

## Overview

### Useful For

Establishing a diagnosis of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM)

Helping distinguish LPL/WM low-grade B-cell lymphoma from other subtypes

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

### Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CXCFX	CXCR4, Gene Mutation, Reflex	Yes, (order CXLPL), Bill Only	No

### Testing Algorithm

The algorithm starts with the sensitive *MYD88* L265P testing by allele-specific polymerase chain reaction. If a *MYD88* L265P variant is detected, *CXCR4* testing will be performed at an additional charge. If a *MYD88* L265P variant is not detected, the algorithm ends, and no further testing is necessary.

### Special Instructions

- [Hematopathology Patient Information](#)

### Highlights

This test offers highly sensitive detection of the well-characterized hotspot variants c.1013C->G/A, p.S338X and routine Sanger sequencing for other variants in the C-terminus region.

### Method Name

Allele-Specific Polymerase Chain Reaction (AS-PCR)/Bridged Nucleic Acids (BNA) Clamp Sanger Sequencing/Routine Sanger Sequencing (BNAclamp is utilized pursuant to a license agreement with BNA Inc.)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

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**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:** Bone marrow aspirate

**Container/Tube:**

**Preferred:** Lavender top (EDTA)

**Acceptable:** Yellow top (ACD), green top (sodium heparin)

**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) 10 days/Refrigerated 10 days

**Specimen Type:** Whole blood

**Container/Tube:**

**Preferred:** Lavender top (EDTA)

**Acceptable:** Yellow top (ACD), green top (sodium heparin)

**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) 10 days /Refrigerated 10 days

**Specimen Type:** Paraffin-embedded tissue

**Container/ Tube:** Paraffin block

**Collection Instructions:**

1. Decalcified specimens (eg, bone marrow core biopsies) are not acceptable.
2. Indicate specimen source.

**Specimen Stability Information:** Ambient

**Additional Information:** If the quality of the biopsy specimen is poor, testing should not be ordered. Testing may be

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canceled if DNA requirements are inadequate.

**Acceptable:****Specimen Type:** Tissue slide**Slides:** 20 unstained slides**Container/ Tube:** Transport in plastic slide holders.**Collection Instructions:**

1. Send 20 unstained, nonbaked slides with 5-micron thick sections of tissue.
2. Decalcified specimens (eg, bone marrow core biopsies) are not acceptable.
3. Indicate specimen source.

**Specimen Stability Information:** Ambient**Additional Information:** Testing may be canceled if resultant extracted DNA does not meet concentration requirements.**Specimen Type:** Frozen tissue**Container/Tube:** Plastic container**Specimen Volume:** 100 mg**Collection Instructions:**

1. Freeze tissue within 1 hour of collection
2. Indicate specimen source.

**Specimen Stability Information:** Frozen**Specimen Type:** Extracted DNA**Container/Tube:** 1.5- to 2-mL tube**Specimen Volume:** Entire specimen**Collection Instructions:**

1. DNA must be extracted within 7 days of collection.
2. Label specimen as extracted DNA and source of specimen.
3. Provide volume and concentration of DNA on label.

**Specimen Stability Information:** Frozen (preferred)/Refrigerated/Ambient**Additional Information:** DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). 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.**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 aspirate: 0.5 mL; Frozen tissue: 50 mg; Extracted DNA: 50 mL at 20 ng/mL; Tissue slides:  
10 unstained slides

## Reject Due To

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

## Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies	10 days	

## Clinical & Interpretive

### Clinical Information

The *MYD88* L265P abnormality is highly associated (>90%) with the pathologic diagnosis of lymphoplasmacytic lymphoma and the clinical syndrome of Waldenstrom macroglobulinemia (LPL/WM), particularly in the setting of an elevated IgM serum monoclonal paraprotein.

*CXCR4* mutations are identified in approximately 30% to 40% of LPL/WM patients and are almost always in association 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 variants) 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 intermediated 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

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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 variant.

**Reference Values**

*MYD88* L265P: Mutation present or absent based on expected variant polymerase chain reaction product size for the *MYD88* gene (NCBI accession NM\_002468.4).

*CXCR4*: Mutation 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 *MYD88* test is a targeted assay and will not detect any alteration at the *MYD88* codon 265 that does not result in the L>P (leucine to proline) amino acid change. It will also not detect additional *MYD88* variants, including insertion or deletion events. The analytical sensitivity of the assay (1% *MYD88* L265P in a wildtype background) can be affected by a variety of factors, including biologic availability (ie, tumor burden), fixation of paraffin-embedded specimens, or nonspecific polymerase chain reaction (PCR) interferences. Rare cases of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM) have been reported to lack the *MYD88* L265P abnormality, so a negative result would not completely exclude this diagnosis but would make the possibility of LPL/WM more unlikely.

The reflexed 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 variants 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 (BNA) clamped Sanger sequencing, and DNA that does not meet the established criteria can lead to false-negative results. In the extremely rare event that a rare benign variant (ie, polymorphism), insertion, or deletion occurs at the Sanger sequencing primer binding sites, in cis with a 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 benign variants, insertions, or deletions at the primer binding sites or nonspecific PCR interferences.

**Clinical Reference**

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2. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121(13):2522-2528. doi:10.1182/blood-2012-09-457101
3. Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. *Blood*. 2013;121(11):2051-2058. doi:10.1182/blood-2012-09-454355
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9. Poulain S, Roumier C, Venet-Caillault A, et al. Genomic landscape of *CXCR4* mutations in Waldenstrom macroglobulinemia. *Clin Cancer Res*. 2016;22(6):1480-1488. doi:10.1158/1078-0432.CCR-15-0646
10. 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;123(26):4120-4131. doi:10.1182/blood-2014-03-564583
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12. Treon SP, Cao Y, Xu L, et al. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. *Blood*. 2014;123(18):2791-2796. doi:10.1182/blood-2014-01-550905
13. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med*. 2015;372(15):1430-1440. doi:10.1056/NEJMoa1501548
14. Gertz MA. Waldenstrom macroglobulinemia: 2025 Update on diagnosis, risk stratification, and management. *Am J Hematol*. 2025;100(6):1061-1073. doi:10.1002/ajh.27666

## Performance

### Method Description

Extracted DNA from the clinical specimen is subjected to allele-specific polymerase chain reaction (PCR) using *MYD88* exon 5 primers that simultaneously amplify both a wild-type sequence fragment and a fragment containing the specific nucleotide change resulting in L265P if present. PCR products are visualized by capillary electrophoresis and the presence of mutated and wildtype amplicons is determined according to the expected specific PCR product sizes.(Unpublished Mayo method)

The C-terminal end of *CXCR4* (NM\_003467.2, c. 898-1059) is amplified from extracted genomic DNA by PCR, 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

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No

**Day(s) Performed**

Monday through Friday

**Report Available**

7 to 10 days

**Specimen Retention Time**

Bone marrow aspirate/Whole blood/Fresh/Frozen Tissue: 2 weeks; Extracted DNA: 3 months; FFPE tissue: Unused portions of blocks will be returned to the client. Unstained slides: Not retained

**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**

81305

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
LPLFX	Reflex Testing of MYD88 and CXCR4	82140-5

Result ID	Test Result Name	Result LOINC® Value
MP042	Specimen Type	31208-2
601511	LPLFX Reflex Result	82140-5
601510	Final Diagnosis	50398-7