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| **Patient** **PATIENT\_IN**  **URN** URN\_IN  **DOB** DOB\_IN  **Sex** SEX\_IN | **Lab No** LAB\_NO\_IN  **Ext Ref** EXT\_REF\_IN  **Collected** COLLECTED\_IN  **Received** RECEIVED\_IN  **Specimen** SPECIMEN\_IN | **Requester** REQUESTER\_IN  **Referral Lab** REFERRAL\_LAB\_IN |

**Clinical Indication** CLINICAL\_INDICATION\_IN

**Correlative Morphology** CORRELATIVE\_MORPHOLOGY\_IN

**Specimen Details** SPECIMEN\_DETAILS\_IN

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| **HAEMATOLOGICAL MALIGNANCY GENE PANEL REPORT** |

**Test Description** Somatic variant analysis of 57 genes with clinical significance in haematological malignancy plus analysis of potential germline variants in the DDX41 gene. Refer to Panel Summary for gene list.

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| **Result Summary NO VARIANTS DETECTED.** RESULTS\_SUMMARY\_IN  **Clinical Interpretation** CLINICAL\_INTERPRETATION1\_IN  CLINICAL\_INTERPRETATION2\_IN |

**Test Methodology**

DNA is analysed by targeted gene sequencing of coding regions and flanking splice sites (within 2 bp) of the genes listed below. Libraries are prepared using a custom QIAGEN QIAseq single primer extension-based panel (Peter MacCallum Cancer Centre AllHaem v1) and sequenced on an Illumina NextSeq500 with 150 bp paired end reads. A customised CLC bioinformatics pipeline including QIAGEN CLC enterprise solutions is used to generate aligned reads and call variants (single nucleotide variants and short insertions or deletions) against the hg19 human reference genome. Variants are analysed using PathOS software (Peter Mac) and described according to HGVS nomenclature version 19.01 (http://varnomen.hgvs.org/) with minor differences in accordance with Peter MacCallum Cancer Centre Molecular Pathology departmental policy. The following population variation and cancer or genetic disease databases are commonly used in addition to literature review to assist with variant interpretation: the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org), the Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk), ClinVar (ncbi.nlm.nih.gov/clinvar) and the IARC TP53 Database (p53.iarc.fr). **Somatic variant analysis –** Variant origin (i.e. somatic or germline) is assumed based on ancillary information (e.g. population databases, literature, variant read frequency) for the purpose of clinical interpretation. All assumed somatic variants are reported (and generally considered clinically significant). Variants of uncertain origin are also reported, as are likely benign germline polymorphisms if sufficiently rare and otherwise undescribed. Testing of a non-haematological specimen may be recommended to evaluate variant origin. Recurrent population variants are not reported. When performed, FLT3-ITDs are tested for by fragment length analysis using capillary electrophoresis. The FLT3 allelic ratio is calculated by peak height ITD/peak height WT. The detection limit for FLT3-ITDs is approximately 1%. **Germline variant analysis** – All rare germline variants in DDX41 are classified according to ACMG guidelines for the interpretation of sequence variants1 with class 3 (uncertain significance), class 4 (likely pathogenic) and class 5 (pathogenic) variants reported only. Please note however that germline confirmation is required for all potential clinically significant DDX41 variants.

**Test Limitations**

The detection limit of this assay for specimens sequenced to the target read depth of 500x is a variant allele frequency (VAF) of approximately 2% with the exception of CEBPA (detection limit ~ 10%) and ASXL1 c.1934dup;p.Gly646Trpfs\*12 (detection limit ~ 5%). This assay is primarily qualitative however, the variant read frequency (VRF) is provided to assist with variant interpretation and is assumed to approximate VAF in most instances (noting that the VAF of some insertions/deletions may be underrepresented due to assay-based allele bias). The measurement of uncertainty provided as a percentage relative standard uncertainty (i.e. CV%) for variants with VAFs of 5%, 10%-20%, 30%-40% and 50% are on average, 10.2%, 10.4%, 3.5% and 4.4%, respectively. Copy number variations, loss of heterozygosity, structural rearrangements or aneuploidies are not reported. Insertions or deletions (particularly those > 25 bp in length), including FLT3-ITDs, are not reliably detected by this assay. Genes are analysed using the reference transcripts listed below; coding exons found in alternative transcripts are not assessed by this assay. This assay does not distinguish between somatic and germline variants. In addition, the clonal origin of somatic variants (i.e. disease compartment or cell lineage) cannot be determined. **For somatic variant analysis**, synonymous variants are not routinely reported. **For germline variant analysis**, variant zygosity is assumed to be either heterozygous or homozygous in the germline based on allele frequency for the purpose of clinical interpretation however, the possibilities of hemizygosity or somatic acquisition are not excluded. In haematological specimens, the possibility of a false negative germline result due to loss of the mutant allele through a somatic reversion event cannot be excluded. Please note Peter Mac assumes sample identification, family relationships, and clinical diagnoses are as stated on the request. Our clinical recommendations may be based on evidence from third-party data sources and should be interpreted in the context of all other clinical and laboratory information for this patient.

**Panel Summary**

Gene coverage in this sample is as follows

\* Please note FLT3-ITDs are not detected with this assay. A separate assay may have been performed, result included in Result Summary if sample tested. ^Partial coverage of region

Please note variants may not be optimally detected in genes with less than 100% coverage. The gene coverage above is considered acceptable given the available information about the clinical context, however please contact the laboratory for further advice should specific genes covered at less than 100% require full coverage. A list of regions with suboptimal coverage is available upon request.

Please contact the laboratory on 03 8559 7284 if you wish to discuss this report further.

**Reported by REPORTED\_BY1\_IN**

**REPORTED\_BY2\_IN**

**Authorised by AUTHORISED\_BY\_IN**

**Reported 10-Jan-2023**

**References**

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**(5): 405-23.

**CLINICAL\_CONTEXT\_IN**