Overview

Useful For
Diagnosing systemic mastocytosis in extracted DNA specimens

Special Instructions
- Hematopathology Patient Information

Method Name
Allele-Specific Oligonucleotide Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Specimen must arrive within 168 hours of collection.

Necessary Information
The following information is required:

1. Pertinent clinical history
2. Clinical or morphologic suspicion
3. Date of collection
4. Specimen source

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Extracted DNA from blood or bone marrow

Container/Tube: 1.5- to 2-mL tube

Specimen Volume: Entire specimen

Collection Instructions:

1. Label specimen as extracted DNA from blood or bone marrow
2. Indication volume and concentration of DNA

Specimen Stability Information: Refrigerated/Ambient
Test Definition: KITAS

KIT Asp816Val Mutation Analysis, V

Forms
1. Hematopathology Patient Information (T676) in Special Instructions

2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume
Extracted DNA: 50 mcL at 20 ng extracted DNA/mcL

Reject Due To

<table>
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<tr>
<th>Other</th>
<th>Bone marrow biopsies</th>
<th>Paraffin-embedded bone marrow clots</th>
<th>Paraffin-embedded tissue</th>
<th>Slides</th>
<th>Paraffin shavings</th>
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Specimen Stability Information

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Clinical and Interpretive

Clinical Information

Systemic mastocytosis is a hematopoietic neoplasm that can be included in the general category of chronic myeloproliferative disorders (CMPDs). These neoplasms are characterized by excessive proliferation of one or more myeloid lineages, with cells filling the bone marrow and populating other hematopoietic sites. In systemic mastocytosis, mast cell proliferation is the defining feature, although other myeloid lineages and B-cells are frequently part of the neoplastic clone.

Function-altering point mutations in KIT, a gene coding for a membrane receptor tyrosine kinase, have been found in myeloid lineage cells in the majority of systemic mastocytosis cases. The most common KIT mutation is an adenine-to-thymine base substitution (A->T) at nucleotide position 2468, which results in an aspartic acid-to-valine change in the protein (Asp816Val). Much less frequently, other mutations at this same location are found and occasional cases with mutations at other locations have also been reported. Mutations at the 816 codon are believed to alter the protein such that it is in a constitutively activated state. The main downstream effect of KIT activation is cell proliferation.

Detection of a mutation at the 816 codon is included as one of the minor diagnostic criteria for systemic mastocytosis in the World Health Organization (WHO) classification system for hematopoietic neoplasms and is also of therapeutic relevance, as it confers resistance to imatinib, a drug commonly used to treat CMPDs. It is now clear that individual mast cell neoplasms are variable with respect to the number of cell lineages containing the mutation; some having positivity only in mast cells and others having positivity in additional myeloid and even lymphoid lineages. The mutation has not been reported in normal tissues.

Reference Values

An interpretive report will be provided indicating the mutation status as positive or negative.

Interpretation
The test will be interpreted as positive or negative for KIT Asp816Val.

**Cautions**

Some systemic mastocytosis cases may have the mutation only in mast cells. Since these cells rarely circulate in blood and are difficult to obtain in significant numbers from bone marrow aspirate specimens, false-negative results may occur if neoplastic cells are present below the sensitivity of the assay (fewer than 0.01% mutated alleles).

The test is qualitative only. Reliable quantitative results cannot be issued.

**Supportive Data**

The analytic sensitivity of this test is 0.01% and was determined by the dilution of a cell line containing homozygous KIT mutation. This means that 0.01% or greater of the KIT alleles present in the specimen must contain the mutation to be detected by the assay. The analytic specificity was 100% in assay validation.

**Clinical Reference**


**Performance**

**Method Description**

The KIT mutation assay developed for clinical use in the Mayo Molecular Hematopathology Laboratory detects the KIT mutation responsible for Asp816Val. The technique used is allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) with fragment analysis on an ABI3100 genetic analyzer. Briefly, DNA is extracted from whole bone marrow or blood and PCR is used to amplify across the mutation site in 2 separate tubes; 1 contains a reverse primer complementary to the unmutated sequence and the other contains a reverse primer complementary to the mutated sequence. Each of these reverse primers is labeled with a fluorescent tag and both tubes contain an identical, nonlabeled forward primer. Both primer sets amplify a 200-bp fragment that differs only at the mutation site. The unmutated fragment should be amplified in all samples. Samples negative for KIT Asp816Val will not have an amplified fragment in the mutated reaction tube. Positive samples will have amplified fragments in both the unmutated and mutated tubes. The test gives a qualitative (positive or negative) result only, as the end point PCR used is not reliable for quantification.(Unpublished Mayo method)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Friday

**Analytic Time**

4 days

**Maximum Laboratory Time**

7 days
Specimen Retention Time
DNA 3 months

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
81273

LOINC® Information

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