MAYO CLINIC LABORATORIES

Test Definition: LPLFX

Reflex Testing of MYD88 and CXCR4

Overview

Useful For

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

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

Aiding in the prognostication and clinical management of LPL/WM

Testing Algorithm

The algorithm starts with the sensitive *MYD88* L265P testing by allele-specific polymerase chain reaction. If a *MYD88* L265P variant is detected, additional *CXCR4* testing will be performed. 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.

Reflex Tests

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

Method Name

Allele-Specific Polymerase Chain Reaction (AS-PCR), Bridged Nucleic Acids (BNA) Clamp Sanger Sequencing and Routine Sanger Sequencing

(BNAClamp is utilized pursuant to a license agreement with BNA Inc.)

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Peripheral 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

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD solution B)

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

Specimen Type: Paraffin-embedded tissue

Container/Tube: Paraffin block

Specimen Stability: Ambient

Specimen Type: Paraffin-embedded bone marrow aspirate clot

Container/Tube: Paraffin block

Specimen Stability: Ambient

Specimen Type: Tissue

Slides: Unstained slides

Specimen Volume: 10 slides

Additional Information: Tissue must demonstrate involvement by a hematologic neoplasm (eg, acute myelocytic leukemia), not solid tumors.

Specimen Stability Information: Ambient

Acceptable:

Specimen Type: Peripheral blood

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD solution B)

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: Extracted DNA

Container/Tube: 1.5- to 2-mL tube with indication of volume and concentration of the DNA

Specimen Volume: Entire specimen

Collection Instructions: Label specimen as extracted DNA and list the specimen source. Include indication of volume and concentration of the DNA.

Specimen Stability Information: Frozen (preferred)/Refrigerated/Ambient

Forms

1. Hematopathology Patient Information (T676) in Special Instructions

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

Reject Due To

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

Specimen Minimum Volume

Blood, Bone marrow: 1 mL

Extracted DNA: 20 mcL with a concentration of at least 10 nanograms per mcL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)	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 variants 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* variants 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 (wild type) 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: Variant present or absent based on expected variant PCR product size for the *MYD88* gene (NCBI accession NM_002468.4).

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

Interpretation

Reportable variant present or not detected; an interpretive report will be provided.

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 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 wild type 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 lacking 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 variants 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 may occur 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 variants 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

1. Treon SP, Xu L, Yang G, et al: MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med. 2012 Aug 30;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 Mar 28;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 Mar 14;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 May 30;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 Jan;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 Sept;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 Mar 13;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 Mar 15;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 Jun 26;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 Jun;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 May 1;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 Apr 9;372(15):1430-1440. doi: 10.1056/NEJMoa1501548

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

PDF Report

No

Specimen Retention Time

DNA: 3 months; Peripheral blood, bone marrow: 2 weeks

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test 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	Reporting Name	LOINC®
MP042	Specimen Type	31208-2
601511	LPLFX Reflex Result	82140-5
601510	Final Diagnosis	50398-7