Validation of the EGFR Uncommon Variants Multiplex ddPCR Assay for Blood-Based Testing in NSCLC

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Background

Lung cancer is the leading cause of cancer-related deaths in the United States with the majority of diagnoses falling under the subtype of non-small cell lung cancer (NSCLC). Surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapies – alone or in combination – are used to treat NSCLC. 10-15% of NSCLC patients express a somatic mutation in the epidermal growth factor receptor (EGFR) which is a main pathogenic driver and therapeutic target. While most of the EGFR-mutated NSCLC cases present with common mutations, either deletions in exon 19 (del19) or a point mutation in exon 21 (L858R), up to 10% of patients with EGFR alterations present with uncommon mutations such as G719X, S768I, and L861Q. To enhance the GeneStrat droplet digital PCR (ddPCR™) test service at Biodesix, which has covered the sensitizing EGFR mutations (del19 and L858R) as well as the T790M resistance mutation in patient blood, a new ddPCR assay for the EGFR uncommon variants (UCV) has been validated, approved and commercialized. The inclusion of these additional EGFR variants expands its clinical relevance.

Methods

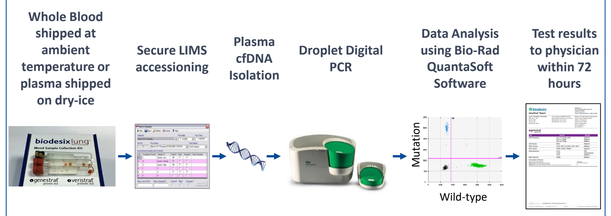
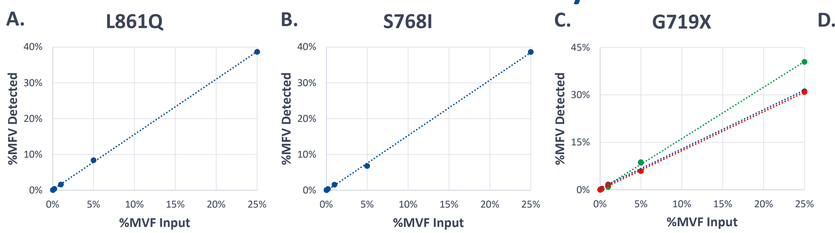


Figure 1. Overview of the GeneStrat® Test Sample Processing for cfDNA in the Biodesix Laboratory. Sample testing is initiated when whole blood is drawn and shipped to the Biodesix Laboratory. Samples for testing are accessioned into the Laboratory Information Management System (LIMS). Following nucleic acid extraction, samples are processed using the Bio-Rad QX200™ Droplet Digital PCR (ddPCR) system. Numbers of mutant and wild-type copies are assessed using QuantaSoft™ Software, and Test Result Reports (TRRs) are generated from the secure LIMS. Based on this workflow, a multiplex ddPCR assay was designed to detect five EGFR uncommon variants (G719A, G719C, G719S, S758I, L861Q) in a non-discriminating manner.

Analytical Results



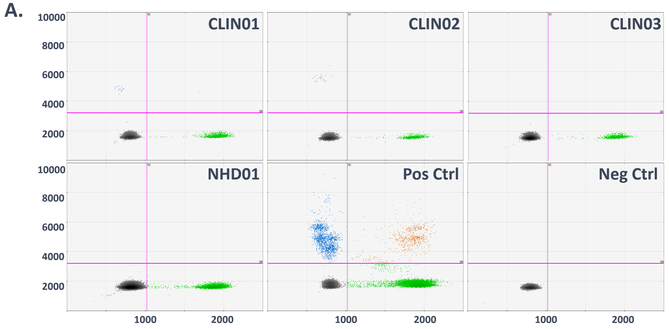
D.

EGFR Variant	Total Mut Copies	Total WT Copies	Result
T790M gBlock	0	388	Negative
L858R gBlock	0	434	Negative
C797S gBlock	0	470	Negative
Del19 gBlock	0	456	Negative
Positive Ctrl	126	886	Positive
Negative Ctrl	0	0	Negative

Figure 2. Results of analytical sensitivity and specificity experiments using the EGFR UCV assay. (A-C) Each EGFR UCV gBlock was diluted into a background of normal human gDNA to achieve six different percent minor variant frequencies (%MVF). %MVF detected by the EGFR UCV assay (y-axis) is plotted relative to input %MVF ranging from 0.02% to 25% (x-axis). (C) G719X variants are overlaid: G719A (blue), G719C (green), G719S (red). $R^2 \geq 0.99$ for all targets. (D) Analytical specificity was tested with gBlocks of four common EGFR variants. There was no off-target detection.

Clinical Results

A.



B.

Specimen	Total Mut Copies	Total WT Copies	Result	%MVF
CLIN01	29	1880	Positive	1.52%
CLIN02	34	1310	Positive	2.53%
CLIN03	0	1380	Negative	0%
CLIN04	1.8	2734	Negative	0%
CLIN05	0	1390	Negative	0%
CLIN06	0	1010	Negative	0%
CLIN07	0	1086	Negative	0%
CLIN08	0	1488	Negative	0%
CLIN09	1.8	1612	Negative	0%
CLIN10	0	3160	Negative	0%
CLIN11	0	1310	Negative	0%
CLIN12	0	422	Negative	0%
NHD01	0	872	Negative	0%
NHD02	0	732	Negative	0%
NHD03	0	632	Negative	0%
NHD04	0	820	Negative	0%
NHD05	0	942	Negative	0%
NHD06	0	1338	Negative	0%
NHD07	0	1034	Negative	0%
NHD08	0	780	Negative	0%
NHD09	0	1408	Negative	0%
NHD10	0	802	Negative	0%
Pos Ctrl	3640	17600	Positive	17.14%
Neg Ctrl	0	0	Negative	N/A

Figure 3. Results of validation using clinical specimens. QuantaSoft 2D plot examples for (A) EGFR UCV positive clinical specimens (CLIN01 & CLIN02), a representative EGFR UCV negative clinical specimen (CLIN03), a representative normal healthy donor (NHD01), and the positive and negative controls are shown. (B) All validation specimen results. Positive call cutoff is 2 or more copies.

EGFR UCV Concordance		NGS Reference Result		
		UCV +	UCV -	Total
ddPCR Result	UCV +	2	0	2
	UCV -	0	10	10
	Total	2	10	12

Table 1. EGFR UCV Concordance. ddPCR results were compared to a reference result generated using Next-Generation Sequencing (NGS) on the Ion Torrent GeneStudio S5 Prime.

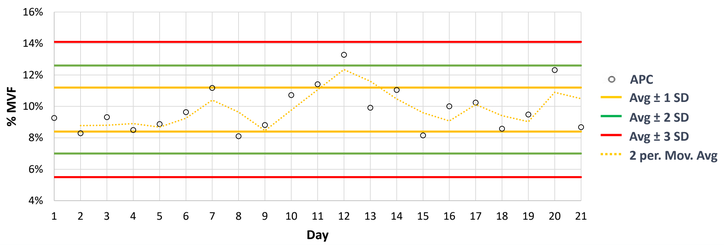


Figure 4. 21 Day Robustness Data. The %MVF over the 21 days of the study. The limits are shown as ± 3 standard deviations with the average %MVF of 9.8% ± 4.2% (Mean ± 3 Standard Deviations).

Precision Results

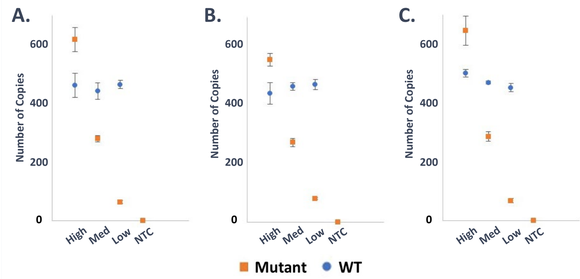


Figure 5. Graphical Representation of Precision. EGFR UCV (orange) and EGFR WT (blue) copies detected for positive control specimens at high, medium and low mutant copy number as well as the NTC are shown. Data represent average ± standard error of the mean for (A) intra-day, (B) inter-day, and (C) inter-operator precision studies.

Conclusions

- The EGFR UCV assay has demonstrated:
  - High sensitivity and specificity with an analytic LOD of 0.02%.
  - Within Lab Precision over multiple days with multiple operators.
  - 100% clinical sensitivity, clinical specificity, and concordance.
- The additional EGFR UCV are NYS CLEP approved as part of the GeneStrat® test and are available to ordering physicians and Pharmaceutical companies for trial use.

References

- Yang, *et al.* Clinical Activity of Afatinib in Patients with Advanced Non-Small Cell Lung Cancer Harbouring Uncommon EGFR Mutations: A Combined Posthoc Analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. The Lancet Oncology. 2015; 16: 830-838.
- Mellert, *et al.* Development and Clinical Utility of a Blood-based Test Service for the Rapid Identification of Actionable Mutations in NSCLC. Journal of Molecular Diagnostics. 2017; 19(3): 404-416.

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