Test Definition: CXLPL

CXCR4 Mutation Analysis, Somatic, Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia, Varies

Overview

Useful For
Aiding in the prognosis and clinical management of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia

Genetics Test Information
This test detects gene mutations within the C-terminal end of the *CXCR4* gene that are commonly found in association with *MYD88* L265P mutations in cases of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia.

Special Instructions
- Hematopathology Patient Information

Highlights
This test offers highly sensitive detection of the well-characterized hotspot mutations c.1013C>G/A, p.S338X and routine Sanger sequencing for other mutations in the C-terminus region. It is strongly recommended that this test be used in the context of the MYD88 / MYD88, L265P, Somatic Gene Mutation, DNA Allele-Specific PCR, Varies. If MYD88 has not been previously performed, consider LPLFX . Reflex Testing of MYD88 and CXCR4 assay during evaluation of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia.

Method Name
Bridged Nucleic Acids (BNA) Clamp Sanger Sequencing Technology/Routine Sanger Sequencing
(BNAClamp is utilized pursuant to a license agreement with BNA Inc)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Whole blood or bone marrow specimens must arrive within 10 days of collection.

Necessary Information
The following information is required:
1. Pertinent clinical history
2. Clinical or morphologic suspicion
3. Date and time of collection

4. Specimen source

Specimen Required

Submit only 1 of the following specimens:

Preferred
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 blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
3. Label specimen as blood.
Specimen Stability Information: Ambient (preferred)/Refrigerated

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

Acceptable
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. Provide volume and concentration of the DNA
Specimen Stability Information: Frozen (preferred)/Refrigerated/Ambient

Specimen Type: Paraffin-embedded tissue
Container/Tube: Paraffin block
Specimen Stability Information: Ambient

Specimen Type: Tissue
Slides: Unstained slides

Specimen Volume: 10 to20 slides
Additional Information: Tissue must demonstrate involvement by a hematologic neoplasm (eg, acute myelocytic leukemia), not solid tumors.
Specimen Stability Information: Ambient

Forms

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

Specimen Minimum Volume

Whole blood, Bone marrow: 1 mL
Extracted DNA: at least 50 mcL with a concentration of at least 20 nanograms per mcL
Other specimen types: See Specimen Required

Reject Due To

Gross hemolysis	Reject
B5-fixed tissues Decalcified bone marrow core biopsies Frozen tissue Methanol acetic acid (MAA)-fixed pellets Moderately to severely clotted Paraffin shavings	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies	10 days	

Clinical & Interpretive

Clinical Information

Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM) is a B-cell lymphoma characterized by an aberrant accumulation of malignant lymphoplasmacytic cells in the bone marrow, lymph nodes, and spleen. It is a B-cell neoplasm that can exhibit excess production of serum IgM symptoms related to hyperviscosity, tissue filtration, and autoimmune-related pathology. *CXCR4* mutations are identified in approximately 30% to 40% of patients with LPL/WM and are almost always associated with *MYD88* L265P, which is highly prevalent in this neoplasm. The status of *CXCR4* mutations in the context of *MYD88* L265P is clinically relevant as important determinants of clinical presentation, overall survival, and therapeutic response to ibrutinib. A *MYD88*-L265P/*CXCR4*-WHIM (C-terminus nonsense/frameshift mutations) molecular signature is associated with intermediate to high bone marrow disease burden and serum IgM levels, less adenopathy, and intermediate response to ibrutinib in previously treated patients. A *MYD88*-L265P/*CXCR4*-WT (wildtype) molecular signature is associated with intermediate bone marrow disease burden and serum IgM levels, more adenopathy, and highest response to ibrutinib in previously treated patients. A *MYD88*-WT/*CXCR4*-WT molecular signature is associated with inferior overall survival, lower response to ibrutinib therapy in previously treated patients, and lower bone marrow disease burden in comparison to those harboring a *MYD88*-L265 mutation.

Reference Values

Mutations present or absent in the test region c. 898-1059 (amino acids 300-353) of the *CXCR4* gene (NCBI NM_003467.2, GRCh37)

Interpretation

Mutation present or not detected; an interpretive report will be issued.

Cautions

This test is a targeted assay for the C-terminal end of the *CXCR4* gene only. It examines c.898-1059 of the *CXCR4* gene (NCBI NM_003467.2 GRCh37) and does not detect mutations outside this region. A 1% analytical sensitivity was established at 50 ng DNA input for the hotspot mutations c.1013C>G/A only, which uses bridged nucleic acids-clamped Sanger sequencing, and DNA not meeting established criteria can lead to false-negative results. In the extremely rare event that a rare polymorphism, insertion, or deletion occurs at the Sanger sequencing primer binding sites, in cis with c.1013C>G/A, data can yield a failed result. Routine Sanger sequencing is used to interrogate other mutations in the tested region with a 15% to 20% analytical sensitivity. The analytical sensitivity of the assay can be affected by a variety of factors, including biologic availability (ie, tumor burden), fixation of paraffin-embedded specimens, rare polymorphisms, insertions, or deletions at the primer binding sites, or nonspecific polymerase chain reaction interferences.

Clinical Reference

1. Hunter Z, Xu L, Yang G, et al: The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood. 2014 Mar 13;123(11):1637-1646. doi: 10.1182/blood-2013-09-525808

2. Landgren O, Tageja N: MYD88 and beyond: novel opportunities for diagnosis, prognosis and treatment in Waldenstrom's Macroglobulinemia. Leukemia. 2014 Sep;28(9):1799-1803. doi: 10.1038/leu.2014.88

3. Poulain S, Roumier C, Venet-Caillault A, et al: Genomic Landscape of *CXCR4* Mutations in Waldenstrom Macroglobulinemia. Clin Cancer Res. 2016 Mar 15;22(6):1480-1488. doi: 10.1158/1078-0432.CCR-15-0646

4. Roccaro A, Sacco A, Jimenez C, et al: C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of

drug resistance in lymphoplasmacytic lymphoma. Blood. 2014 Jun 26;123(26):4120-4131. doi: 10.1182/blood-2014-03-564583
5. Schmidt J, Federmann B, Schindler N, et al: MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. Br J Haematol. 2015 Jun;169(6):795-803. doi: 10.1111/bjh.13361
6. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR: Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood. 2014 May 1;123(18):2791-2796. doi: 10.1182/blood-2014-01-550905
7. Treon SP, Tripsas CK, Meid K, et al: Ibrutinib in previously treated Waldenstrom's macroglobulinemia. N Engl J Med. 2015 Apr 9;372(15):1430-1440. doi: 10.1056/NEJMoa1501548
8. Xu L, Hunter ZR, Tsakmaklis N, et al: Clonal architecture of CXCR4 WHIM-like mutations in Waldenstrom Macroglobulinaemia. Br J Haematol. 2016 Mar;172(5):735-744. doi: 10.1111/bjh.13897

Performance

Method Description

The C-terminal end of *CXCR4 (*NM_003467.2, c.898-1059) is amplified from extracted genomic DNA by polymerase chain reaction, followed by Sanger sequencing and capillary electrophoresis analysis. Review of the sequence data is performed using a combination of automated calls and manual inspection.(Unpublished Mayo method)

The hotspot mutations c.1013C>G/A (p.S338X) are examined using bridged nucleic acids clamped Sanger sequencing with an analytic sensitivity of 1%. All other genetic mutations in the test region are examined by routine Sanger sequencing with an analytic sensitivity of 15% to 20%.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

7 to 10 days

Specimen Retention Time

Blood/Bone marrow: 2 weeks; Extracted DNA: 3 months

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

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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

81479-Unlisted molecular pathology procedure

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CXLPL	CXCR4 Mutation in B-cell Lymphoma	In Process

Result ID	Test Result Name	Result LOINC® Value
MP032	Specimen Type	31208-2
113436	CXLPL Result	59465-5
38287	Final Diagnosis	50398-7