**Methods**

141 genes were selected for the panel based on review of clinical guidelines for myeloid diseases including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN), and lymphoid diseases including acute lymphoblastic leukemia, chronic lymphocytic leukemia, follicular lymphoma, and other lymphoid subtypes. Custom hybridisation probes (Twist Biosciences) were designed to interrogate all coding exons of the 141 genes. The assay detects single nucleotide variants (SNVs), insertions/deletions (in/dels) in 141 genes, FISH internal tandem duplications (FTD-ITDs), select non-coding pathogenic variants, and gender. The assay also identifies CNAs in a subset of 16 genes that were selected based on the frequency of gene-level CNAs observed in a population of >10,000 myeloid or lymphoid samples with confirmed myeloid or lymphoid indications. The custom hybridisation-based assay utilizes genomics libraries, created from 250 ng DNA extracted from peripheral blood, bone marrow, or flow cytometry cell suspensions, followed by sequencing on Illumina NextSeq 550. Sequencing analysis was performed using an in-house analysis pipeline to detect variants and perform robust filtering of technical artifacts. Concordance studies were performed on clinical samples previously assessed using an orthogonal NGS-based laboratory developed test for SNVs/in/dels and FTD-ITDs (ArcherDx) or digital multiplex ligation-dependent probe amplification (dMLPA) (MRM Holland) for CNAs. Small indels were defined as <25bp while long indels are ≥25bp.

**Results and Figures**

**Figure 1. Concordance of SNVs, small indels, and long indels.** Figure shows pairwise absolute frequency (VF) of variants from clinical samples detected by the assay compared to expected VF of variants detected by Illumina dMLPA at 90% mean coverage. SNVs were detected at an expectedVF=0.002±0.001 (P=0.82), long indels were detected at an expected VF=0.006±0.002 (P=0.66). The green and red graphs (highlighted in yellow) show high agreement. The yellow box highlights a population of >10,000 microarray (141 genes, 2,072 probes (Twist Biosciences) were designed to lymphoma subtypes.

**Figure 3. Dilution series of SNVs, small indels, and long indels.** Clinical samples with variants previously identified by an orthogonal method were diluted in gender-matched normal control samples to assess analytical sensitivity. The assay figure shows VF detected by the assay compared to the expected VF based on power method and field dilution, colored by mutation type. Shaded lines show linear fit (adjusted R^2=0.99, slope=0.94). A dilution series of 21 positive clinical samples at 4 different dilutions was performed. For all 3 evaluated SNVs in 1 sample, the VF was detected at 0.6% and 1.0% (10/11 samples). For long indels (≥25bp), 4 in total had long indels ranging from 9.0% to 12.0% (10/11 long indels in genes, n=12 samples). For small indels <25bp, 1 sample was excluded that listed sequencing QC.

**Figure 4. Concordance of CNAs.** CNAs were selected based on their diagnostic and prognostic significance for myeloid and lymphoid malignancies. 12 of these genes were previously reported as CNAs that were selected for the panel (highlighted in yellow), and are listed with a blue outline. Genes highlighted in red are those whose recognition of these regions of genetic alterations with a red outline are additionally isolated for CNAs.

**Figure 5. Dilution series of CNAs.** Cell line DNA (GM03) with known CNAs were diluted in gender-matched normal control cell DNA. Figure shows CNAs detected by the assay compared to the expected CNAs detected by dMLPA for sex chromosome abnormalities. The assay figure shows agreement of CNAs detected by the assay compared to the expected CNAs detected by dMLPA at a 0.05% threshold (P=0.82). Male and Female samples showed detection of 77.6% at VAF ≥3% (45/58 small indels in 10 genes; n=28 samples). 4/11 samples had small indels <25bp and detection was 100% (4/4). A model for gender classification was created using the ratio of each CMS’s female (ChrX) and male (ChrY) gene counts. Genes were selected from a genome-wide Catalogue of Sex Chromosome Abnormalities (dMLPA) (MRM Holland) for CNAs. Small indels were defined as <25bp while long indels are ≥25bp.

**Conclusions**

These data are a preliminary estimate of the performance of the developed NGS assay. Taken together, they show that the NGS assay is sensitive, specific, and accurate when detecting SNVs, indels and CNAs. The panel content includes genes with clinical relevance for a broad range of hematological diseases including those recommended in recent updates by the UN-International Classification of American and MDS/AML with myelodysplasia-related gene mutations.

The clinical utility of the assay is increased by the ability to simultaneously detect clinically relevant CNAs concurrently with SNVs and indels from the same sequence data.

References

1. Severson EA, et al.; Diagnosis and management of AML in adults: 2023 recommendations from an international expert panel on behalf of the work group 1049:1177 (2023)


Corresponding author: Eric A Severson, hoggg@labcorp.com

**Table 1. Concordance of SNVs, small indels, and long indels.** Figure shows pairwise absolute frequency (VF) of variants from clinical samples detected by the assay compared to expected VF of variants detected by Illumina dMLPA at 90% mean coverage. Base calls for whole genome sequencing data is available for comparison. Base calls for small indels <25bp at VAF ≥ 3% (19/19 indels in 11 genes; n=28 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples). For long indels ≥ 25bp, PPA varied for each gene, 47.9% (23/48 small indels in 10 genes; n=12 samples).