

TECHNICAL SHEET IDYLLA™ ctNRAS-BRAF MUTATION TEST



The Idylla™ **ctNRAS-BRAF Mutation Test**, performed on the Biocartis Idylla™ System, is an *in vitro* diagnostic test for the qualitative detection of mutations in **codons 12, 13, 59, 61, 117, 146** of the *NRAS* gene and **codon 600** of the *BRAF* gene.

The Idylla™ ctNRAS-BRAF Mutation Test, from **sample to result**, starts with 1 ml of plasma from patients with metastatic human colorectal cancer (mCRC) to isolate circulating DNA for subsequent real-time PCR amplification and detection of mutations in ctDNA.

FEATURES

NRAS mutation detection		
Codon 12 (exon 2)	G12C	(c.34G>T)
	G12S	(c.34G>A)
	G12D	(c.35G>A)
	G12A	(c.35G>C)
	G12V	(c.35G>T)
Codon 13 (exon 2)	G13D	(c.38G>A)
	G13V	(c.38G>T)
	G13R	(c.37G>C)
Codon 59 (exon 3)	A59T	(c.175G>A)
Codon 61 (exon 3)	Q61K	(c.181C>A)
	Q61R	(c.182A>G)
	Q61L	(c.182A>T)
	Q61H	(c.183A>C; c.183A>T)
Codon 117 (exon 4)	K117N	(c.351G>C; c.351G>T)
Codon 146 (exon 4)	A146T	(c.436G>A)
	A146V	(c.437C>T)

NRAS Total (acting as Sample Processing Control)

BRAF mutation detection		
Codon 600	BRAF V600E	(c.1799T>A; c.1799_1800delinsAA)
	BRAF V600D	(c.1799_1800delinsAC)
	BRAF V600K	(c.1798_1799delinsAA)
	BRAF V600R	(c.1798_1799delinsAG)

BRAF Total (acting as Sample Processing Control)

Specimen requirements

Sample Type 1 ml of plasma

Supported blood collection tubes

Cell-free DNA BCT® tubes (Streck) Blood must be centrifuged within 10 days after collection

Total turnaround time

Time 110 minutes

Result Reporting

Report Qualitative genotype call

Performance

Analytical Sensitivity $\leq 5\%$ for mutations in exons 2, 3 and 4 of the *NRAS* oncogene
 $\leq 1\%$ for mutations in codon 600 of the *BRAF* oncogene

Between Laboratory Reproducibility (496 results at 3 sites) 99.2% agreement for 10% *NRAS* G12D
100% agreement for 10% *NRAS* G12V
99.2% agreement for 10% *NRAS* Q61K
99.2% agreement for 10% *NRAS* Q61R
100% agreement for 5% *BRAF* V600E

Between Lot Reproducibility (296 results on 3 lots) 100% agreement for 10% *NRAS* G12D
100% agreement for 10% *NRAS* G12V
100% agreement for 10% *NRAS* Q61K
100% agreement for 10% *NRAS* Q61R
98.3% agreement for 5% *BRAF* V600E

ACCURACY - CLINICAL PERFORMANCE EVALUATION

Performance of the Idylla™ ct*NRAS*-*BRAF* Mutation Test was assessed along with the Idylla™ ct*KRAS* Mutation Test by evaluating 201 samples from mCRC patients enrolled in the RASANC clinical study¹ (Clinical trial identifier: NCT02502656).

Idylla™ RAS and *BRAF* results were compared with both plasma and tissue reference testing methods.

1. PLASMA - PLASMA COMPARISON (Idylla™ versus deep NGS)

Performance of the Idylla™ ct*KRAS* Mutation Test and ct*NRAS*-*BRAF* Mutation Test as compared to a plasma Reference Method (deep NGS, 0.2% sensitivity²)

Idylla™ Plasma RAS result	Plasma NGS RAS status		
	Mutation detected	WT	Totals
Mutation detected	77	10	87
WT	9	94	103
Totals	86	104	190

Measure	Rate	Point estimate (%)	95% Lower limit (1-sided)
Overall agreement	171/190	90.00	85.84
Positive agreement	77/86	89.53	82.85
Negative agreement	94/104	90.38	84.56

2. PLASMA – TISSUE COMPARISON (Idylla™ versus SOC tissue testing)

Performance of the Idylla™ ctKRAS and ctNRAS-BRAF Mutation Test as compared to standard of care (SOC) tissue testing (NGS, Pyrosequencing, PCR-HRM, Mass Spectrometry, allele-specific PCR)

On 185 samples with results from both methods available, overall RAS agreement between Idylla™ and standard of care tissue tests was 78.9%.

Recently, the RASANC¹ and CAPRI-GOIM³ studies have shown that RAS testing shows an excellent concordance between plasma and tissue, especially in the most important group of liver metastases where ctDNA is usually present. These studies have also shown that about 10-20% of mCRC cases do not harbor detectable ctDNA in their plasma, and these cases include patients with their tumors resected (or metachronous presentation), patients without liver metastases, and patients with metastases confined to the lungs or peritoneum.

An analysis including only **mCRC patients with liver metastases (synchronous and metachronous combined)** revealed an overall RAS concordance of 88.3%, with sensitivity and specificity of 85.2% and 93.6%, respectively. In these patients, overall BRAF agreement with Tissue testing was 99.3% with a sensitivity of 100% and specificity of 99.2%.

RAS agreement

Idylla™ Plasma RAS result	Tissue RAS result (SOC)		
	Mutation detected	WT	Totals
Mutation detected	69	3	72
WT	12	44	56
Totals	81	47	128

Measure	Rate	Point estimate (%)	95% Lower limit (1-sided)
Overall agreement	113/128	88.3	82.8
Positive agreement	69/81	85.2	77.6
Negative agreement	44/47	93.6	85.1

BRAF agreement

Idylla™ Plasma BRAF result	Tissue BRAF result (SOC)		
	Mutation detected	WT	Totals
Mutation detected	11	1	12
WT	0	124	124
Totals	11	125	136

Measure	Rate	Point estimate (%)	95% Lower limit (1-sided)
Overall agreement	135/136	99.3	96.8
Positive agreement	11/11	100.0	80.3
Negative agreement	124/125	99.2	96.5

In the **mCRC population with synchronous liver metastasis**, a RAS agreement of 90.4% with sensitivity and specificity of 87.7% and 95.1%, respectively, was obtained.

RAS agreement

Idylla™ Plasma RAS result	Tissue RAS result (SOC)		
	Mutation detected	WT	Totals
Mutation detected	64	2	66
WT	9	39	48
Totals	73	41	114

Measure	Rate	Point estimate (%)	95% Lower limit (1-sided)
Overall agreement	103/114	90.4	84.8
Positive agreement	64/73	87.7	80.0
Negative agreement	39/41	95.1	86.3

REFERENCES

- (1) Bachet J. B., Bouche O., Taïeb J., Dubreuil O., et al. RAS mutations concordance in circulating tumor DNA (ctDNA) and tissue in metastatic colorectal cancer (mCRC): RASANC, an AGEO prospective multicenter study. *Journal of Clinical Oncology* 2017 35:15_suppl, 11509-11509
- (2) Pécuchet N, et al. Analysis of Base-Position Error Rate of Next-Generation Sequencing to Detect Tumor Mutations in Circulating DNA. *Clin Chem.* 2016 Nov;62(11):1492-1503.
- (3) Normanno N., Esposito Abate R., Lambiase M., Forgione L., et al. Analysis of liquid biopsies from metastatic colorectal carcinoma (mCRC) patients (pts) enrolled in the CAPRI GOIM clinical trial. *Annals of Oncology*, Volume 28, Issue suppl_5, 1 September 2017, mdx393.066, <https://doi.org/10.1093/annonc/mdx393.066>

IDYLLA™ ctRAS PUBLICATIONS

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