

## 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 to 20 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

**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