Overview

Useful For
Diagnosing systemic mastocytosis in bone marrow specimens

Special Instructions
- [Hematopathology Patient Information](#)

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

NY State Available
Yes

Specimen

Specimen Type
Bone Marrow

Shipping Instructions
Specimen must arrive within 7 days (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

**Container/Tube:** Bone marrow

**Preferred:** EDTA (lavender top)

**Acceptable:** ACD-B (yellow top)

**Specimen Volume:** 2 mL

Collection Instructions:

1. Invert several times to mix bone marrow.
2. Send specimen in original tube.
3. Label specimen as bone marrow.
**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**

1 mL

**Reject Due To**

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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<tbody>
<tr>
<td>Other</td>
<td>Moderately to severely clotted</td>
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**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tbody>
<tr>
<td>Bone Marrow</td>
<td>Ambient (preferred)</td>
<td>7 days</td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>7 days</td>
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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 1 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 1 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-KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)

LOINC® Information

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<td>KIT Asp816Val Mutation Analysis, BM</td>
<td>In Process</td>
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<table>
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