ctKRAS CE IVD

TECHNICAL SHEET IDYLLA™ ctKRAS MUTATION TEST

The **Idylla™ ctKRAS Mutation Test,** performed on the Biocartis Idylla™ system, is an *in vitro* diagnostic test for the qualitative detection of 21 mutations in **codons 12, 13, 59, 61, 117 and 146** of the *KRAS* gene.

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

FEATURES

KRAS mutation detection		
Codon 12 (exon 2)	G12C	(c.34G>T)
	G12R	(c.34G>C)
	G12S	(c.34G>A)
	G12A	(c.35G>C)
	G12D	(c.35G>A)
	G12V	(c.35G>T)
Codon 13 (exon 2)	G13D	(c.38G>A)
Codon 59 (exon 3)	A59E	(c.176C>A)
	A59G	(c.176C>G)
	A59T	(c.175G>A)
Codon 61 (exon 3)	Q61K	(c.181C>A; c.180_181delinsAA)
	Q61L	(c.182A>T)
	Q61R	(c.182A>G)
	Q61H	(c.183A>C; c.183A>T)
Codon 117 (exon 4)	K117N	(c.351A>C; c.351A>T)
Codon 146 (exon 4)	A146P	(c.436G>C)
	A146T	(c.436G>A)
	A146V	(c.437C>T)

KRAS 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	130 minutes
Result Reporting	
Report	Qualitative genotype call
Performance	
Analytical Sensitivity	≤ 1% for mutations in exons 2 and 3 of the *KRAS* oncogene ≤ 5% for mutations in exon 4 of the *KRAS* oncogene
Between Laboratory Reproducibility (478 results at 3 sites)	100% agreement for 5% KRAS G12D 99.2% agreement for 5% KRAS G12S 100% agreement for 5% KRAS G12V 99.2% agreement for 5% KRAS G13D
Between Lot Reproducibility (239 results on 3 lots)	100% agreement for 5% KRAS G12D 100% agreement for 5% KRAS G12S 100% agreement for 5% KRAS G12V 100% agreement for 5% KRAS G13D

ACCURACY – CLINICAL PERFORMANCE EVALUATION

Performance of the Idylla™ ctKRAS Mutation Test was assessed along with the Idylla™ ctNRAS-BRAF Mutation Test by evaluating 201 samples from mCRC patients enrolled in the RASANC clinical study[1] (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™ ctKRAS Mutation Test and ctNRAS-BRAF Mutation Test as compared to a plasma Reference Method (deep NGS, 0.2% sensitivity[2])

	Plasma NGS RAS status		
Idylla™ Plasma RAS result	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 RASANC[1] and CAPRI-GOIM[3] 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 concordance of 88.3%, with sensitivity and specificity of 85.2% and 93.6%, respectively.

RAS agreement

	Tissue RAS result (SOC)		
Idylla™ Plasma RAS result	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

In the mCRC population with synchronous liver metastases, an agreement of 90.4% with sensitivity and specificity of 87.7% and 95.1%, respectively, was obtained.

RAS agreement

	Tissue RAS result (SOC)		
Idylla™ Plasma RAS result	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

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