Detection of TERT C228T and C250T Promoter Mutations Across Different Cancer Types Using IDNAPTEX Assays on QIAcuity

Abstract

This study assesses the analytical performance of the IDNAPTEX® TERT assay on the QIAcuity® Digital PCR System. Performance metrics include limit of blank (LoB) and comparison to a reference detection method (digital droplet PCR, ddPCR) for formalin-fixed, paraffin-embedded (FFPE) samples.

The IDNAPTEX TERT assay, used in combination with the QIAcuity Digital PCR System, intended for research use only applications, provides a robust, sensitive and specific solution for detecting well-characterized TERT C228T and C250T promoter mutations across multiple tumor types.

Introduction

TERT promoter mutations (primarily C228T and C250T) are recurrent genetic alterations found in multiple cancer types (5), including gliomas (1, 3 ,7), thyroid carcinoma (1, 6, 9), melanomas (2, 8) and bladder cancer (1, 4, 10). Their presence is often associated with aggressive clinical behavior and poor prognosis. In thyroid cancer and melanoma, TERT mutations may have enhanced predictive value when they co-occur with BRAF V600E (8, 9). Sensitive detection of these mutations, whether in tumor DNA or plasma cell-free DNA (cfDNA), enables better risk stratification and tailored clinical decisions (4, 10).

To ensure robust analytical performance of the IDNAPTEX TERT assay for detecting C228T and C250T variants in TERT promoter, the following analysis was carried out:

- Established analytical performance by assessing sensitivity, precision and accuracy using a diverse panel of mutation-positive and wild-type (WT) samples across a range of allele frequencies.
- Compared results against the reference method to assess the concordance, which was determined using metrics such as positive predictive value (PPV), negative predictive value (NPV) and overall percent agreement (OPA).

- Determined the LoB with sufficient replicates of WT samples (≥30 for LoB).

Materials and methods

The analysis for this study was performed by the Istituti Fisioterapici Ospitalieri (IFO) in Rome, Italy.

Sample types: Genomic DNA from glioma FFPE tissue

DNA extraction kits: Manual isolation with the AllPrep® DNA/RNA FFPE Kit (QIAGEN)

Extraction kits and IDNAPTEX assays were used according to the manufacturers' instructions.

Assays: IDNAPTEX TERT, for Research Use Only applications

Digital PCR platform: QIAGEN QIAcuity Digital PCR System, for Research Use Only applications

Analytical limit determination

To determine the detection capabilities of the assays, the CLSI EP17-A2 guideline was followed for estimating the LoB and limit of detection (LoD). These detection limits are verified using matrix-matched samples and replicate measurements, ensuring robustness and compliance in a research setting.

Limit of blank (LoB): For mutation detection assays in a WT background, analytical noise will increase in proportion to the amount of WT DNA present. Therefore, instead of using pure blanks (no template controls), LoB was calculated using WT-only samples at concentrations representative of clinical conditions.

The CLSI EP17-A2 guideline recommendation is to use at least 15 WT samples that do not carry the target mutations. These should be analyzed in duplicate across two independent sessions, resulting in a total of 30 determinations. The number of false positive partitions (mutation channel) is recorded. The LoB is then calculated and expressed in variant allele frequency (VAF) based on the determined signal for the mutation and the WT. The LoB can be estimated using either a parametric or non-parametric approach, as outlined in CLSI EP17-A2. The parametric method assumes a normal distribution of blank measurements and calculates LoB as the mean plus 1.645 times the standard deviation. In contrast, the non-parametric approach does not rely on distributional assumptions and defines LoB as the 95th percentile of blank replicates. Based on the samples available in this study, the parametric approach was chosen.

This VAF-based LoB provides a practical detection threshold for interpreting results under typical sample conditions.

Limit of detection (LoD): The LoD is calculated as the lowest concentration of mutant DNA that yields ≥95% positive detection above the established LoB. The LoD is typically determined using spiked-in synthetic mutants at low frequencies in a high WT background and validated on ≥20 replicates to confirm the required detection performance.

To achieve reliable detection of mutations at low frequencies (e.g., 0.1% VAF), a minimum of 20,000 total copies per reaction is generally required. If necessary, the sensitivity can be further enhanced by merging the data from multiple wells (technical replicates), effectively increasing the number of partitions and improving the detection probability of rare events.

In this study, samples with low mutation frequencies were not available. Therefore, no data can be shown for the LoD.

Comparison of detection methods used in this study

For this study, 15 matched samples were tested using the IDNAPTEX TERT assay.

Results were classified as positive or negative for each targeted TERT mutation.

Concordance metrics: PPV, NPV, OPA

VAF values were compared quantitatively via regression analysis.

Statistical analysis

LoB was determined using the parametric approach where LoB = Mean (WT) + 1.645 × SD using the VAF.

Comparison of detection methods:

The qualitative metrics PPA (positive percentage agreement), NPA (negative percentage agreement), and OPA (overall percentage agreement) were determined using the data from the WT and mutated samples, and linear regression was applied for quantitative comparison.

Results

1. Method concordance assessed by linear regression

To compare the IDNAPTEX TERT assay run on the QIAcuity dPCR system with the reference ddPCR method, the VAF values determined with the IDNAPTEX TERT assay were plotted against the VAF values obtained by ddPCR. In this study, 15 mutation-positive samples were measured in duplicate. For the linear regression analysis, the mean value was used. The linear regression plot in Figure 1 shows a strong correlation between the IDNAPTEX TERT assay and ddPCR across both C228T and C250T mutations. In Figure 2, results for the two mutations are shown separately. Detection of the TERT C228T mutation showed higher concordance compared to C250T.

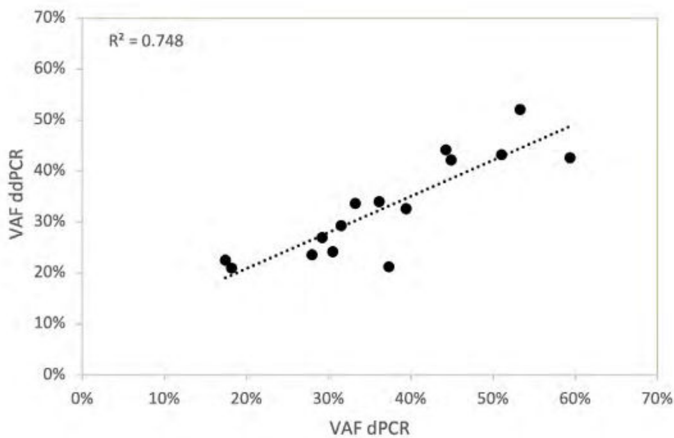
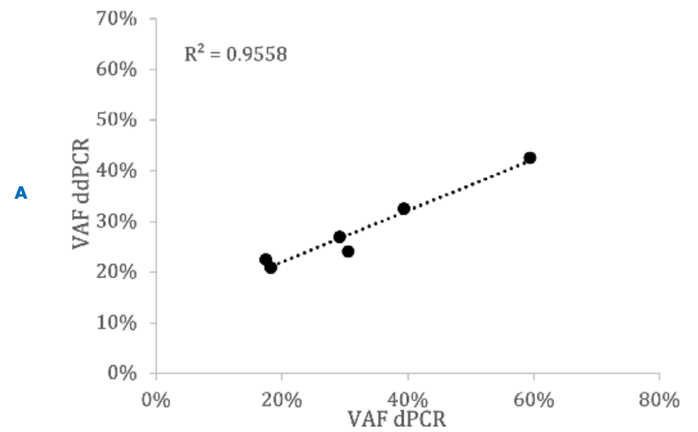


Figure 1

Linear regression between dPCR and ddPCR for the VAF.

Correlation plot showing a positive correlation between dPCR and ddPCR, displaying the agreement between the two methods.

A



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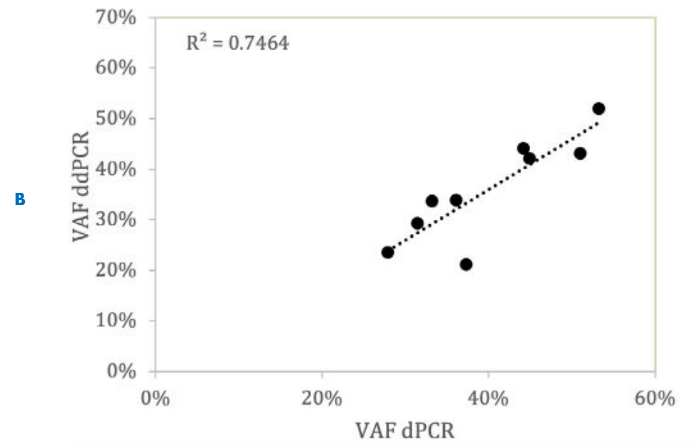

Figure 2

Linear regression between dPCR and ddPCR for the VAF for TERT C228T (A) and C250T (B).

Correlation plot showing a positive correlation between dPCR and ddPCR, displaying the agreement between the two methods.

2. Performance metrics

To confirm the assay's analytical performance, especially for mutation screening in FFPE material, performance metrics were determined (Table 1). All mutation-positive samples were correctly identified, and no mutations were missed in this study, resulting in PPA of 100% and NPV of 100%. Furthermore, with the exception of one sample with a VAF under 1% and close to the determined LoB, 13 WT samples were correctly classified by the calculated LoB for both mutations. Therefore, the NPA in this study was 92.86% and the predictive value 93.75%, confirming a high accuracy in identifying true negatives and high confidence in positive calls. Overall, the metrics showed a strong overall classification with an accuracy of 96.55%.

Table 1

PPA, NPA and predictive values for TERT.

The statistical measures of performance were calculated with the proportion of actual positives and actual negatives.

Classification	True Negative	True Positive	False Positive	False Negative
Samples n	13	15	1	0
NPA	92.86%			
PPA	100.00%			
Positive predictive Value	93.75%			
Negative predictive Value	100.00%			
Accuracy	96.55%			

Conclusion

The IDNAPTEX TERT assay run on the QIAcuity digital PCR platform demonstrated excellent agreement with the reference method, ddPCR, for both mutation classification and quantitative VAF measurement. The assay's performance supports its use in translational research settings. Furthermore, except for a single sample with a VAF below 1% and close to the LoB, all 13 WT samples were correctly classified as negative for both mutations. Such borderline variation is commonly observed with FFPE-derived DNA and could be further minimized by defining the LoB using a larger set of WT samples.

Mutations in the TERT promoter, particularly C228T and C250T, are recurrent in several solid tumors and can serve as clinically actionable biomarkers for diagnosis, prognostic stratification or disease monitoring. While most studies rely on tumor tissue, access to high-quality FFPE samples can be limited or invasive. Exploring the detection of TERT promoter mutations in alternative, less invasive matrices such as urine (for bladder cancer), cerebrospinal fluid (CSF, for gliomas) or fine-needle aspiration (FNA, for thyroid nodules or lymph nodes) could broaden the applicability of such assays and support personalized patient management when surgical biopsy is not feasible.

A future study could evaluate the performance of the IDNAPTEX TERT assay on the QIAcuity dPCR platform for detecting promoter mutations in cfDNA or cellular DNA extracted from these alternative sources. By comparing mutation detection rates and allelic frequencies across matrices and correlating them with known tumor mutation status, the study would assess the feasibility and added value of TERT mutation testing beyond conventional tumor biopsies.

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