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Performance of a 24-hour turnaround, guideline-complete NSCLC assay, optimized for sparse samples

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Introduction

Based on a 2020 ACS CAN survey of cancer patients and survivors, only 33% had received biomarker testing. Of those patients not tested, more than 25% of those reported it was because of high cost or insurance issues. A low cost and more accessible alternative to NGS is necessary so all cancer patients can have access to biomarker testing. Based on its high sensitivity, faster turnaround, and familiar workflow, digital PCR (dPCR) is an emerging platform for detecting genomic variants. dPCR is traditionally limited in multiplexing capabilities by the number of color channels on the instrument, limiting practical applications in complex fields like oncology. ChromaCode has leveraged their High-Definition PCR (HDPCR™) technology to increase the multiplexing of dPCR instruments by ten-fold[1,2].

ChromaCode has developed a research use only (RUO) dPCR assay, for multiplexed detection of 10 DNA variants and 5 RNA fusion variants relevant in non-small cell lung cancer (NSCLC) samples. The assay is constructed using both amplitude modulation and multi-channel resilient signal encoding methods. Amplitude modulation enables different variants to generate signals at different intensity levels in single color channel, allowing for greater than *n* targets in *n* color channels. In contrast, resilient encoding generates a signal in more than one color channel to create a form of error detecting code. The combination of these two techniques allows for unique fluorescence signatures to be associated with partition populations inhabited by specific targets as seen in Figure 1.

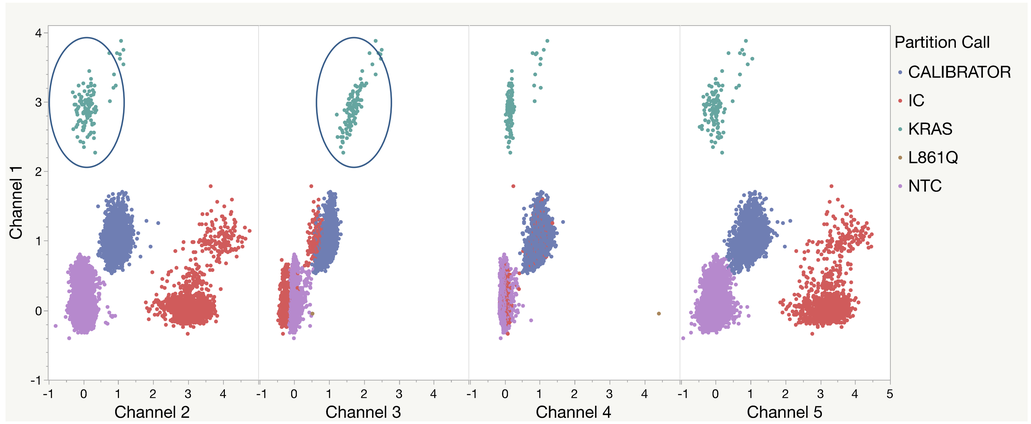

Figure 1. Example of amplitude modulation and resilient coding, where the KRAS target is at a normalized fluorescence value of 2 in Channel 3, a normalized fluorescence value of 3 in Channel 1, and a 0 in Channels 2, 4, and 5. The redundance of the KRAS target in multiple channels allows for increased target specificity.

Methods

The ChromaCode HDPCR NSCLC RUO assay is composed of three wells, two for detection of DNA variants, and one for RNA fusion detection. Each well contains a calibrator, internal control and five unique targets. A total of fifty-two (52) FFPE specimens from tissue resections were evaluated for DNA targets, and sixty (60) were evaluated for RNA fusion targets using the HDPCR NSCLC Panel. DNA and RNA were extracted from a single 10µm section of specimen, with a sister section previously characterized with the Oncomine Focus assay. The DNA/RNA eluates (20ng DNA per well, 20ng RNA) were run with the HDPCR NSCLC Panel on the Qiagen QIAcuity dPCR instrument. Data analysis was conducted using ChromaCode Cloud™, which includes a proprietary algorithm that can detect and quantify six targets (five targets and one internal control) for each of the three wells. DNA samples discordant with the comparator assay (Oncomine Focus), were then evaluated using the Archer DNA VariantPlex panel, while discordant RNA samples were evaluated using the Archer RNA FusionPlex panel.

Results

Fifty-two (52) FFPE specimens were evaluated for DNA targets and sixty (60) were evaluated for RNA fusion targets using the HDPCR NSCLC RUO assay with the results showing a high-level of agreement, 98.9% across the panel, after discordant resolution. Of the fifty-two (52) samples evaluated for DNA targets, forty-three (43) were concordant with the comparator NGS. Three false negative calls for EGFR T790M and three false negative calls for EGFR L858R were resolved in discordant resolution when the Archer DNA VariantPlex panel did not detect EGFR T790M or EGFR L858R in those samples. The false positive result for Exon 19 Deletion was also detected when tested with the Archer VariantPlex. After discordant resolution, all DNA targets showed >95% sensitivity and specificity across all samples tested (Table 2).

Of the sixty (60) samples evaluated for RNA fusion targets, fifty-one (51) were concordant with the comparator NGS result. Results have not yet been received for three discordant RNA samples run on the Archer RNA FusionPlex panel. One false negative NTRK sample was not detected by the Archer assay, and another was determined to be a novel NTRK fusion and outside the scope of reported inclusivity of the NSCLC panel (Table 2).

Table 1. Summary of clinical sample performance using the HDPCR NSCLC Panel

Target	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)
EGFR Exon19 Deletion	2	49	1[1]	0	100.0% (15.8 - 100.0)	98.0% (89.4 - 100.0)
EGFR T790M	1	47	1*	3[2,3,4]	25.0% (0.63 - 80.6)	97.9% (88.9 - 100.0)
EGFR S768I	2	50	0	0	100.0% (15.8 - 100.0)	100.0% (92.9 - 100.0)
EGFR L858R	3	46	0	3[4,5,6]	50.0% (11.8 - 88.2)	100.0% (92.3 - 100.0)
BRAF V600E	1	51	0	0	100.0% (2.5 - 100.0)	100.0% (93.0 - 100.0)
EGFR Exon 20 Insertion	7	43	1*	1*	87.5% (47.4– 99.7)	97.7% (88.0 - 99.9)
EGFR G719X	3	49	0	0	100.0% (29.2 - 100.0)	100.0% (92.8 - 100.0)
KRAS G12C	3	49	0	0	100.0% (29.2 - 100.0)	100.0% (92.8 - 100.0)
ERBB2 Exon 20 Insertion	8	44	0	0	100.0% (63.1 - 100.0)	100.0% (92.0 - 100.0)
EGFR L861Q	2	49	1*	0	100.0% (15.8 - 100.0)	98.0% (89.4 - 100.0)
ALK	4	54	1**	1**	80.0% (28.4 - 99.5)	98.2% (90.3 - 100.0)
ROS1	3	55	2**	0	100.0% (29.2 - 100.0)	96.5% (87.9 - 99.6)
RET	2	57	0	1[7]	66.7% (9.4 - 99.2)	100.0% (93.7 - 100.0)
NTRK	0	58	0	2[8,9]	N/A	100.0% (93.8 - 100.0)
MET	3	56	1[10]	0	100.0% (29.2 - 100.0)	98.3% (90.6 - 100.0)

*Sample not sent for discordant sequencing
**Sample sent for discordant sequencing but results not yet received at time of publishing

Table 2. Discordant testing results for samples tested in Table 1

Footnote Table 1	Oncomine Result	ChromaCode Result	Archer Result	ChromaCode Result Concordant with Archer
1	Exon 19 Deletion Not Detected	Exon 19 Deletion Detected	Exon 19 Deletion Detected	✓
2, 3	T790M Detected	T790M Not Detected	T790M Not Detected	✓
4	T790M (SNV); L858R, Exon 19 Deletion Detected	Exon 19 Deletion Detected	Exon 19 Deletion Detected	✓
5, 6	L858R Detected	L858R Not Detected	L858R Not Detected	✓
7	RET Detected	RET Not Detected	RET Not Detected	✓
8	NTRK Detected	NTRK Not detected	NTRK Not Detected	✓
9	NTRK Detected	NTRK Not detected	Novel NTRK Fusion Detected	X
10	MET Not Detected	MET Detected	MET Not Detected	X

Conclusion

The HDPCR NSCLC RUO assay includes relevant research biomarkers in current National Comprehensive Cancer Network (NCCN) guidelines, including variants in EGFR, BRAF, KRAS, ERBB2, ALK, ROS1, RET, MET, and NTRK, and provides comparable performance to validated NGS assays with a turnaround time that enables adherence to guideline-recommended treatment, and a low sample requirement that maximizes the number of patients for molecular testing. The standard variants can be reported with the click of a button and in 24 hours, allowing for an easily adopted solution to testing needs. The standard protocols used by the assay and the use of a cloud-based analysis pipeline enables easy deployment of this assay in any lab. This assay leverages a proprietary bioinformatics suite, the ChromaCode Cloud, to accurately and quickly report relevant NSCLC markers.

References

1. Jacky, Lucien, et al. "Virtual-Partition Digital PCR for High-Precision Chromosomal Counting Applications." Analytical Chemistry (2021).
2. Jacky, Lucien, et al. "Robust Multichannel Encoding for Highly Multiplexed Quantitative PCR." Analytical Chemistry 93.9 (2021): 4208-4216