cell immortality.[1,2]
- *TERT* promoter mutations are relatively enriched in larger, aggressive thyroid cancers and these malignancies carry an independently decreased rate of disease free and disease specific survival when compared to thyroid cancers with wild type *TERT* promoter gene sequences.[3]
- High quality analytical validation of a diagnostic test promotes confidence in the results which inform clinical decision making. *TERT* promoter mutation testing is now offered as a complement to Afirma Genomic Sequencing Classifier (GSC) testing in thyroid nodules that are not designated as molecularly benign.

METHODS

- Assay development is based on the AmpliSeq-for-Illumina technology to detect *TERT* promoter variants C228T and C250T in genomic DNA (gDNA) extracted from patient thyroid fine needle aspirates (FNAs) sent for Afirma GSC testing.
- Determinations included:
  - The analytical sensitivity of the Afirma *TERT* test to varied input DNA amounts and the limit of detection (LOD) of variant allele frequency (VAF).
  - The negative percent agreement (NPA) and the positive percent agreement (PPA) of the Afirma *TERT* test against a reference primer pair.
  - The analytical specificity from potential interfering substances such as RNA and blood gDNA.
  - The intra-run, inter-run and inter-laboratory reproducibility of the assay.

RESULTS

- Analytical sensitivity analysis:
  - The Afirma *TERT* promoter test is tolerant to variation in DNA input amount (7–13 ng) and can detect expected positive *TERT* promoter variants down to 5% VAF LOD at 7ng DNA input with > 95% sensitivity **(Figure 1)**.
- Both NPA and PPA were 100% against the reference primer pair.
- Analytical specificity analysis:
  - The Afirma *TERT* promoter test remains accurate in presence of 20% RNA or 80% blood gDNA contamination for an average patient sample that typically has 30% VAF **(Figure 2)**.

RESULTS — Cont'd.

- Reproducibility studies demonstrated that the Afirma *TERT* promoter test is highly reproducible across different operators, runs, reagent lots, and laboratories.
- There was a 100% confirmation rate when compared with an external NGS-based reference assay executed in a non-Veracyte laboratory **(Figure 3)**.
- The red line in each graph indicates the threshold above which there is a positive result called. In figure 1a, the orange line represents the 5% VAF.

FIGURE 1. ANALYTICAL SENSITIVITY RESULTS

a. 2 *TERT* positive cell line gDNAs diluted to 5% VAF, 11 replicates at each input level (7ng, 10ng, 13ng) 10ng is the nominal input
b. 4 *TERT* positive patient samples, 5%-17.5% VAF, 3 replicates at each input level (7ng, 10ng, 13ng)

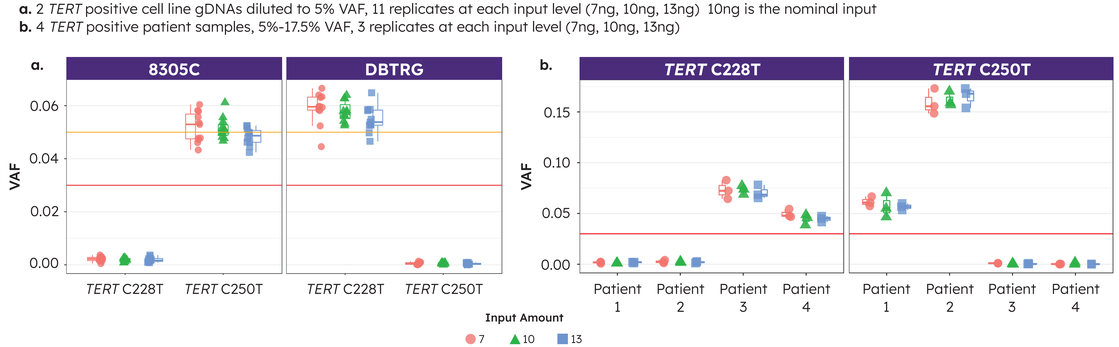


FIGURE 2. ANALYTICAL SPECIFIC RESULTS

a. *TERT* variant detection status of all patient sample replicates at 10% RNA and 20% RNA contamination are concordant with expectation.
b. *TERT* variant detection status of all replicates at all added blood levels (0%, 20%, 40%, 60%, 80%) are concordant with expectation for patient samples with ≥30% VAF.

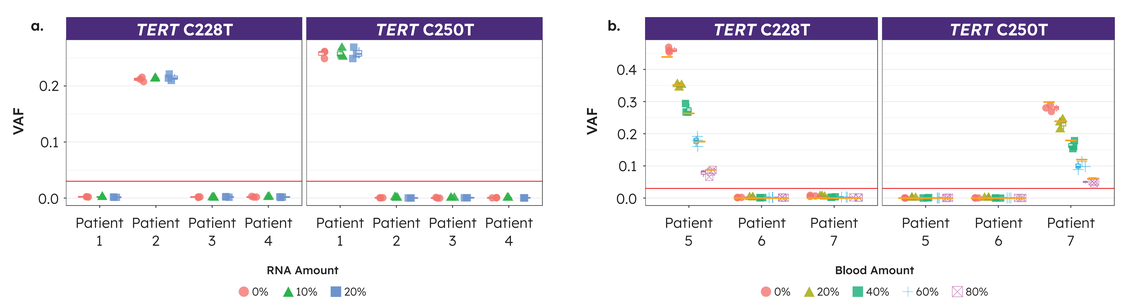
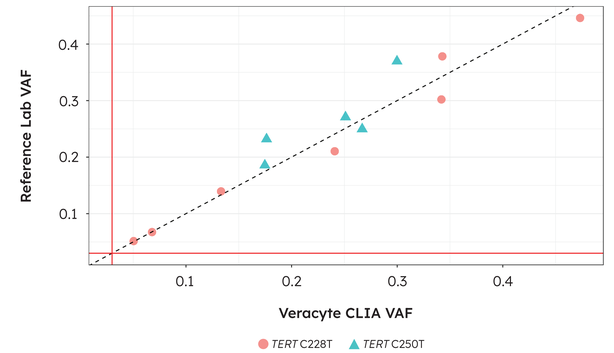


FIGURE 3. EXTERNAL CONFIRMATION

The reference lab uses a capture-based NGS assay interrogating 638 genes. In 12 patient samples, 100% of C228T and C250T *TERT* promoter mutations were detected and correlated with the Veracyte assay.



CONCLUSION

The analytical robustness and reproducibility of the Afirma *TERT* test support its routine clinical use among thyroid nodules with indeterminate cytology that are Afirma GSC suspicious or Bethesda V/VI nodules.

REFERENCES

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