FLT3 Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) Mutation Testing

FLT3 mutation status helps refine prognosis and guide therapy in patients with acute myeloid leukemia (AML). Two basic categories of mutation are recognized in *FLT3:* (1) internal tandem duplication (ITD) within the *FLT3* juxtamembrane domain and (2) activating missense mutations affecting the tyrosine kinase domain (TKD). Both categories of mutation cause constitutive activity of the mutant FLT3 protein. The resulting constitutive FLT3 kinase activity is thought to promote cell proliferation and anti-apoptotic signaling via the JAK/STAT, PI3K/AKT and RAS pathways.

FLT3 ITD results from an in-frame insertion in exons 14 and 15 of the juxtamembrane domain of FLT3 that can vary in size (3~400 nucleotides). *FLT3* ITD mutations are observed in approximately 15% of pediatric, 30% of young adult/adult, and 25% of older adult AML patients, more commonly in the presence of normal cytogenetics.

FLT3 TKD mutations occur at exon 20 of the kinase domain and can result from point mutations, insertions or deletions. This assay identifies 90% of reported mutations in the *FLT3* tyrosine kinase domain by interrogating two commonly mutated codons- Asp835 (D835) and Ile836 (I836)- associated with response to midostaurin. TKD mutations have been reported in 7-10% of AML patients.

The presence of either a *FLT3* ITD or TKD mutation may be associated with response to the tyrosine kinase inhibitor, midostaurin.[1] In addition, *FLT3*-ITD mutations are associated with inferior prognosis in patients with AML, particularly when the allelic ratio (mutant allele:wild-type allele ratio) is elevated.[2] The threshold for a high allelic ratio has varied in publications, with cut-offs ranging from 0.5 to 0.8.

Orderable tests and clinical indications:

1. Combined *FLT3* (ITD) and *FLT3* (TKD) DNA assay panel: Indicated in new AML patients to refine prognosis and to identify those patients likely to respond to the tyrosine kinase inhibitor, midostaurin. **The combined *FLT3*-ITD/TKD assay is included as a reflex order with the Myeloid Mutation Panel- AML.** For samples positive for a *FLT3*-ITD mutation, the ratio of the mutant to wild-type allele is reported (*FLT3*-ITD allelic ratio).

2. Stand-alone *FLT3* (ITD) DNA analysis: Indicated in patients with relapsed or persistent AML, which was previously shown to harbor a *FLT3*-ITD mutation. Stand-alone *FLT3*-ITD testing may be utilized to confirm that the persistent clone continues to harbor an ITD mutation. When positive, an allelic ratio is reported.

Turn around time:
Less than 7 days (the assay is run twice per week).

Specimen Requirements:
Bone marrow aspirate (1 mL, EDTA) and peripheral blood (3mL, EDTA) having at least 10% neoplastic cells and refrigerated up to 24 hours. However, Wright-stained or unstained bone marrow aspirate smears are also accepted. The assay is generally sensitive to variants above 5% allele fraction (10% clonal cells). Therefore, a minimum percentage of 10% neoplastic cells is required. This test is NOT appropriate for monitoring minimal residual disease. Results are reported as positive or negative to a sensitivity of 5% of DNA.

References:

1. Stone RM, *et al. N Engl J Med.* 2017; 377(5):454-464. PMID: 28644114.
2. Schlenk RF, *et al. Blood.* 2014; 124(23):344-3449. PMID: 25270908.
3. Stirewalt DL, *et al. Nat Rev Cancer*. 2003;3(9):650-665. PMID:12951584.
4. Thiede C, *et al. Blood*. 2002;99(12):4326-4335. PMID: 12036858.

To consult a pathologist about indications for testing or the significance of a result, call the Molecular Genetics Lab at (984) 974-1825 or Dr. Nathan Montgomery at (919) 445-6414.
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