Clinical and Analytical Validation of the CE IVD ONCO/Reveal™ Dx Lung and Colon Cancer Assay (ORLCCA)

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Abstract

Introduction Targeted therapies for KRAS in colorectal cancer (CRC) for G12/G13 wild-type patients and for EGFR in non-small cell lung cancer (NSCLC) for patients with a L858R mutation or an Ex19 in-frame deletion are available. ORLCCA, an NGS based assay, detects DNA variants across 22 genes including EGFR and KRAS commonly mutated in NSCLC or CRC. The assay also includes the proprietary PiVAT software which detects and generates patient reports of KRAS G12 and G13, and EGFR L858R and Ex19 deletion status for potential companion diagnostic (CDx) purposes. KRAS Q61X and A146T and BRAF V600E in CRC and EGFR T790M, G719X, and Ex20 insertions in NSCLC are also reported in the CE IVD product. Here, we report the results of the clinical and analytical validation experiments used to support CE IVD claims.

Methods Analytical Validation- Clinical samples positive for representative targeted mutations were used in the analytical validation experiments. DNA input range was evaluated at 5, 10, 20, 40, 80, and 160 ng input from 15 clinical samples. Limit of detection (LoD) was determined by serial dilutions of KRAS, EGFR, or BRAF positive FFPE samples with normal tissues to achieve variant allele frequency (VAF) between 1.0 - 10.0% at 5 levels. Accuracy was assessed by comparing detected variants with an externally validated comparator method (CompO) across 208 samples. Reproducibility was determined across multiple sites, assay runs, operators, reagent lots and thermocyclers by a total of 360 libraries. Clinical Validation- Retrospective clinical studies using NSCLC and CRC samples were conducted. The non-inferiority (NI) statistical tests were used to compare the ORLCCA with an FDA approved companion diagnostic assay: for EGFR Ex19 del and L858R mutations (CompQ) and for KRAS G12 and G13 mutations (CompC).

Results Analytical- We determined the DNA input range to be between 10-80 ng for CE IVD approval. LoD for KRAS G12 and G13, EGFR L858, and Ex19 del, and BRAF V600E were established <4%. In the accuracy study, PPA and NPA between ORLCCA and CompO >99%. Across all variables tested in reproducibility, the APA and ANA were >95%. Clinical- Non-inferiority margin <10% was observed between ORLCCA and CompQ across all NSCLC samples and between ORLCC and CompC across all CRC samples.

Conclusions ORLCCA is a highly accurate CE IVD approved assay for the detection of clinically relevant KRAS variants in CRC and EGFR variants in NSCLC.

CDx Claims

Indication	Gene	Variant	Targeted Therapy
Colorectal Cancer (CRC)	KRAS	KRAS Wide-type (absence of mutations in codons 12 and 13)	Erbitux (Certuximab), or Vectibix (Panitumumab)
Non-Small Lung Cancer (NSCLC)	EGFR	Exon 19 Deletion Exon 21 L858R	Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib), or Vizimpro (dacomitinib)

***Table 1** – Variants with supported CDx claims in ORLCCA*

Assay Design and Additional Claims

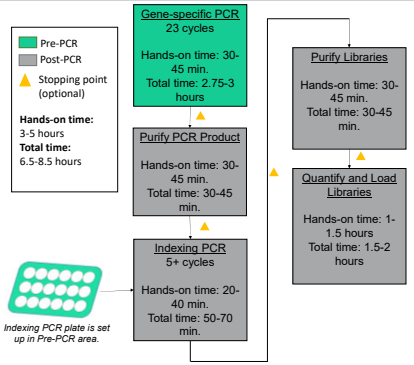

***Figure 1** – Overview of ORLCC workflow. The library preparation process can be completed in one day with as little as 3 hours of hands-on bench time.*

Gene	Variant ID	Cancer	Nucleotide Change
EGFR	T790M	NSCLC	c.2369C>T
EGFR	G719A	NSCLC	c.2156G>C
EGFR	G719C	NSCLC	c.2154_2155delinsTT; c.2155G>T
EGFR	G719D	NSCLC	c.2156G>A
EGFR	G719S	NSCLC	c.2155G>A
EGFR	Exon 20 In-frame Insertions	NSCLC	Multiple
BRAF	V600E	NSCLC	c.1799T>A; c.1799_1800delinsAA
KRAS	Exon 2 Mutation	NSCLC	Multiple

***Tables 2 (top) and 3 (right)** – Additional non-CDx variants reported by ORLCC in NSCLC (top) and CRC (right). KRAS Q61X and A146T and BRAF V600E in CRC and EGFR T790M, G719X, and Ex20 insertions in NSCLC are reported in the CE IVD product. BRAF V600E variant was used in analytical validation experiments to assess ORLCCA performance.*

Gene	Variant ID	Cancer	Nucleotide Change
KRAS	A59E	CRC	c.176C>A
KRAS	A59G	CRC	c.176C>G
KRAS	A59T	CRC	c.175G>A
KRAS	A59S	CRC	c.175G>T
KRAS	Q61E	CRC	c.181C>G
KRAS	Q61H	CRC	c.183A>C ; c.183A>T
KRAS	Q61K	CRC	c.180_181delinsAA ; c.180_181inv ; c.181C>A
KRAS	Q61L	CRC	c.182A>T ; c.182_183delinsTC ; c.182_183delinsTG ; c.182_183inv
KRAS	Q61R	CRC	c.182A>G ; c.182_183delinsGC ; c.182_183delinsGT
KRAS	K117N	CRC	c.351A>C ; c.351A>T
KRAS	A146T	CRC	c.436G>A
KRAS	A146P	CRC	c.436G>C
KRAS	A146V	CRC	c.437C>T
BRAF	V600E	CRC	c.1799T>A ; c.1799_1800delinsAA

Results and Conclusions

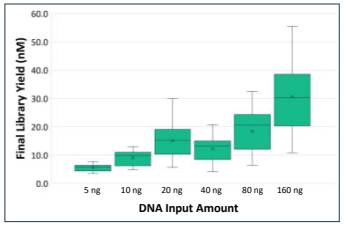

***Figure 2** – Final library yield was assessed across DNA input ranging from 5 ng to 160 ng. At 5 ng of DNA input, 8 out of 30 samples failed to generate sequencing libraries that meet the library yield requirement of ≥ 3.5 nM.*

DNA input (ng)	# TP	# TN	# FP	# FN	PPA	NPA
5	32	6,867	1	0	(32/32) 100%	(6,867/6,868) 100%
10	32	6,868	0	0	(32/32) 100%	(6,868/6,868) 100%
20	32	6,867	1	0	(32/32) 100%	(6,867/6,868) 100%
40	32	6,868	0	0	(32/32) 100%	(6,868/6,868) 100%
80	32	6,868	0	0	(32/32) 100%	(6,868/6,868) 100%
160	32	6,868	0	0	(32/32) 100%	(6,868/6,868) 100%
Overall (10 - 80)	128	27,471	1	0	(128/128) 100%	(27,471/27,472) 100%

***Table 4** - The DNA input range was evaluated at 5, 10, 20, 40, 80, and 160 ng in duplicate using DNA extracted from 15 FFPE samples containing representative SNVs, insertions and deletions. The expected variants (KRAS G12D, KRAS G12S, KRAS G12V, KRAS G13D, KRAS A146T, BRAF V600E, EGFR Exon 19 deletion, EGFR L858R, EGFR Exon 20 insertion, and EGFR G719S) present in all 15 samples were called correctly at DNA inputs of 5-160 ng.*

Gene	Variant	Variant Category	Estimated VAF%
KRAS	G13D	SNV	2.8
KRAS	G12D	SNV	1.9
EGFR	L858R	SNV	1.6
EGFR	Exon 19 Del	Dellns	1.7
BRAF	V660E	SNV	1.4
EGFR	G719C	SNV	2.4
EGFR	Exon 20 Ins	Insertion	2.2
EGFR	T790M	SNV	3.0
KRAS	Q61L	SNV	2.2
KRAS	A146T	SNV	2.9

***Table 5** - The LoD is based on the highest VAF% with 95% or more correct calls observed for the variant being tested and claimed LoD for each variant was calculated as the average VAF% across replicates for that level. Measured LoDs varied between 1.4% and 3.0% for SNV variants, 1.7% for the Delins variant, and 2.2% for the Insertion variant. The sample set contained two different FFPE specimens containing the same variant.*

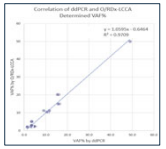

***Figure 3** – VAF values for samples used in LoD analysis were verified with ddPCR. A high correlation between VAFs measured by ddPCR and ORLCCA was observed.*

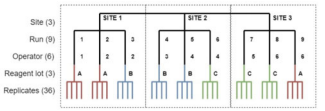

***Figure 4** - Overview of reproducibility experiment. Each panel member was tested across 3 sites, 9 runs, 6 operators, 3 reagent lots and 3 thermocyclers in the study (Table 1) resulting in a total of 360 libraries.*

	TP	TN	FP	FN	APA	LB	UB	ANA	LB	UB
Overall	360	82440	0	0	100%	98.9%	100%	100%	100%	100%
RGNT_LOT_1	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
RGNT_LOT_2	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
RGNT_LOT_3	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
SITE_1	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
SITE_2	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
SITE_3	120	27480	0	0	100%	96.9%	100%	100%	100%	100%
SNV	288	65952	0	0	100%	98.7%	100%	100%	100%	100%
DELETION	72	16488	0	0	100%	94.9%	100%	100%	100%	100%

***Table 6** - The reproducibility of the O/RDx-LCCA was evaluated using a total of 10-member sample panel: 2 FFPE CRC specimens with KRAS mutations (Gly12Asp and Gly13Asp), 1 FFPE CRC specimen with BRAF mutation (Val600Glu) and 2 FFPE NSCLC specimens with EGFR mutations (Glu746_Ser752delinsVal and Leu858Arg) were prepared at high and low variant frequency levels. Overall APA and ANA for the experiment are shown along with results from the three reagent lots and three sites used in testing. TP=True positive; TN=True negative; FP=False Positive; FN=False Negative; APA=Average Positive Agreement; ANA=Average Negative Agreement; LB=Lower bound of 95% confidence interval; UB= Upper bound of 95% confidence interval*

Note: All specifications are for the CE IVD product. This product is not FDA approved or available for sale in the US.

	CompO+	Pillar+	PPA	LB	UB	CompO-	Pillar-	NPA	LB	UB
Variant	228	219	96.1%	92.7%	97.9%	60372	60366	100.0%	100.0%	100.0%
SNV	208	199	95.7%	92.0%	97.7%	20192	20187	100.0%	99.9%	100.0%
Short indel (<6 nt)	5	5	100.0%	56.6%	100.0%	10595	10595	100.0%	100.0%	100.0%
Medium indel (6-50 nt)	15	15	100.0%	79.6%	100.0%	29585	29584	100.0%	100.0%	100.0%

***Table 7** – Results from accuracy experiments using CompO as a direct comparator. A total of 208 samples (84 CRC and 124 NSCLC) were tested of which 6 yield invalid results with the validated NGS comparator method and 2 yield invalid results with ORLCCA. Among the 200 valid samples, 168 (84.0%) were identified as being positive for at least one variant with the comparator method. The samples included simple SNVs, short and medium indels that are targeted by the ORLCCA. PPA and NPA were >95% for all variants as well as SNVs only, short indels (<6 nt), medium indels (6-50 nt).*

	Enrollment CCD+ (CCD1+)			Enrollment CCD- (CCD1-)		
	CCD2 +	CCD2 -	Total	CCD2 +	CCD2 -	Total
FCD+	91	0	91	0	3	3
FCD -	0	0	0	0	163	163
Total	91	0	91	0	166	166

	Enrollment CCD+ (CCD1+)			Enrollment CCD- (CCD1-)		
	CCD2 +	CCD2 -	Total	CCD2 +	CCD2 -	Total
FCD+	79	2	81	0	1	1
FCD -	2	2	4	0	103	103
Total	81	4	85	0	104	104

***Tables 8 (top) and 9 (bottom)** – Table 8 NI analysis comparing ORLCCA with an FDA approved companion diagnostic assay for EGFR Ex19 del and L858R mutations (CompQ) Table 9 NI analysis comparing ORLCCA with an FDA approved companion diagnostic assay for KRAS G12 and G13 mutations (CompC). Confidence intervals were determined using a 1000x bootstrap in SAS. Upper bounds of the 95% confidence intervals are less than 4% for CompQ and upper bounds of the 95% confidence intervals are less than 6% for CompC.*