Lab Dept: Anatomic Pathology

Test Name: FLT3 ASSAY, QUALITATITVE

General Information

Lab Order Codes:	FLT3
Synonyms:	FLT3 Gene Mutation Analysis, Qualitative
CPT Codes:	81245 – Chromosome analysis for breakage syndromes; baseline Sister Chromatid Exchange (SCE), 20-25 cells
Test Includes:	All patient samples are tested with two controls for assay quality and accuracy. The polymerase chain reaction (PCR) consisted of ~10 ng of genomic DNA being amplified using two consensus primer pairs that are fluorescently labeled. These primer pairs correspond to the two regions surrounding the mutation sites in the FLT3 gene (ITD and D835). The products of the PCR reaction (35 cycles) are digested with the restriction endonuclease EcoRV, which cleaves wild type D835, but not any mutant counterparts. The resulting amplicons are then identified using capillary electrophoresis.

Logistics

Test Indications:	Used as a prognostic factor in Acute Myeloid Leukemia (AML). An internal tandem duplication within exons 14 and 15 has been found to be an independent prognostic factor for poor outcome in both pediatric and adult AML patients. Prognostic significance of FLT3/ITD may be modified by the allelic ratio of the FLT3/ITD. The test is free of charge for the initial diagnostic sample for all **on study** patients (typically bone marrow samples only). Please indicate by filling in the “On Study” check box from the Bone Marrow Submission Form. Patients not on study will be billed as well as subsequent samples from on study patients.
Lab Testing Sections:	Chemistry - Sendouts
Referred to:	Molecular Hematopathology @ Seattle Cancer Care Alliance, 825 Eastlake Ave, E Room G7801, Seattle, Washington 98109
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Daily, 24 hours
Turnaround Time:	8 – 10 days

Special Instructions:	Restricted draw times. Please draw samples Sunday through Friday only.

Specimen

Specimen Type:	Blood or Bone Marrow
Container:	Lavender top (EDTA) tube
Draw Volume:	5 mL (Minimum: 5 mL) blood 2 mL (Minimum: 1 mL) bone marrow
Processed Volume:	Same as Draw Volume
Collection:	Routine venipuncture or bone marrow collection. Clearly label specimen as blood or bone marrow. Invert specimen gently to mix.
Special Processing:	Lab Staff: **Do Not** centrifuge specimen. Submit specimen in original collection container. Store and ship specimen at ambient temperature. Specimen must be received within 48 hours of collection. Mark for Overnight delivery. Do not ship on Friday for Saturday delivery. If sample must be drawn for weekend delivery, please contact the lab ahead of time, and include tracking number, to ensure receipt and processing of sample. Forward promptly. Note: To credit billing for patients “on study” use code CFLT.
Patient Preparation:	None
Sample Rejection:	Mislabeled or unlabeled specimens

Interpretive

Reference Range:

Possible results are found below:	
Positive	ITD of FLT3 gene is present
Negative	ITD of FLT3 gene is not detected
Inconclusive	The duplicate results were not in concordance. A repeat sample is recommended as clinically indicated.
Inadequate Sample	The DNA extracted from the sample was insufficient and/or degraded. A repeat sample is recommended.

Allelic Ratio	Mutant to wild type ratio. Ratios of 0.4 or greater in pediatric patients and 0.78 or greater in adults may be a significant prognostic factor for poor outcomes.

Critical Values: N/A

Limitations: The FLT3 Assay does not detect an internal duplication (ITD) or a point mutation at D835. A negative result does not rule out the presence of a small population of cells containing either FLT3 mutation [FLT3(+)] that fall below the sensitivity of the assay (5% allele fraction). The clinical significance of small populations of FLT3(+) cells is unknown.

FDA required disclaimer: This test was developed and it performance characteristics determined by the SCCA Molecular Oncology Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined, however, that in most cases, such approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research.

Methodology: PCR (Polymerase Chain Reaction)

References: Molecular Hematopathology @ Seattle Cancer Care Alliance (January 2011)

Updates: 1/24/2011: Testing moved from Fred Hutchinson to Seattle Cancer Care Alliance.
2/5/2013: CPT update