

Conference abstract

Validation of an Updated 51-Gene Hybrid-Capture NGS Assay for Myeloid Neoplasms: Improved Variant Detection and Laboratory Efficiency

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INTRODUCTION

Next-generation sequencing (NGS) is widely used in the evaluation of myeloid neoplasms; however, earlier assays may have limitations in gene coverage and detection of complex variants. Our laboratory previously utilized a 2015-era NGS assay that required ancillary testing for select targets (e.g., CEBPA, FLT3-ITD, CALR). We sought to validate an updated hybrid-capture NGS panel with enhanced bioinformatics to improve analytical performance and workflow efficiency.

METHODS

Validation was performed using a 51-gene hybrid-capture assay (Sophia Genetics) with the Sophia DDM analysis platform. A total of 52 specimens (whole blood, bone marrow, FFPE) were tested across five runs, including reference control material (HD829). Variant types included single nucleotide variants, insertions/duplications, deletions, and splice-site variants, with variant allele frequencies (VAF) ranging from ~2% to 100%. Variant interpretation followed established guidelines (J Mol Diagn. 2017;19:423). Performance characteristics assessed included accuracy, precision, analytical sensitivity, and specificity.

RESULTS

A total of 201 clinically significant variants were identified (127 SNVs, 47 insertions/duplications, 27 deletions). Concordance with reference results was 100% for variants with

VAF >5%. Precision studies demonstrated 100% intra- and inter-run reproducibility. Analytical sensitivity testing showed 100% detection (22/22 variants) at ≥5% VAF. At ~2.5% VAF, 21 of 22 variants were consistently detected; a single FLT3-ITD variant was detected in two of three replicates. Sequencing depth exceeded 5000 across all genes, with no low-coverage regions, including CEBPA. Sequencing artifacts >2% VAF were rare and readily distinguishable.

CONCLUSIONS

This updated NGS assay demonstrates excellent analytical performance, with high sensitivity, specificity, and reproducibility for clinically relevant variants in myeloid neoplasms. Improved coverage of CEBPA eliminates the need for supplemental Sanger sequencing, while NGS-based detection of FLT3-ITD and CALR enables variant allele frequency quantification for disease monitoring. Expanded gene content, including DDX41 and UBA1, enhances diagnostic yield and supports identification of germline predisposition and VEXAS syndrome. Implementation also improved laboratory workflow through reduced reliance on ancillary testing, decreased repeat analyses due to low artifact rates, and an estimated 0.75 FTE savings. Overall, this assay represents a significant advancement in both diagnostic capability and operational efficiency.



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