Molecular Mutation Sequencing for Hematological Malignancies

Providence Health & Services is pleased to offer the following Next Generation Sequencing (NGS) panels for the molecular profiling of hematological malignancies, focusing on leukemia and Myeloproliferative disorders effective June 1, 2016.

<table>
<thead>
<tr>
<th>Sequencing Panel Order</th>
<th>Gene Coverage</th>
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<tbody>
<tr>
<td>Myeloid AML Targeted Panel, Sequencing</td>
<td>CEBPA, FLT3, IDH1, IDH2 and NPM1</td>
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<tr>
<td>Myeloid AML Comprehensive Panel, NGS</td>
<td>ASXL1, BCOR, BRAF, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2 Exon 12+14 (includes JAK2 V617F), KIT, KRAS, MLL, NPM1, NRAS, PDGFRA, PHF6, PTPN11, RUNX1, SETBP1, STAG2, TET2, TP53 and WT1</td>
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<tr>
<td>JAK2 V617F with Reflex to JAK2 Exon 12/14, NGS</td>
<td>If JAK2 V617F is not detected, sequencing results are reanalyzed and reviewed for JAK2 Exon 12+14</td>
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<tr>
<td>Myeloid MPN Targeted Panel, NGS</td>
<td>JAK2 Exon 12+14 (includes JAK2 V617F), CALR, MPL</td>
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<tr>
<td>Myeloid MPN/MDM/CMMV Comprehensive Panel, NGS</td>
<td>ABL1, ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2 Exon 12+14 (includes JAK2 V617F), KIT, KRAS, MPL, NPM1, NRAS, PDGFRA, PTEN, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, and ZRSR2</td>
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Clinical Indications:

These new panels are designed to target frequently mutated genes that are used in the diagnosis, prognosis, and risk stratification of Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS), and Myeloproliferative Neoplasms (MPN). NGS technologies can simultaneously test for mutations in multiple genes associated with myeloid neoplasias, streamlining the ordering and reporting process.

As noted in NCCN® Guidelines, genetic biomarkers such as FLT3, NPM1, CEBPA and KIT can be important for risk assessment and prognostications, and may also guide treatment decisions in AML. It has also been reported that other molecular markers may provide additional prognostic impact, such as IDH1 & IDH2 and DNMT3A in AML.

WHO diagnostic criteria for myeloproliferative disorders recognizes the significance of mutations in JAK2, as well as other gene variants, such as MPL and CALR. Mutations is KIT, PDGFRA, PDGFRB and FGFR1 may be used to identify Tyrosine kinase inhibitor (TKI) sensitivity. Mutations in ASXL1, TP53, ETV6, EZH2, RUNX1 and TET2 can be used to assess prognosis in MDS.

May 2016
**Performed:** Batched once per week

**Reported:** 7-14 days

**Collect:** Whole, peripheral blood Lavender (EDTA) OR Bone Marrow aspirate Lavender (EDTA)

NOTE: Genomic DNA also accepted: 50 ng (recommended), A260/A280 = 1.8-2.0

**Transport:** 3 mL whole blood or bone marrow aspirate at 2 to 8°C (minimum 0.5 mL)

**Storage / Stability Blood and bone marrow:**
- Ambient: up to 1 day
- Refrigerated (2 to 8°C): 7 days
- Frozen: unacceptable

**Comments:**
Mutation detection is performed using targeted NGS methods based on the Illumina TruSight® Myeloid Sequencing Panel. CEBPA gene coverage is supplemented with Sanger Sequencing to provide adequate coverage for detection of all possible variants. Single genes included in the above panels may also be requested. Post-sequencing analysis of tumor-associated variants are filtered through bioinformatic pipelines and results for genes not requested by the ordering provider are masked and excluded from the annotation and review processes.

The assay targets somatic mutations with variable full exon, partial region, or hot spot coverage (depending on specific locus). This test will detect single base substitutions (i.e., point mutations), as well as small insertion or deletions, but it does not detect gene rearrangements (i.e., translocations), gene fusions or copy number alterations. Mutations outside the targeted regions will not be detected. This test does not reliably differentiate between somatic and germline alterations. Additional testing may be necessary to clarify the significance of results if there is a potential hereditary risk.

The results of this test should not be used as the sole means for clinical diagnosis or patient management decisions and must always be interpreted within the clinical context of clinical findings, tumor sampling, histopathology, and other laboratory data, and should not be used alone for a diagnosis of malignancy.

**References:**

For more information, please contact Providence Laboratory Services at 503.215.6660.  

May 2016