MAYO CLINIC LABORATORIES

# 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 assay during evaluation of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia.

### Method Name

BNAClamp Sanger Sequencing Technology 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

**Specimen 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:**

**Specimen Type:** 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

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

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**Specimen Stability Information:** 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.

## Specimen Minimum Volume

Blood, Bone marrow: 1 mL
Extracted DNA: at least 20 mcL with a concentration of at least 10 nanograms per mcL

## Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| B5-fixed tissues<br>Decalcified bone marrow core biopsies<br>Frozen tissue<br>Methanol acetic acid (MAA)-fixed pellets<br>Moderately to severely clotted<br>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* va mutations riants 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 (wild type) 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 variant.

### Reference Values

Variants present or absent in the test region of the *CXCR4* gene (NCBI NM_003467.2, GRCh37).

### Interpretation

Mutations detected 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 may occur 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

DNA: 3 months; Blood, bone marrow: 2 weeks

### Performing Laboratory Location

Rochester

## 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 Regional Manager. 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. This test 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 |