Test Definition: KITQ

KIT p.Asp816Val Variant Analysis, Quantitative, Varies

Overview

Useful For
Diagnosing systemic mastocytosis

Genetics Test Information
This test uses droplet digital polymerase chain reaction to detect the *KIT* NM_000222.3:c.2447A>T(p.Asp816Val) variant.

Testing Algorithm
For more information see:
-Mast Cell Disorder: Diagnostic Algorithm, Bone Marrow
-Eosinophilia: Bone Marrow Diagnostic Algorithm

Special Instructions
- Hematopathology Patient Information
- Mast Cell Disorder: Diagnostic Algorithm, Bone Marrow
- Eosinophilia: Bone Marrow Diagnostic Algorithm

Method Name
Droplet Digital Polymerase Chain Reaction (ddPCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Whole blood or bone marrow specimens must arrive within 14 days of collection.

Necessary Information
Specimen type is required to perform testing.

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:

Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix.
2. Send specimen in original tube. **Do not aliquot.**
3. Label specimen as blood.
Specimen Stability Information: Ambient (preferred) 14 days/Refrigerate 14 days

Specimen Type: Bone marrow aspirate
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix.
2. Send specimen in original tube. **Do not aliquot.**
3. Label specimen as bone marrow.
Specimen Stability Information: Ambient (preferred) 14 days/Refrigerate 14 days

Specimen Type: Extracted DNA
Container/Tube: 1.5- to 2-mL tube
Specimen Volume: Entire specimen
Collection Instructions:
1. Label specimen as extracted DNA
2. Provide indication of volume of DNA.
Specimen Stability Information: Frozen (preferred)/ Refrigerate/Ambient
Additional Information: We cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed, and a charge will be applied.

Specimen Type: Paraffin-embedded bone marrow aspirate clot
Container/Tube: Paraffin block
Specimen Stability Information: Ambient

Specimen Type: Tissue (FFPE)
Container/Tube: Paraffin block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block.
Specimen Stability Information: Ambient
Additional Information: Decalcified core biopsies are not accepted.

Forms

1. Hematopathology Patient Information (T676)
2. If not ordering electronically, complete, print, and send an Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Blood, bone marrow: 1 mL; Extracted DNA: 50 mcL at 10 ng/mcL concentration; Paraffin block: 1 block

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject
Moderately to severely clotted Bone marrow core biopsies (decalcified embedded) Slides Paraffin shavings	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Systemic mastocytosis (SM) is a hematopoietic neoplasm that is now recognized as a distinct entity in the current World Health Organization and International Consensus Classifications. SM is characterized by a proliferation of neoplastic mast cells in the bone marrow and rarely in extramedullary sites. SM may present with variable degrees of clinical severity and can sometimes be associated with a non-mast cell hematologic neoplasm. SM is diagnosed using a combination of major and minor criteria, encompassing morphologic, biochemical and molecular genetic features. An important minor criterion is the presence of an activating somatic mutation in the *KIT* gene, encoding the tyrosine kinase receptor for stem cell factor, which is a critical growth factor in early myeloid cell proliferation and development. In SM, the most common *KIT* alteration is a missense change in exon 17 at codon 816, p.Asp816Val (D816V). Much less frequently, other missense mutations involving the D816 codon or adjacent amino acids are encountered and rarely, *KIT* genetic alterations can occur in other exons. A subset of acute myeloid leukemias (AML) with core-binding factor gene fusions can also acquire activating *KIT* gene mutations, including D816V in many cases. Detection of *KIT* D816V is critical to help establish a diagnosis of SM and is optimally determined by molecular testing. Because mast cell lesions are typically sparse and fibrotic in bone marrow and circulating tumor mast cells are in low abundance, highly sensitive and specific assays are required for optimal detection of *KIT* D816V. This can be achieved using quantitative allele-specific polymerase chain reaction (PCR) or droplet digital PCR (ddPCR) methods. The presence of *KIT* D816V mutation in the

appropriate clinical and pathologic context is highly supportive of SM. In addition, although the D816V in SM is insensitive to targeted therapy with imatinib, other tyrosine kinase inhibitors such as avapritinib have demonstrated significant therapeutic efficacy in advanced SM, indicating that this mutation may also be a theranostic marker for these patients.

Reference Values

An interpretive report will be provided indicating the status as positive or negative for *KIT* p.Asp816Val (D816V).
KIT gene (NCBI accession NM_000222.3)

Interpretation

The test will be interpreted as positive or negative for *KIT* p.Asp816Val and a quantitative result will be included if positive.

Cautions

Some cases of systemic mastocytosis (SM) may have sparse representation in bone marrow aspirate or blood samples containing too few neoplastic mast cells to successfully detect the *KIT* p.D816V (below limit of assay detection). Paraffin embedded tissues may be prone to sampling effects and DNA degradation, which may also compromise assay performance. Rare cases of SM may have other *KIT* mutations involving D816 (such as p.D816Y), or in other exon 17 codons that are not specifically targeted by this D816V ddPCR assay.

Assay sensitivity may be impacted by variability in tumor cell distribution or limited overall DNA quantity or quality.

Clinical Reference

1. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719
2. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228
3. Reiter A, George TI, Gotlib J. New developments in diagnosis, prognostication, and treatment of advanced systemic mastocytosis. Blood. 2020;135(16):1365-1376
4. Arock M, Sotlar K, Broesby-Olsen S, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. Leukemia. 2015;29:1223-1232
5. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood. 2017;129(11):1420-1427
6. Munoz-Gonzalez JI, Alvarez-Twose I, Jara-Acevedo M, et al. Frequency and prognostic impact of KIT and other genetic variants in indolent systemic mastocytosis. Blood. 2019;134(5):456-468
7. Gotlib J, Reiter A, DeAngelo DJ. Avapritinib for advanced systemic mastocytosis. Blood. 2022;140(15):1667-1673

Performance

Method Description

This test is performed using droplet digital polymerase chain reaction (ddPCR) to detect the *KIT*:c.2447A>T, p.Asp816Val (NM_000222.3:g.55599321A>T) variant. DNA extracted from patient samples is PCR-amplified using oligonucleotide primers and mutant- and wild type-specific fluorescently labeled probes. Results are analyzed using dedicated software

and Poisson statistics to provide absolute quantification of mutant target and wild type copies. Calculated results are reported as *KIT* p.D816V mutant fractional abundance (variant allele fraction %). The analytical sensitivity of this assay is 0.1%; however, sensitivity may be impacted by variability in tumor cell distribution or limited overall DNA quantity or quality.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
4 to 7 days

Specimen Retention Time
Whole blood/Bone marrow: 2 weeks; Extracted DNA: 3 Months; Paraffin blocks: Unused portions of blocks will be returned 1 week after testing is complete

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81273

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
KITQ	KIT D816V Variant Analysis Quant, V	55201-8

Result ID	Test Result Name	Result LOINC® Value
MP089	Specimen Type	31208-2
622375	Interpretation	69047-9

622376	Signing Pathologist	19139-5