LeukoStrat CDx FLT3 Mutation Assay

Versiti offers the LeukoStrat® CDx FLT3 Mutation Assay in vitro diagnostic test designed to detect internal tandem duplication (ITD) mutations and the tyrosine kinase domain (TKD) mutations in acute myeloid leukemia (AML).

The fms related tyrosine kinase 3 (FLT3) is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis[1]. Although generally associated with normal cytogenetics where patients have standard risk of relapse, FLT3 mutations have also been identified in sub-groups of patients with chromosomal abnormalities that are associated with high risk of disease relapse[2-3]. The most prevalent type of FLT3 mutation is an internal tandem duplication (ITD) in the juxtamembrane domain[4]. The second most common mutation type in the FLT3 gene is a tyrosine kinase domain (TKD) point mutation in the codon for an aspartate (D835) or an isoleucine (I836) residue. TKD mutations result in constitutive autophosphorylation and activation of FLT3[5-6] and have also been linked to poor overall survival, but to a lesser extent as compared to ITD mutations[7]. To determine the best treatment options, it is recommended that patients with AML be screened for the presence of FLT3 mutations.

Indications for testing:

The LeukoStrat CDx FLT3 Mutation Assay is a PCR-based, in vitro diagnostic test designed to detect internal tandem duplication (ITD) mutations and the tyrosine kinase domain (TKD) mutations D835 and I836 in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myeloid leukemia (AML).

The LeukoStrat CDx FLT3 Mutation Assay is the only FDA approved predictive test for the efficacy of midostaurin (RYDAPT®) therapy in all acute myeloid leukemia (AML) patients, regardless of cytogenetics. AML Patients with a detectable FLT3 mutation above the clinical cut-off of signal ratio 0.05 are indicated for midostaurin (RYDAPT®) therapy.

The LeukoStrat CDx FLT3 Mutation Assay is the only FDA approved predictive test for the efficacy of gilteritinib (XOSPATA®) therapy in relapsed or refractory acute myeloid leukemia (AML) patients. Relapsed or refractory AML Patients with a detectable FLT3 mutation above the clinical cut-off of signal ratio 0.05 are indicated for gilteritinib (XOSPATA®) therapy.

Assay Description:

The LeukoStrat CDx FLT3 Mutation Assay is a PCR based test designed to detect internal tandem duplications (ITD) and tyrosine kinase (TKD) mutations in the FLT3 gene.

ITD: The duplication and insertion of a portion of the FLT3 gene that includes the region in and around the juxtamembrane region of the FLT3 gene.

TKD: Nucleotide(s) changes at codon 835 and/or 836 that are detected by inactivation of the EcoRV restriction digestion site within the tyrosine kinase domain for the FLT3 gene.

The polymerase chain reaction is performed on DNA isolated from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myeloid leukemia. Fluorescently labeled primers are used to amplify the sequences of interest. The TKD PCR product is digested with the EcoRV restriction enzyme. The ITD PCR products and the digested TKD PCR products are run on an ABI 3500xL Genetic Analyzer and their sizes determined.

The LeukoStrat CDx FLT3 Mutation Assay is performed at the Laboratory for Personalized Molecular Medicine (LabPMM) LLC and the interpretive comments are provided by Versiti.

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Assay sensitivity and limitations:

The assay measures the ratio of signals from mutation against a background of signal from wild type. The mutant:wild-type signal ratio (SR) cut-off (medical decision point) is 0.05. The SR is the peak area of the mutant signal, if present, divided by the peak area of the wild-type signal[8].

Reporting of results:

If the mutant:wild type SR for either ITD or TKD in a valid sample is at or above the clinical cut-off of 0.05, the result will be interpreted and reported as Positive (DETECTED). Below the clinical cut-off of 0.05, the result will be interpreted and reported as Negative (NOT DETECTED). The overall FLT3 mutation will also be reported as DETECTED or NOT DETECTED. When mutations are detected, the signal ratio (SR) will be reported for each FLT3 ITD mutations and/or FLT3 TKD mutations.

Specimen requirements:

Recommended Specimen Volume (Preservative)

- 2mL of peripheral blood (Sodium Heparin)
- 0.5mL of bone marrow (Sodium Heparin)

Minimum Specimen Volume (Preservative)

- 1mL of peripheral blood (Sodium Heparin)
- 0.25 mL of bone marrow (Sodium Heparin)

Specimens can be stored at 2-8°C for up to 7 days prior to testing. Do not freeze.

Frozen samples are not acceptable.

SHIP

Shipping requirements:

Ship the specimen(s) with cold packs in boxes via overnight carrier. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Versiti Client Services/Molecular Oncology Laboratory
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

Versiti Molecular Oncology Requisition.

Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround time:

Test Code: 4675

CPT codes: PLA codes 0023U, 0046U

Turnaround time: 5-7days

CPT and Order Codes are provided for reference purposes only and are subject to change. They are not intended as a guide for internal billing procedures. Institution is solely responsible for identification of correct billing codes.

For additional information related to shipping, billing, or pricing, please contact Versiti Client Services: 414-937-6396 or 800-245-3117 Option 1, or LabInfo@versiti.org.

References:

1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, (v.2.2014) National Comprehensive Cancer Network.
2. Lowenberg, B. et al. (1999) "Acute myeloid leukemia." N Engl J Med 341(14):1051-62.
3. Thiede, C. et al. (2002) "Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." Blood 99(12): 4326-35.
4. Nakao, M. et al. (1996) "Internal tandem duplication of the FLT3 gene found in acute myeloid leukemia." Leukemia 10(12):1911-18.
5. Yamamoto, Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R, Naoe T. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood, 2001, 97(8):2434-9.
6. Gilliland, D.G. and Griffin, JD. (2002) The roles of FLT3 in hematopoiesis and leukemia. Blood 100(5):1532-1542.
7. Yanada M, et al. (2005) Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. Leukemia 19 (8): 1345-1349. Leukemia 19 (8): 1345-1349.
8. PMA number P160040 on www.fda.gov

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