Patient ID / MRN	Patient Name		Birth Date	Gender	Age
	LAST, FIRST		DD MM YYYY		
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Test		Test Indication			
17243207 - AML, NGS, Varies		Text			

AML, NGS, Varies

ONCOGENIC OR LIKELY ONCOGENIC (TIER I/II) VARIANTS DETECTED

None Detected

See below for the Variants of Unknown Clinical Significance and Additional Notes. Please see the section "PANEL GENE LIST" section below for the complete list of genes tested.

INTERPRETATION

No clinically actionable variants were identified in this individual. This result does not rule out the presence of a mutation occurring at an allele fraction below the limit of detection of this assay, or any other mutations present outside of the targeted regions. This result should be interpreted in context of clinical, histopathological and other laboratory data.

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

None Detected

The VUS variants listed in this section (with approximate VAF) have insufficient evidence of oncogenicity or clinical significance. They are listed here for future reference in the event they become clinically significant in the light of new scientific evidence.

METHODOLOGY

The total genomic DNA was extracted from the provided sample using a bead-based EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). DNA quantity and quality were assessed using the Denovix DS-11 Spectrophotometer/Fluorometer system. The DNA was then randomly fragmented, and sequencing libraries were prepared using the Agilent Magnis NGS Prep system.

Target enrichment of regions of interest was performed using a hybridization-based capture method with the SureSelect CD Glasgow Cancer Haem Panel Kit (Agilent Technologies, CA, USA). Captured libraries were sequenced on an Illumina NextSeq 2000 platform using 2 × 150 bp paired-end reads, achieving an average sequencing depth of ≥500×. Primary data processing, including image analysis, base calling, and generation of quality scores, as well as secondary analysis involving read alignment to the human reference genome (GRCh38/hg38) and variant calling, were performed using Illumina DRAGEN software v4.2.7. Sequencing quality control metrics were evaluated using QualiMap v2.3 (Max Planck Institute, Germany) and VarSeq v3.0.0 (Golden Helix, MT, USA). Copy number variation (CNV) analysis was performed using ClinCNV v1.18.3, CNVkit v0.9.12, DRAGEN-CNV v4.2.7 and VarSeq v3.0.0, while structural variant (SV) calling was performed using DRAGEN-SV v4.2.7. Variant annotation, filtering, and clinical interpretation were carried out using VarSeq v3.0.0.

Variants are annotated according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Variant classification was performed in accordance with the AMP/ASCO/CAP and ClinGen recommendations (Li et al., 2017, PMID: 27993330; Horak et al., 2022, PMID: 35101336). Under the AMP/ASCO/CAP framework, variants are classified into four tiers based on their clinical significance: Tier I: Variant of Strong Clinical Significance, Tier II: Variant of Potential

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Clinical Significance, Tier III: Variant of Unknown Clinical Significance, and Tier IV: Benign or Likely Benign Variants. According to the ClinGen guidelines, variants are classified based on oncogenicity into five categories: Oncogenic, Likely Oncogenic, Variant of Uncertain Significance, Likely Benign, and Benign.

Variants classified as Likely Benign or Benign are not reported.

PERFORMANCE CHARACTERISTICS OF NGS PANEL

Single base substitution: accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). **Small insertion/deletion events (up to 52 bp):** accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). Larger single gene insertion/deletion events with size ≥52 bp including (meeting the minimum coverage of 500X and variant allele fraction ≥5%) will also be reported.

COVERAGE METRICS

Average target coverage: 1783X
Coverage of target region ≥50X in 99.39%
Coverage of target region ≥100X in 98.77%
Coverage of target region ≥250X in 96.27%
Coverage of target region ≥500X in 91.54%

ADDITIONAL NOTES

None

CLINICAL TRIALS

Information regarding possible clinical trials for this patient can be found at the following sites:

1. ClinicalTrial.gov: https://clinicaltrials.gov/
2. National Cancer Institute: https://www.cancer.gov/research/participate/clinical-trials-search
3. The Leukemia and Lymphoma Society's Clinical Trial Support Center: https://www.hematology.org/education/clinicians/clinical-trial-support-center

LIMITATIONS

This test does not detect gene fusions, balanced translocations, complex inversions, copy-neutral loss of heterozygosity (CN-LOH), and intronic variants deeper than ±10 base pairs from the exon-intron boundary unless otherwise indicated. Additionally, this test may not reliably detect the following: indels larger than 52bp, and single exon deletions or duplications.

The depth of sequencing coverage may be variable for some target regions, and they will be noted if they are below the minimum acceptable criteria (minimum reads <50). Low tumor cell percentage in the sample may affect the true variant allele fraction (VAF) and/or sensitivity. Multiple additional factors may impact the observed VAF including copy number alterations of the genetic locus, chromosome ploidy changes or subclonal composition. This assay does not distinguish

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between somatic and germline mutations, particularly those with variant allele fractions near 50% or 100%. Similarly, prior treatment for hematological malignancy can affect the results obtained in this assay.

Low coverage exons/regions (minimum reads <50): None

DISCLAIMER

Hematopoietic cells in some individuals may harbor age-related mutations associated with myeloid neoplasms, a phenomenon referred to as age-related clonal hematopoiesis (ARCH) or clonal hematopoiesis of indeterminate potential (CHIP). These mutations are most frequently observed in genes such as DNMT3A, TET2, and ASXL1, but may also occur in other myeloid-associated genes. The distinction between ARCH/CHIP and an overt myeloid malignancy requires correlation with clinical, morphologic, cytogenetic, and additional laboratory findings.

This is a **qualitative** next-generation sequencing assay. Reported variant allele fractions (VAFs) are provided for information only and do not represent a validated measure of disease burden or assay sensitivity for individual variants. The overall analytical sensitivity of this panel is as stated in the "Performance characteristics of NGS panel" section. If a detected variant is suspected to be a germline alteration associated with hereditary predisposition, and there is compatible clinical context and/or family history, confirmatory testing on non-hematopoietic tissue and formal genetic counseling should be considered.

The results of this test must always be interpreted in conjunction with clinical findings and other relevant hematologic, morphologic, and laboratory data, and should **not** be used in isolation to establish or exclude a diagnosis of malignancy. This assay is **not intended or validated for measurable residual disease (MRD) detection or monitoring**. This test was developed and its performance characteristics determined by the Molecular Diagnostics & Genomics Laboratory of NRL. It is used for clinical purposes and should not be regarded as investigational or for research.

PANEL GENE LIST

Gene	Region covered	Gene	Region covered	Gene	Region covered	Gene	Region covered
ANKRD26	Exons 1-34, exon 1 starting from c.-168	DNMT3A	Exons 2-23	KRAS	Exons 2-5	SETBP1	Exons 2-5, exon 6 (partial, amino acids 1391-1521, 1544-1597)
ASXL1	Full gene, introns and exons 1-13	ETV6	Exons 1-8 including partial coverage of introns 2&7 and 3' UTR	NF1	Exons 1-58	SF3B1	Exons 1-25
BCOR	Exons 2-15	EZH2	Exons 2-20	NPM1	Exons 1-11	SRSF2	Exons 1-2
BCORL1	Exons 2-14	FLT3	Exons 2-24 including introns 8 & 13-14	NRAS	Exons 2-5	STAG2	Exons 2-35
BRAF	Exons 2-18	GATA2	Exons 2-6	PHF6	Exons 2-10	TET2	Exons 3-11
CBL	Exons 1-16 including introns 7-8	IDH1	Exons 3-10	PPM1D	Exons 1-6	TP53	Full gene, introns and exons 1-11

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CEBPA	Exon 1	IDH2	Exons 1-11	PTPN11	Exons 2-15	U2AF1	Exons 1-8
CSF3R	Exons 3-17	JAK2	Exons 3-25	RAD21	Exons 2-14	WT1	Full gene, introns and exon 1 (partial, amino acids 27-100, 140-221), exons 2-10
DDX41	Exons 1-17	KIT	Exons 1-21	RUNX1	Exons 2-9 including partial coverage of introns 2, 5-7	ZRSR2	Exons 1-11

Unless otherwise indicated, coverage of the intronic regions flanking the exons is ±10 bp. For all genes, standard MANE Select transcript IDs were used.

REVIEWED BY

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