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 of *CXCR4*.

### 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

### Ordering Guidance

It is strongly recommended that this test be used in the context of results of MYD88 / *MYD88*, L265P, Somatic Gene Mutation, DNA Allele-Specific PCR, Varies. If MYD88 has not been previously performed, consider LPLFX / Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia, *MYD88* L265P with Reflex to *CXCR4*, Varies during evaluation of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia.

### 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:**
1. Testing may be canceled if resultant extracted DNA does not meet concentration requirements.
2. Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

**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
**Additional Information:** Testing may be canceled if resultant extracted DNA does not meet concentration requirements.

**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 |
| Broken or cracked stained slides that cannot be repaired | 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.

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.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

## 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;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;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;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;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;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;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;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;172(5):735-744. doi:10.1111/bjh.13897
9. 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

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

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

# Test Definition: CXLPL

CXCR4 Mutation Analysis, Somatic, Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia, Varies

## 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

### 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 |