# Genetic Diagnosis of Acute Myeloid Leukaemia by Next Generation Sequencing


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## Introduction

Acute myeloid leukemia (AML) is a cancer that affects the blood and bone marrow, characterised by an overproduction of immature white blood cells. Molecular genetic analysis of AML-associated genes is assuming increasing importance in the diagnosis, prognosis and treatment of AML. The presence and pattern of gene mutations in AML can provide critical prognostic information and may assist in guiding therapeutic management decisions by clinicians, particularly if targeted therapies are available.

Next generation sequencing (NGS) is a massively parallel sequencing technology that allows for comprehensive analysis of multiple genes in a single assay that has led to changes in the classification, risk stratification, treatment and response assessment of AML.

In the absence of a proficiency testing program, RCPAQAP received requests to develop an external quality assurance for NGS analysis of AML in 2018. A trial study was introduced the same year to laboratories from the Australasian region. In 2019, the first pilot external quality assurance (EQA) for AML NGS Panel Testing was offered to laboratories from the Australasia region. Following a successful introduction of the pilot program, the AML NGS Panel Testing EQA has now been added to the RCPAQAP scope of accreditation and is offered worldwide.

This study presents the findings from the 2019 and 2020 AML NGS Panel Testing surveys.

## Method

- Two patient-derived DNA samples were provided to participating laboratories in each survey.
- A total of 1µg DNA per sample was provided for NGS analysis.
- Participating laboratories were required to perform NGS analysis of each DNA sample using their routine AML NGS gene panel and submit a copy of the clinical report for assessment.
- A report outlining the NGS methods used, gene variants reported, and laboratory detection rate (number of laboratories reporting / number of laboratories performing the test) was issued.
- A total of 11 laboratories participated in this program in 2019 and 2020 (Figure 1). Ninety-one percent (91%) of laboratories re-enrolled in the subsequent year following their initial participation in the program.

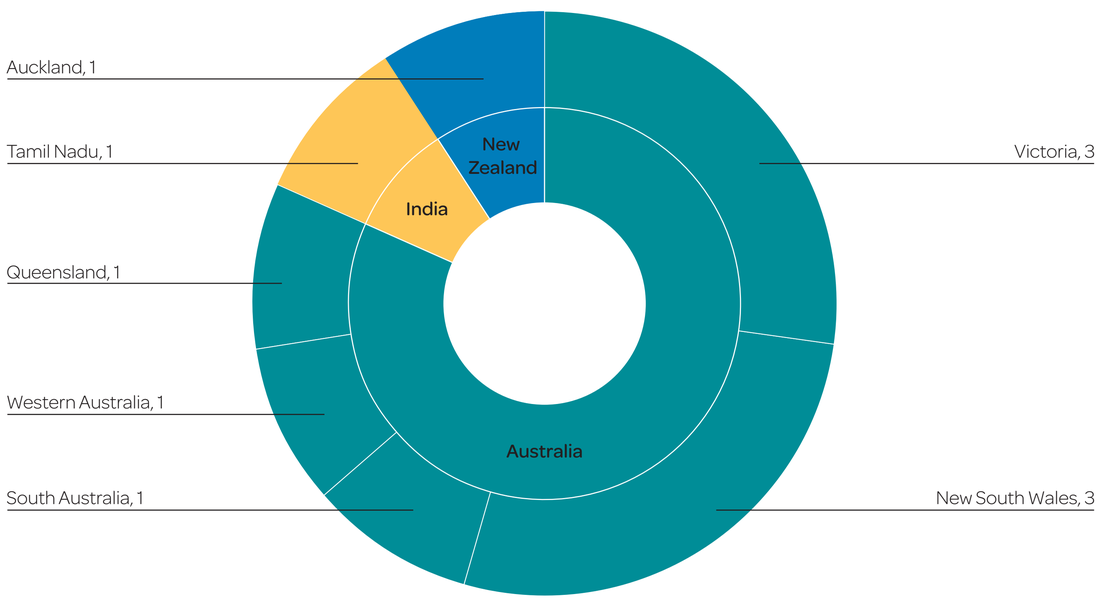


**Figure 1.** Geographical origins of laboratories enrolled in the 2019 and 2020 Acute Myeloid Leukaemia Next Generation Sequencing Panel Testing program.

- The most common NGS platforms used in this program are the Illumina systems (MiSeq, MiniSeq, NextSeq 500 and NextSeq 550). Most assays have a limit of detection of 5 to 10%.
- A total of 146 genes were screened in the 2019 survey; 103 genes were screened in the 2020 survey.
- The total number of genes tested by each participating laboratory are presented in Table 1.
- Common genes included in the NGS panels of all participating laboratories are presented in Table 2.

**Table 1.** Total number of genes tested by each laboratory.

| Laboratory | 2019 | 2020 |
|---|---|---|
| 1 | 37 | 39 |
| 2 | 39 | n/a |
| 3 | 30 | 30 |
| 4 | 31 | 28 |
| 5 | n/a | 57 |
| 6 | 30 | 30 |
| 7 | 41 | 41 |
| 8 | 86 | 50 |
| 9 | 26 | 26 |
| 10 | n/a | 37 |
| 11 | 78 | 78 |

**Table 2.** Table 2. Common genes included in all next generation sequencing gene panels for the 2019 and 2020 surveys.

| 2019 | 2020 |
|---|---|
| *ASXL1* | *ASXL1* |
| *CALR* | *CALR* |
| *CSF3R* | *CSF3R* |
| *DNMT3A* | *DNMT3A* |
| *EZH2* | *EZH2* |
| *FLT3* | *FLT3* |
| *IDH1* | *IDH1* |
| *IDH2* | *IDH2* |
| *JAK2* | *JAK2* |
| *KIT* | *KIT* |
| *MPL* | *KRAS* |
| *NRAS* | *MPL* |
| *TET2* | *NPM1* |
| *CBL* | *NRAS* |
| *SETBP1* | *RUNX1* |
| *SF3B1* | *TET2* |
| *SRSF2* | *TP53* |
| *U2AF1* | – |

## Results

- Assessment of participant results takes into consideration the genes and gene regions screened, and the limit of detection.
- Variants identified in each sample and the median variant allele frequency (VAF) for each variant are listed in Table 3. Consensus variants were reported by at least 80% of participating laboratories. Non-consensus variants (<80%) are presented in red in Table 3.
- All consensus variants reported are from the Tier I or II variant classification groups. Of the participating laboratories, 7/11 (65%) were able to identify all consensus variants in both surveys.
- There were 4/11 participating laboratories (35%) that received at least one 'Discordant' assessment for failing to detect a consensus variant.
- Variation in the interpretation and reporting of variants detected is noted, specifically variants of unknown clinical significance (VUS).

**Table 3.** Consensus and non-consensus (in red) variants reported and the associated variant allele frequency presented as median ± standard deviation (SD).

| Sample | Variant | Median VAF ± SD | Laboratory Detection Rate |
|---|---|---|---|
| 1 | FLT3 internal tandem duplication | 34.00 ± 16.65 | 100.0% |
| | WT1 p.(Ser381Leufs*71) | 35.10 ± 3.35 | 100.0% |
| | WT1 p.(Arg385Glyfs*5) | 38.5 ± 7.72 | 86.0% |
| | *ZRSR2 p.(Ser447_Arg448dup)* | n/a | *17%* |
| 2 | DNMT3A p.(Arg882Cys) | 49.00 ± 2.29 | 100.0% |
| | IDH1 p.(Arg132His) | 42.00 ± 1.18 | 100.0% |
| | NPM1 p.(Trp288Cysfs*12) | 40.50 ± 6.76 | 100.0% |
| | NRAS p.(Gly12Asp) | 7.50 ± 1.21 | 89.0% |
| | TET2 p.(Cys1289Tyr) | 47.00 ± 3.03 | 89.0% |
| | *PTPN11 p.(Ala72Thr)* | *4.50 ± 0.50* | *60%* |
| | *JAK3 p.(Val772Ile)* | *49.00* | *25%* |
| 3 | DNMT3A p.(Arg882His) | 8.00 ± 0.74 | 100.0% |
| | FLT3 internal tandem duplication | 10.00 ± 5.76 | 100.0% |
| | IDH2 p.(Arg172Lys) | 36.04 ± 1.72 | 100.0% |
| | RUNX1 p.(Gly408Valfs*193) | 32.00 ± 4.43 | 90.0% |
| | U2AF1 p.(Ser34Phe) | 36.70 ± 0.75 | 88.9% |
| | *FLT3 p.(Ala680Val)* | *3.00 ± 0.95* | *50%* |
| | *NRAS p.(Gly12Asp)* | *2.80 ± 0.86* | *40%* |
| | *TET2 p.(Pro1728Ser)* | *48* | *10%* |
| | *TET2 p.(Tyr867His)* | *52* | *10%* |
| 4 | FLT3 p.(Asp835Glu) | 41.00 ± 2.04 | 100.0% |
| | NPM1 p.(Trp288Cysfs*12) | 38.47 ± 7.45 | 100.0% |
| | WT1 p.(Ala382Glyfs*3) | 47.27 ± 5.29 | 100.0% |
| | DNMT3A p.(Arg736Cys) | 48.00 ± 2.10 | 90.0% |
| | *FLT3 p.(Lys623Ile)* | *4.97 ± 0.29* | *60%* |
| | *ZRSR2 p.(Pro383Ala)* | *53.5 ± 4.95* | *25%* |

## Discussion

Developing an EQA program for NGS presents a variety of problems as the identification of clinically relevant genes is challenging. Due to the wide breadth of strategies used by laboratories, it was not ideal to restrict the EQA to particular diagnostic testing scenarios as this would significantly limit the number of participating laboratories.

Genetic variants detected between 5% and 10% VAF may indicate low-level (i.e. subclonal) tumour populations, although these findings' clinical significance may not be apparent. Correlations with clinical, histopathologic and additional laboratory findings are required for final interpretation of the results.

The major limitations of the NGS methodology of detecting AML-associated variants are related to the limited sensitivity and specificity of the assays. The NGS approach should be validated to establish the expected performance characteristics within each laboratory.

### References

1. Aguilera-Diaz, V., Vazquez, I., Ariceta, B., et al., 2020. Assessment of the clinical utility of four NGS panels in myeloid malignancies. Suggestions for NGS panel choice or design. *PLOS ONE*, 15(1).
2. Bullinger, L., Dohner, K. and Dohner, H. 2017. Genomic of Acute Myeloid Leukemia Diagnosis and Pathways. *J Clin Oncol*, 35(9):934-946.
3. Cai, S. F. and Levine, R. 2019. Genetic and epigenetic determinants of AML pathogenesis. *Semin Hematol*, 56(2):84-89.
4. Leisch, M., Jansko, B., Zaborsky, N., et al. 2019. Next Generation Sequencing in AML – On the Way to Becoming a New Standard for Treatment Initiation and/or Modulation?. *Cancers*, 11(2): 252.
5. Papaemmanuil, E., Gerstung, M., Bullinger, L., et al., 2016. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Eng J Med*, 374(23):2209-2221.
6. Yoest, J. M., Shirai, C. L. and Duncavage, E. J. 2020. Sequencing-Based Measurable Residual Disease Testing in Acute Myeloid Leukemia. *Front Cell Dev Biol*, 8:249.

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