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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

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| **CEBPA GERMLINE VARIANT ANALYSIS REPORT** |

**Test Description** Germlinevariantanalysis of all coding regions of the CEBPA gene.

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| **Result Summary RESULTS\_SUMMARY1\_IN** **See Reportable Variants table below for details.**  **Clinical Interpretation** CLINICAL\_INTERPRETATION1\_IN  CLINICAL\_INTERPRETATION2\_IN |

**Test Results**

**Reportable Variants** Please note, variant origin (somatic or germline) cannot be determined by this assay. Variant origin is assumed here based on ancillary information (e.g. population databases, literature, variant read frequency) for the purpose of clinical interpretation however testing of a germline sample may be recommended in some circumstances.

VRF – variant read frequency

**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. **Germline variant analysis** – All rare germline variants in CEBPA are classified according to ACMG guidelines for the interpretation of sequence variants[1](#_ENREF_1) with class 3 (uncertain significance), class 4 (likely pathogenic) and class 5 (pathogenic) variants reported only. 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. **Somatic variant categorisation (modified from AMP/ASCO/CAP guidelines**1**) –** Variants are curated and categorised according to the clinical context of the patient and categorised as **DIAGNOSTIC** (the variant either defines a diagnostic category or is sufficiently specific for the clinical context to contribute to diagnostic subcategorisation), **PROGNOSTIC** (the variant has been associated in large trials/series with inferior or superior outcomes in either the context of a specific therapy or independent of therapy. Note this does not take into account interaction between prognostic variants present in the individual patient. Relevant pairwise interactions are presented in the clinical summary), **DRUG TARGET** (the variant or variant class is specifically targeted by a therapeutic agent, this category only includes therapeutic agents that are clinically advanced and generally available through either reimbursement or clinical trials [i.e. not early stage investigational agents]), **DRUG RESISTANCE** (the variant is specifically associated with resistance to a targeted agent [i.e. does not include non-specific resistance to non-targeted therapies]), **MRD MARKER** (the variant is an established biomarker for which assessment at MRD sensitivity after therapy is accepted practice). If the variant is not categorised into any of the above categories it is assigned **CLONAL MARKER** indicating its utility in defining the presence of a clonal haematopoietic process in the specimen. These categorisations are general in nature and may not be applicable to the specific clinicopathological context of the patient.

**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 ~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 10%-20%, 30%-40% and 50% are on average, 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) are not reliably detected by this assay. CEBPA is analysed using the reference transcript listed below; coding exons found in alternative transcripts are not assessed by this assay. **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. 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 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.