MAYO CLINIC LABORATORIES

Test Definition: LPLFX

Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia, MYD88 L265P with Reflex to CXCR4, Varies

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

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

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 mcL at 20 ng/mcL; 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
Broken or cracked unstained slides	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 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.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Clinical Reference

1. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med. 2012;367(9):826-833. doi:10.1056/NEJMoa1200710
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
4. Poulain S, Roumier C, Decambron A, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood. 2013;121(22):4504-4511. doi:10.1182/blood-2012-06-436329
5. Gachard N, Parrens M, Soubeyran I, et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia. 2013;27(1):183-189. doi:10.1038/leu.2012.257
6. Ondrejka SL, Lin JJ, Warden DW, et al. MYD88 L265P somatic mutation: its usefulness in the differential diagnosis of bone marrow involvement by B-cell lymphoproliferative disorders. Am J Clin Pathol. 2013;140(3):387-394. doi: 10.1309/AJCP10ZCLFZGYZIP
7. 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;123(11):1637-1646. doi:10.1182/blood-2013-09-525808
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
11. 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;169(6):795-803. doi:10.1111/bjh.13361
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
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