Multi institutional evaluation of a high sensitive NGS assay for liquid biopsy mutation detection in lung cancer

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Background

The detection of actionable mutations in lung cancer is still a major challenge due to the lack of tissue specimens for molecular profiling of the tumor in approximately 25% of lung cancers samples. Circulating tumor DNA (ctDNA) isolated from plasma of cancer patients is an alternative, minimally invasive source of tumor DNA that allows multiple determination of the mutational tumor status over time. However, the intrinsic low abundance of ctDNA makes the mutation detection and its quantification in plasma a challenging task. Here we report a multi-institutional validation of the Oncomine cfDNA Lung assay for the analyses of ctDNA in molecular diagnostics laboratories.

Material & Methods

Table 1: Targets included in the assay

Genes	Hotspots
ALK, BRAF,	>169 hotspots including:
EGFR, ERBB2,	*EGFR:* T790M, C797S, L848R, Exon 19 del
KRAS, MAP2K1,	*KRAS:* G12X, G13X, Q61X
MET, NRAS,	*BRAF:* V600E
PIK3CA, ROS1,	*ALK:* Exon 21-25
TP53	*PIK3CA:* E545K, H1047R, E542K

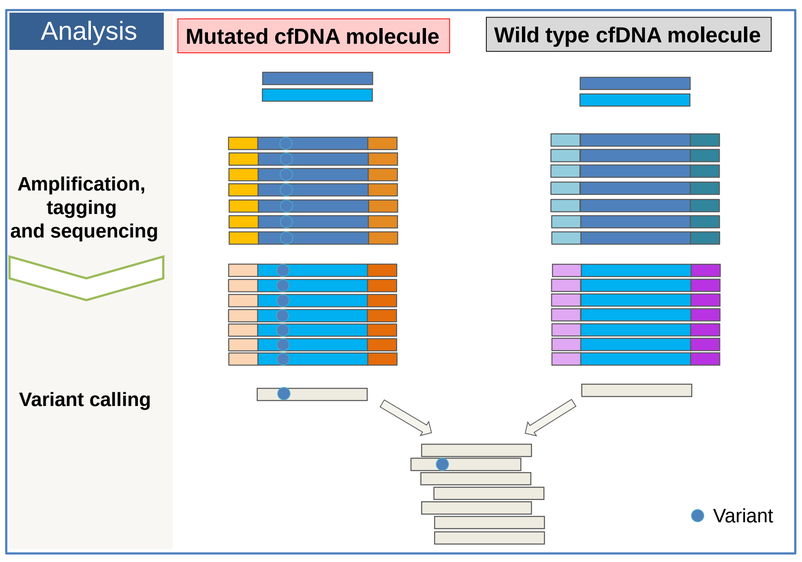
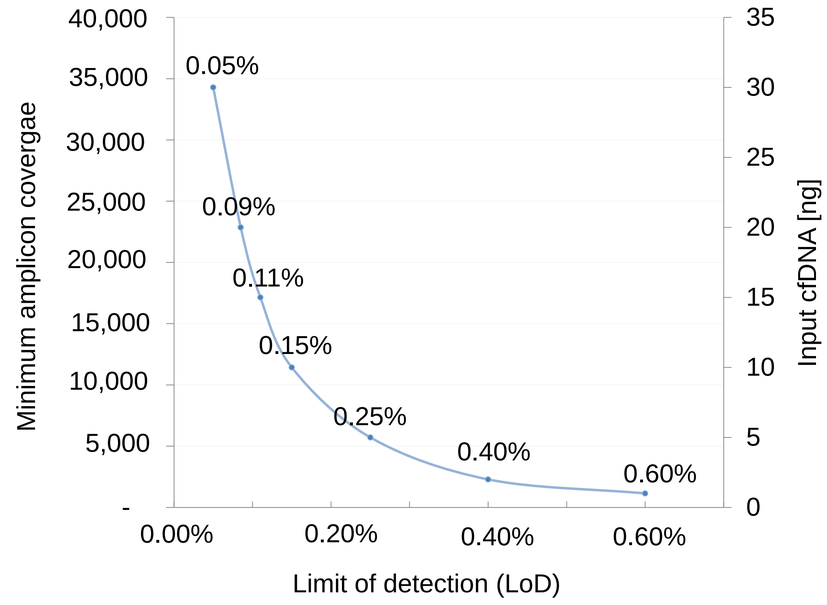


Figure 1: The assay includes unique tags to label individual DNA molecules used as input material. Tag families are generated after amplification, and consensus sequences are built to eliminate errors and represent the original molecules. This approach allows to distinguish reads amplified from the same original DNA molecule, and identify molecules containing variant by excluding false positives.

The Oncomine cfDNA Lung assay is a multiplexed sequencing assay (Table 1) using unique identifiers to tag each original DNA molecule used as input material (Figure 1). This enables the highly sensitive, quantitative and reproducible detection of low frequency mutations providing an ideal assay for liquid biopsies (Figure 2). To ensure an unbiased and uniform evaluation of the assay, cfDNA reference standard covering eight mutations in *EGFR*, *KRAS*, *NRAS* and *PIK3CA* at 5%, 1%, and 0.1% allele frequency was distributed to the laboratories within the OncoNetwork Consortium. Samples were sequenced twice in each laboratory either as 4 libraries on a 318/520 chip or 8 libraries on a 530 chip using the Ion PGM/Ion S5 systems. A bioinformatics pipeline within the Torrent Server software allowed for automated variant calling.



cfDNA	Limit of Detection
1 ng	0.6% LoD
5 ng	0.25% LoD
10 ng	0.15% LoD
20 ng	**0.1% LoD**
30 ng	0.05% LoD

Figure 2: Limit of detection (LoD) of cfDNA assay is dependent on amplicon coverage and cfDNA input amount.

Results

All eight hotspot base changes and indels present in the reference samples at allele frequencies from 0.1% to 5% were detected by the 11 laboratories with an average of 94.05% sensitivity and 99.87% specificity (Figure 3). At 0.1% allele frequency, the average sensitivity was 83.04% and the average specificity was 99.95%. Notably, at 0.1% allele frequency, all laboratories accomplished to detect the challenging *EGFR* p.T790M.

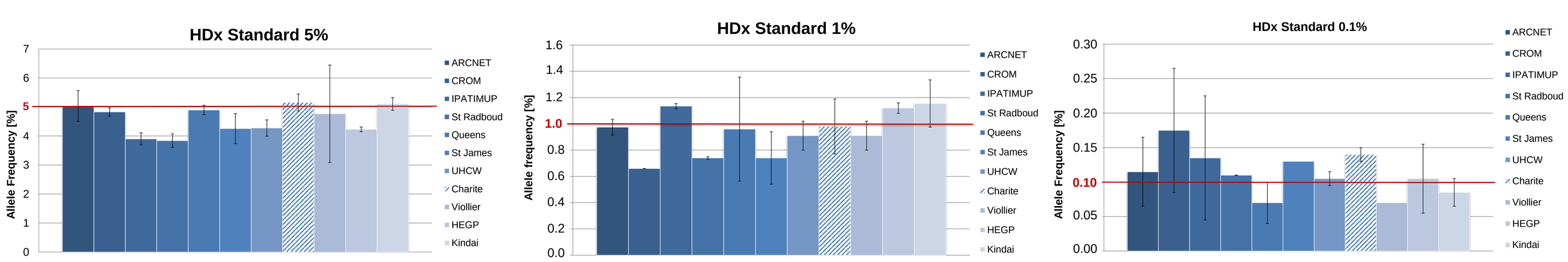


Figure 3: *EGFR* p.T790M mutation at 5%, 1% and 0.1% allele frequency

Conclusions

The data confirm the Oncomine Lung cfDNA assay as a multi biomarker next generation sequencing assay that enables the detection of primary driver and resistance mutations to a level of 0.1%. OncoNetwork Consortia evaluted the assay in a repeatability and reproducibility multicenter study using Horizon cfDNA Reference Set. Results from 11 laboratories demonstrated more than 94% sensitivity and 98% specificity.

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