Detection of KRAS Variants in Exons 2, 3 and 4 from Liquid Biopsy and Tissue Samples Using IDNAPTEX Assays on QIAcuity

Abstract

This study evaluates the analytical performance of two IDNAPTEX® assays, KRAS G12C and KRAS 3X (targeting mutations in exons 2, 3 and 4), on the QIAcuity® Digital PCR System, intended for research use only applications. Analytical performance metrics include limit of blank (LoB) and comparison to a reference detection method (next-generation sequencing, NGS) for formalin-fixed, paraffin-embedded (FFPE) tissue, fresh-frozen tissue and cell-free DNA (cfDNA) samples.

The IDNAPTEX KRAS G12C assay, used in combination with the QIAcuity Digital PCR System, provides a robust, sensitive and precise method for detecting KRAS G12C mutations for research purposes. This enables reliable identification of the KRAS G12C variant, a recurrent driver mutation in non-small cell lung cancer (NSCLC) and colorectal cancer (CRC), that may be matched with targeted therapies. The IDNAPTEX KRAS 3X assay on QIAcuity provides a robust and targeted solution for KRAS mutation profiling in exons 2, 3 and 4. Identification of these variants may support research into treatment stratification and sensitive post-treatment surveillance.

Introduction

KRAS G12C is a recurrent driver mutation in CRC and NSCLC (1-4) that leads to constitutive activation of the RAS/MAPK signaling pathway (3, 4). This mutation is now targetable by novel covalent inhibitors, including sotorasib and adagrasib (4, 5, 8, 9). Accurate detection of KRAS G12C in tumor tissue or cfDNA is essential for identifying patients eligible for these targeted therapies (6). Digital PCR (dPCR) offers a highly sensitive and quantitative approach for detecting this mutation, especially in cfDNA where mutant alleles are present at low frequency (7).

In addition, KRAS mutations in exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146) occur frequently in CRC (11) and are strong negative predictors for the efficacy of anti-EGFR therapy (10, 15, 16). Comprehensive detection of these alterations is essential for treatment planning and prognosis, particularly in metastatic CRC. dPCR enables highly sensitive mutation detection from plasma-derived cfDNA or FFPE samples (12, 14), which is also valuable for longitudinal

monitoring and assessment of minimal residual disease (MRD) following treatment (13).

In CRC, mutations in KRAS – particularly in exons 2, 3 and 4 – are not only predictive biomarkers for anti-EGFR therapy resistance, but also stable clonal markers suitable for disease tracking (17, 18). After surgical resection or systemic therapy, the persistence or reappearance of KRAS-mutated cfDNA in plasma may indicate microscopic residual disease or early relapses, often months before radiological signs (13, 19). Similarly, in metastatic settings, dynamic changes in KRAS mutation burden in cfDNA may reflect therapeutic response or clonal evolution under treatment pressure (14, 18).

To ensure robust analytical performance of the IDNAPTEX KRAS G12C and IDNAPTEX KRAS 3X assays with real-world samples, 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 (NGS) 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 limit of blank (LoB) with sufficient replicates of WT samples (≥30 for LoB).
- Evaluated the assay's ability to distinguish KRAS G12C from other common KRAS codon 12 mutations (e.g., G12D, G12V), ensuring high analytical specificity.

Materials and methods

For this study, two sites performed the analysis for KRAS G12C: The Landeskrankenhaus Feldkirch in Feldkirch, Austria and the Istituti Fisioterapici Ospitalieri in Rome, Italy. The former will be referred to as Site 1 and the latter as Site 2 in the following sections. The analysis for KRAS 3X was solely performed by the Istituti Fisioterapici Ospitalieri.

Sample types: cfDNA extracted from the plasma of CRC patients (metastatic or post-surgical) and DNA extracted from FFPE tumor tissue and fresh-frozen tissue with known KRAS mutations.

DNA extraction kits: The DNA at Site 1 was isolated from FFPE material with the QIAamp® DNA FFPE Advanced Kit and from plasma samples with the QIAamp Circulating Nucleic Acid Kit (QIAGEN).

The DNA at Site 2 was isolated with the AllPrep® DNA/RNA FFPE Kit for FFPE and AllPrep DNA/RNA Kit for fresh frozen tissue (QIAGEN).

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

Assays: IDNAPTEX KRAS G12C, IDNAPTEX KRAS 3X, for Research Use Only applications

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

Analytical limit determination

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 at both sites. Therefore, no data can be shown for the LoD.

Comparison of detection methods used in this study

For Site 1, 27 KRAS G12C-positive samples were tested using the IDNAPTEX KRAS G12C assay.

For Site 2, 11 positive samples were tested using IDNAPTEX KRAS G12C and 15 mutation-positive samples were tested with both IDNAPTEX KRAS 3X assays.

Results were classified as positive or negative for each targeted KRAS 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 for KRAS G12C

1. Method concordance assessed by linear regression

To compare the IDNAPTEX assays with the reference NGS method, the VAF values determined with the IDNAPTEX KRAS G12C assay were plotted against the VAF values obtained by NGS (Figure 1). At Site 1, 27 KRAS G12C-positive samples with VAF values ranging from 9% to 66% were analyzed. An additional 28 samples were tested in which NGS reported 0% VAF for KRAS G12C. At Site 2, 11 KRAS G12C-positive samples (2–66% VAF) were tested, with each sample measured in duplicate; the mean value was used for linear regression. The linear regression plot in Figure 1 shows a positive correlation between the IDNAPTEX KRAS G12C assay and the reference method for both sites.

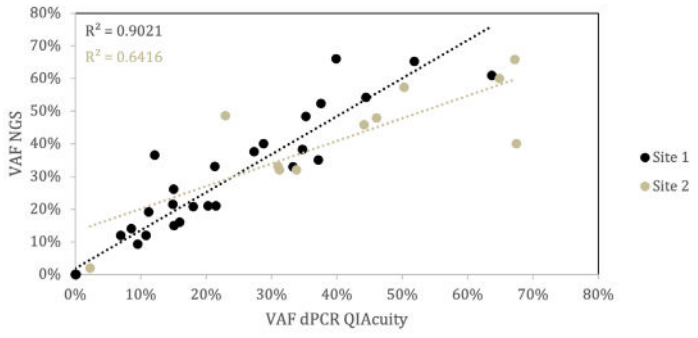

Figure 1
Linear regression comparing dPCR and NGS VAF values from two independent sites.
Correlation plot showing a positive correlation between dPCR and NGS, displaying the agreement between the two methods.

2. Performance metrics for KRAS G12C

Analytical performance metrics were determined for KRAS G12C (Table 1). For both testing sites, the mutation-positive samples were correctly identified as true positives, resulting in a PPA and NPA of 100%. Furthermore, all WT samples were correctly classified as true negatives verified by the calculated LoB. In addition, all samples that were positive for other mutations (other KRAS mutations, EGFR, BRAF, NRAS) could be classified as true negatives according to the calculated LoB. The OPA for both sites was 100%.

Table 1

PPA, NPA and predictive values for Site 1 and Site 2 for KRAS G12C.

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

	Site 1	Site 2
Number of total samples	55	30
True positives	26	11
True negatives	29	19
NPA	100%	100%
PPA	100%	100%
Positive Predictive Value	100%	100%
Negative Predictive Value	100%	100%
Overall Percentage Agreement	100%	100%

3. Cross-reactivity with other KRAS G12 mutations

Site 1 also analyzed 16 samples with alternative mutations in the KRAS gene. The mutation rates for these samples ranged between 10% and 53% VAF, as determined by NGS. Based on the calculated LoB, all tested samples could be classified as negative for the G12C mutation measured with the IDNAPTEX KRAS G12C on the QIAcuity system. This confirms that there is no cross-reactivity with the variants listed below.

VAF % dPCR	VAF % NGS	Mutation
0.03%	26.00%	KRAS G12D, TP53
0.00%	31.00%	KRAS G12A, TP53 L43
0.00%	17.00%	KRAS G13D
0.00%	11.00%	KRAS Q61R
0.02%	10.00%	KRAS G12D
0.03%	21.00%	KRAS A59T
0.01%	14.00%	KRAS G13D, AKT1 E17K, TP53
0.00%	39.00%	KRAS G12V, TP53 V157F
0.09%	13.00%	KRAS G12D
0.05%	37.00%	KRAS G12R
0.01%	53.00%	KRAS G13V
0.12%	34.00%	KRAS G13D, TP53 C135F
0.01%	40.00%	KRAS G12A, TP53 L43
0.00%	23.00%	KRAS G12A, TP53 L43
0.01%	38.00%	KRAS G12D
0.02%	25.00%	KRAS K117N

4. Correlation of DNA input and analyzed concentration

To determine the correlation between DNA input and the concentration in copies/µL, Site 1 analyzed one sample with a VAF of 38% using different DNA inputs ranging from 1 ng to 200 ng. As shown in Figure 2, there was a high correlation between the input amount and the measured concentration for both the WT and the mutation.

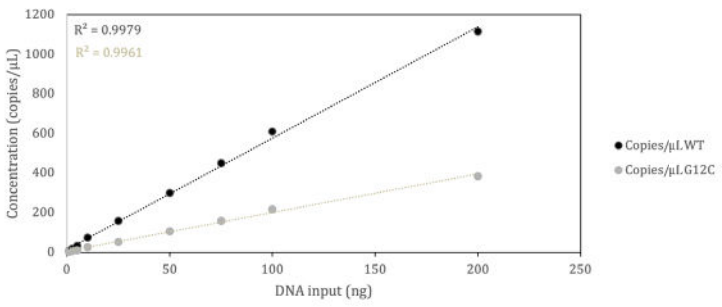

Figure 2

Linear regression between different DNA inputs and the concentration measured by dPCR.

Correlation plot showing a high correlation between DNA input and the measured concentration. Analysis was performed at Site 1 using one of the samples with 38% MAF for KRAS G12C.

Results for KRAS 3X

1. Method concordance assessed by linear regression

The IDNAPTEX KRAS 3X assay was compared against the reference NGS method by plotting the VAF values determined with the IDNAPTEX assay against the VAF values obtained by NGS. Site 2 used 15 samples positive for KRAS mutations in exons 2, 3 and 4, with each sample measured in duplicate. For the linear regression analysis, the mean values were used. The linear regression plot in Figure 3 shows a strong correlation between the IDNAPTEX KRAS 3X assay and the reference method.

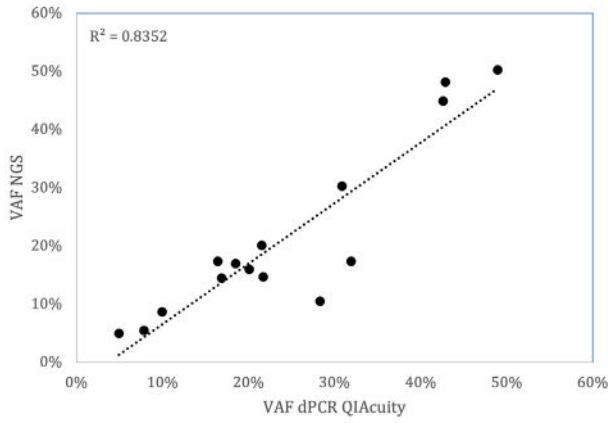

Figure 3
Linear regression between PCR and NGS for the VAF for KRAS 3X.
Correlation plot showing a high correlation between DNA input and the measured concentration. Analysis was performed at Site 1 using one of the samples with 38% MAF for KRAS G12C.

2. Performance metrics

To confirm the assay's analytical performance, especially for mutation screening in FFPE samples and fresh frozen tissues, performance metrics were determined (Table 2). All mutation-positive samples were correctly identified, with no mutations missed, resulting in PPA of 100% and NPV of 100%. Fourteen WT samples were correctly classified as true negatives, with only one sample, whose VAF was close to the calculated LoB, misclassified. The resulting NPA was 93.33% and the PPV was 93.75%, indicating high accuracy in identifying true negatives and strong confidence in positive calls. The overall classification accuracy was 96.67%.

Table 2
PPA, NPA and predictive values for KRAS 3X.
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	14	15	1	0
NPA	93.33%			
PPA	100.00%			
Positive predictive Value	93.75%			
Negative predictive Value	100.00%			
Accuracy	96.67%			

Conclusion

The results of this study demonstrate that the IDNAPTEX KRAS G12C and KRAS 3X assays, when deployed on the QIAcuity digital PCR platform, offer high PPA, NPA and quantitative accuracy for detecting KRAS mutations in CRC and NSCLC for research purposes. The strong concordance with NGS, a common alternative approach for mutation detection, demonstrates that these assays perform reliably across multiple sample types, including FFPE, fresh-frozen tissue and plasma-derived cfDNA, and support their use in translational research settings.

The quantitative accuracy and sensitivity of these assays, combined with the QIAcuity digital PCR platform, suggest that detection of KRAS G12C mutations in early-stage cancers (Stage I–II) using cfDNA may be feasible. This supports potential future applications in early detection or post-surgical MRD monitoring.

A future study could evaluate the utility of KRAS cfDNA quantification using the IDNAPTEX KRAS CRC panel on the QIAcuity dPCR platform for detecting MRD after curative surgery or for tracking mutation dynamics during systemic treatment in metastatic CRC. Serial plasma sampling may enable earlier relapse prediction and provide real-time assessment of therapeutic response.

References

1. Strickler JH, Yoshino T, Stevinson K, Eichinger CS, Giannopoulou C, Rehn M, Modest DP. Prevalence of KRAS G12C Mutation and Co-mutations and Associated Clinical Outcomes in Patients With Colorectal Cancer: A Systematic Literature Review. The Oncologist. 2023;28(11):e981–e994. doi:10.1093/oncolo/oyad138
2. Niesner ICC, Balbi KJ, Poskitt B, et al. Prevalence and breakdown of KRAS driver mutations in a large UK non-small cell lung cancer cohort. J Clin Pathol. 2025;78(5):346–351. doi:10.1136/jcp-2024-209972
3. Araujo LH, Souza BM, Leite LR, et al. Molecular profile of KRAS G12C-mutant colorectal and non-small-cell lung cancer. BMC Cancer. 2021;21:193. doi:10.1186/s12885-021-07884-8
4. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J Med. 2021;384(25):2371–2381. doi:10.1056/NEJMoa2103695
5. Jänne PA, Riely GJ, Gadgeel SM, et al. Adagrasib in Non–Small-Cell Lung Cancer Harboring a KRAS G12C Mutation. N Engl J Med. 2022;387(2):120–131. doi:10.1056/NEJMoa2203690
6. Bauml JM, Li BT, Velcheti V, et al. Clinical validation of plasma cell-free DNA (cfDNA) sequencing in the phase 2 trial of sotorasib in patients with KRAS p.G12C mutated NSCLC. Presented at: AACR Annual Meeting 2021; April 10–15, 2021; Virtual. Poster CT181.
7. Michaelidou K, Koutoulaki C, Mavridis K, et al. Detection of KRAS G12/G13 Mutations in Cell Free-DNA by Droplet Digital PCR Offers Prognostic Information for Patients with Advanced Non-Small Cell Lung Cancer. Cells. 2020;9(11):2514. doi:10.3390/cells9112514
8. Canon J, Rex K, Saiki AY, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature. 2019;575(7781):217–223. doi:10.1038/s41586-019-1694-1
9. Hallin J, Engstrom LD, Hargis L, et al. The KRAS(G12C) inhibitor MRTX849 provides insight toward therapeutic susceptibility of KRAS-mutant cancers in mouse models and patients. Cancer Discov. 2020;10(1):54–71. doi:10.1158/2159-8290.CD-19-1167
10. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer. 2009;101(4):715–721. doi:10.1038/sj.bjc.6605177
11. Takeda M, Yoshida S, Inoue T, et al. The role of KRAS mutations in colorectal cancer: biological insights, clinical implications, and future therapeutic perspectives. Cancers (Basel). 2025;17(3):428. doi:10.3390/cancers17030428
12. Ye P, Cai P, Xie J, Wei Y. The diagnostic accuracy of digital PCR, ARMS and NGS for detecting KRAS mutation in cell-free DNA of patients with colorectal cancer: a systematic review and meta-analysis. PLoS One. 2021;16(3):e0248775. doi:10.1371/journal.pone.0248775
13. Tie J, Wang Y, Tomasetti C, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92. doi:10.1126/scitranslmed.aaf6219
14. Diaz LA Jr, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486(7404):537–540. doi:10.1038/nature11219
15. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369(11):1023–1034. doi:10.1056/NEJMoa1305275
16. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359(17):1757–1765. doi:10.1056/NEJMoa0804385
17. Santini D, Loupakis F, Vincenzi B, et al. High concordance of KRAS status between primary colorectal tumors and related metastases: implications for clinical practice. Oncologist. 2008;13(12):1270–1275. doi:10.1634/theoncologist.2008-0181.
18. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med. 2015;21(7):795–801. doi:10.1038/nm.3870.
19. Tie J, Wang Y, Cohen J, et al. Circulating tumor DNA dynamics and recurrence risk in patients undergoing curative intent resection of colorectal liver metastases. PLoS Med. 2021;18(5):e1003620. doi:10.1371/journal.pmed.1003620.

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