

Clinical Implementation of Next-Generation Sequencing for Diagnostics and Use of Reference Standards to Evaluate Operator Variability

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Introduction

Due to the complex mechanisms driving carcinogenesis, multiple aberrations in important tumor suppressor genes and oncogenes are relevant for tumor diagnostics and therapy. To enhance the understanding of the underlying pathogenesis, extensive investigation of commonly mutated and critical key genes are an efficient way to analyze the oncogenic potential in human cancer samples.

The high-throughput and increasingly affordable nature of Next-Generation Sequencing (NGS) has led to its expanded use in routine clinical procedures. The simplicity of targeted enrichment cancer panels (available from a number of commercial providers), allows routine laboratories to simultaneously analyse the coding (exonic) regions of multiple cancer-related key genes.

In diagnostic laboratories, the technical transition from Sanger sequencing to NGS requires several validation studies, demonstrating the effective and reproducible performance of the NGS workflow. One of the most important steps is to evaluate that there is no run-to-run variability between different operators performing such NGS assays. Within the Gerhard-Domagk Institute of Pathology (University Hospital Muenster), clinical implementation of NGS for routine diagnostics has been successfully facilitated and

validated by use of HDxTM Reference Standards.

The FFPE format of these Reference Standards allows users to start from DNA extraction and proceed through the entire analytical platform to final variant detection.

Results and Conclusions

The highly-characterized nature of FFPE HDx Reference Standards provides additional quantitative checkpoints at specific genomic coordinates, beyond the scope of the metrics provided by the sequencing platform (cluster density, read output, and data quality). Specifically, HDx Reference Standards include variants orthogonally verified using droplet digital PCR (ddPCR) during quality control of each lot manufactured. Those variants that are covered by the customized Qiagen GeneRead DNAseq Mix-n-Match panel were ideal candidates as validation checkpoints.

As shown in Figure 1, NGS results were compared to the expected allelic frequency, as well as the run-to-run performance for each variant listed.

Many of these variants were detected near the expected values, with the average allelic frequencies for the EGFR L858R, KRAS G12D, KRAS G13D, NRAS Q61K, PIK3CA and both CTNNB1 variants falling within the acceptable range for FFPE HDx Reference Standards. Interestingly, those variants that were detected at higher (BRAF V600E) or lower (cKIT D816V, EGFR G719S, and IDH1

S261L) allelic frequencies showed high reproducibility between runs. This indicates the results may be improved with manual optimization of the pipeline, and are not a random error in the analysis.

In line with additional validation steps, the clinical laboratory has successfully implemented the NGS analysis workflow to simultaneously analyse the mutational status of multiple cancer-related key genes and intends to use FFPE HDx Reference Standards to routinely monitor NGS assay performance.

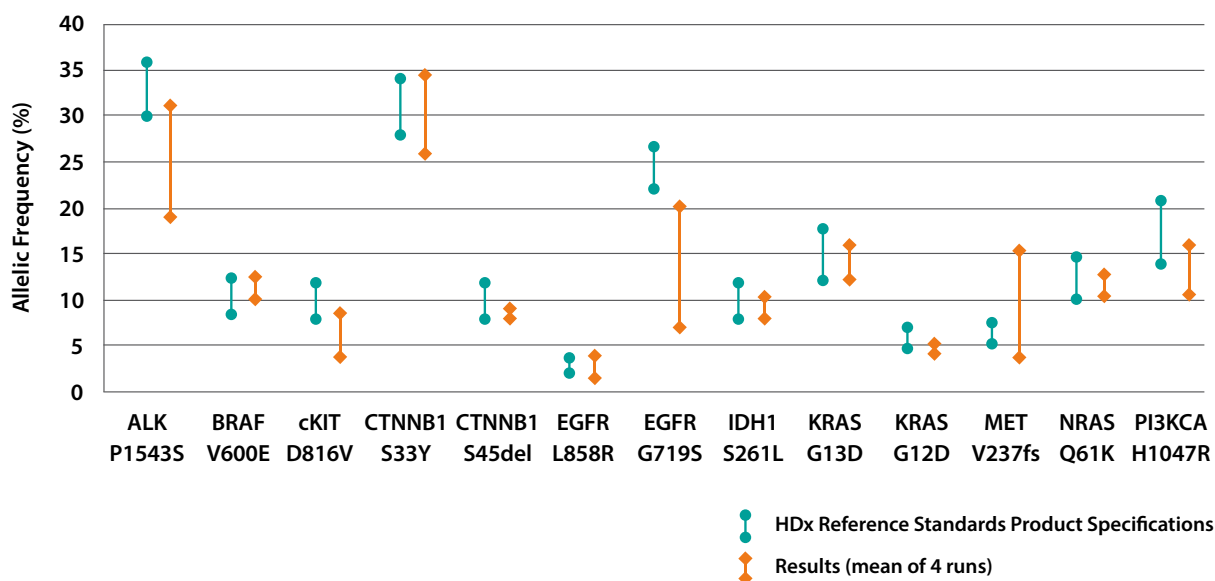


Figure 1. Representative NGS results of the Quantitative Multiplex FFPE HDx Reference Standard including expected and detected allelic frequencies by independent operators (Runs 1–4).

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