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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 |

**COMMENT\_IN**

**Clinical Indication** CLINICAL\_INDICATION\_IN

**Specimen Details** SPECIMEN\_DETAILS\_IN

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| **HAEMATOLOGICAL MALIGNANCY GENE PANEL REPORT** |

**Test Description** Somatic variant analysis of 79 genes with clinical significance in haematological malignancy. Refer to Panel Summary for gene list.

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| **Result Summary NO VARIANTS DETECTED.** RESULTS\_SUMMARY\_IN See comment below regarding the test limitations of cell free DNA analysis.  **Clinical Interpretation** CLINICAL\_INTERPRETATION1\_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 v4) and sequenced on an Illumina NovaSeq 6000 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). 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.

**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 ASXL1 c.1934dup;p.Gly646Trpfs\*12 (detection limit ~ 5%-10%) and CEBPA (detection limit ~ 10%). 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 2%, 5%-10%, 20%-40% and 50% are on average, 15.4%, 8.6%, 4.0% and 1.8%, 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 and UBTF-TDs, 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. Synonymous variants are not routinely reported. 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.

**Please note the quantity of tumour derived cell free DNA within this sample is unknown. In addition, the profile of mutations present within the cell free DNA compartment may differ from that in any given individually assessed tumour sample. Therefore a negative result, or the absence of detection of a particular mutation does not imply its absence from all tumour sites in the patient (Blombery et al. 2018, PMID: 28880377). In addition, the sensitivity of this assay for variant detection is not appropriate for monitoring or minimal residual detection but rather for determining mutation profile at diagnosis/relapse.**

**Panel Summary**

Gene coverage in this sample is as follows

\* Please note FLT3-ITDs are not reliably detected with this assay

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\_BY\_IN**

**Authorised by AUTHORISED\_BY\_IN**

**Reported 15-Mar-2024**

**CLINICAL\_CONTEXT\_IN**