MAYO CLINIC LABORATORIES

Test Definition: NGCLN

MayoComplete Chronic Lymphoid Neoplasms, Next-Generation Sequencing, Varies

Overview

Useful For

Aiding in establishing diagnosis, refining prognosis, and potentially identifying targeted therapies for the optimal management of patients with chronic or low-grade B-cell lymphoid neoplasms

Genetics Test Information

This test includes next-generation sequencing to evaluate the following 25 genes and select intronic regions: *ATM, BCL2, BIRC3, BRAF, BTG1, BTK, CCND1, CDKN2A, CXCR4, DDX3X, EZH2, FBXW7, KLF2, KRAS, MAP2K1, MYD88, NOTCH1, NOTCH2, NRAS, PIK3CA, PLCG2, SF3B1, TNFAIP3, TP53,* and *XPO1*. For a list of genes and exons targeted by this test, see Targeted Genes Interrogated by MayoComplete Chronic Lymphoid Neoplasms Next-Generation Sequencing.

Special Instructions

- Hematopathology Patient Information
- Targeted Genes Interrogated by MayoComplete Chronic Lymphoid Neoplasms Next-Generation Sequencing

Highlights

This test utilizes next-generation sequencing for the detection of somatic mutations with diagnostic, prognostic, or therapeutic value in a set of genes associated with chronic or clinically "low-grade" lymphoid neoplasms.

Method Name

Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Whole blood, bone marrow aspirate, and body fluid specimens must arrive within 14 days of collection.

Specimen Required

Submit only 1 of the following specimens:

Preferred:
Specimen Type: Bone marrow aspirate
Container/Tube:

Preferred: Lavender or pink top EDTA) or yellow top (ACD)
Acceptable: 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) 14 days/Refrigerate 14 days
Additional Information: To ensure minimum volume and concentration of DNA is met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender or pink top (EDTA) or yellow top (ACD)
Acceptable: 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) 14 days/Refrigerate 14 days
Additional Information: To ensure minimum volume and concentration of DNA is met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Paraffin-embedded tissue
Container/Tube: Paraffin block
Collection Instructions:
1. If available, send 1 representative hematoxylin and eosin-stained slide.
2. Minimum amount of tumor nuclei is 20%
3. Required amount of tissue area is at least 25 mm(2)
4. Tissue should be fixed in 10% neutral-buffered formalin. Other fixatives are not acceptable.
5. Decalcified specimens (eg, bone marrow core biopsies) are not acceptable.
Specimen Stability Information: Ambient
Additional Information: If the quality of the biopsy specimen is poor or the target tumor cell population is below 20%, 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. If available, also send 1 representative hematoxylin and eosin-stained slide.
3. Minimum amount of tumor nuclei is 20%

4. Required amount of tissue area is at least 25 mm(2)
5. Tissue should be fixed in 10% neutral-buffered formalin. Other fixatives are not acceptable.
6. Decalcified specimens (eg, bone marrow core biopsies) are not acceptable.

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: Freeze tissue within 1 hour of collection
Specimen Stability Information: Frozen
Additional Information: Testing may be canceled if resultant extracted DNA does not meet concentration requirements.

Specimen Type: Body fluid
Container/Tube: Sterile container
Specimen Volume: 5 mL
Collection Instructions: Specify the type of fluid being submitted.
Specimen Stability Information: Refrigerated 14 days/Frozen 14 days
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 14 days after collection.
2. Label specimen as extracted DNA and source of specimen.
3. Indicate 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

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, body fluid: 0.5 mL; Frozen tissue: 50 mg; Extracted DNA: 100 microliters (mcL) at 20 ng/mcL; Tissue slides: 10 unstained slides

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Specimens that have been decalcified (all methods)	Reject
Bone marrow core biopsies	Reject
Paraffin shavings	Reject
Fixatives other than 10% neutral-buffered formalin for paraffin-embedded tissue	Reject
Moderately to severely clotted bone marrow aspirate	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies	14 days	

Clinical & Interpretive

Clinical Information

This test is intended to evaluate a targeted set of genes involved in a heterogeneous group of chronic lymphoid neoplasms that includes chronic lymphocytic leukemia (CLL) and various low-grade B-cell lymphomas. The test includes actionable targets to aid in the differential diagnosis of low-grade B-cell lymphomas (eg, hairy cell leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma), predict prognosis (eg, risk stratification in CLL), and evaluate therapeutic options or efficacy (eg, ibrutinib therapy in CLL, EZH2 [enhancer of zeste homolog 2] inhibitors in follicular lymphoma). Genomic analysis by next-generation sequencing is complementary to the standard evaluation in the classification and management of patients with chronic lymphoid neoplasms.

Reference Values

An interpretive report will be provided.

Interpretation

Genomic variants detected by this test will be documented in a detailed laboratory-issued report. This report will contain information regarding the detected alterations and their associations with prognosis or possible therapeutic implications in chronic lymphoid neoplasms. The information in the clinical report may be used by the patient's healthcare professional to help guide decisions concerning management. Final interpretation of next-generation sequencing results requires correlation with all relevant clinical, pathologic, and laboratory findings and is the responsibility of the managing healthcare professional.

Cautions

This test is a targeted next-generation sequencing (NGS) panel assay that encompasses 25 genes with variable full exon, partial region (including select intronic or noncoding regions), or hot spot coverage (depending on specific genetic locus). Therefore, this test will not detect other genetic abnormalities in genes or regions outside the specified target areas. The test detects single-base substitutions (ie, point mutations), as well as small insertion or deletion type events. This test is not configured to detect structural genomic rearrangements (ie, translocations), gene fusions, copy number alterations, or large-scale (segmental chromosome region) deletions and other complex genomic changes.

This assay does not distinguish between somatic mutations and germline alterations in analyzed gene regions, particularly with variant allele frequencies near approximately 50% or 100%. If nucleotide alterations in genes associated with germline variant syndromes are present and there is a strong clinical suspicion or family history of malignant disease predisposition, additional genetic testing and appropriate counseling may be indicated. Some apparent mutations classified as variants of undetermined significance may represent rare or low population frequency polymorphisms.

Prior treatment for hematologic malignancy could affect the results obtained in this assay. Particularly, a prior allogeneic hematopoietic stem cell transplant may cause difficulties in either resolving somatic or polymorphic alterations or assigning variant calls correctly to donor and recipient fractions if pertinent clinical or laboratory information (eg, chimerism engraftment status) is not provided.

Inadequate samples (eg, insufficient DNA quantity or quality) will preclude further testing and will be noted in the interpretive report. For formalin-fixed, paraffin-embedded specimens, NGS testing should not be pursued if the quality of the biopsy specimen is poor (eg, limited sample size, presence of extensive necrosis or fibrosis) or the target tumor cell population is low (<20%).

Clinical Reference

1. Swerdlow S, Campo E, Harris NL, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. IARC Press; 2017. WHO Classification of Tumours, Vol 2
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3. Jajosky AA, Havens NP, Sadri N, et al. Clinical utility of targeted next-generation sequencing in the evaluation of low-grade lymphoproliferative disorders. Am J Clin Pathol. 2021;156(3):433-444
4. Davis AR, Stone SL, Oran AR, et al. Targeted massively parallel sequencing of mature lymphoid neoplasms: assessment of empirical application and diagnostic utility in routine clinical practice. Mod Pathol. 2021;34(5):904-921
5. Stewart JP, Gazdova J, Darzentas N, et al. Validation of the EuroClonality-NGS DNA capture panel as an integrated genomic tool for lymphoproliferative disorders. Blood Adv. 2021;5(16):3188-3198
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. Morin RD, Arthur SE, Assouline S. Treating lymphoma is now a bit EZ-er. Blood Adv. 2021;5(8):2256-2263
8. Thangavadivel S, Byrd JC. Gly101Val BCL2 Mutation: One step closer to understanding Venetoclax resistance in CLL. Cancer Discov. 2019;9(3):320-322. doi:10.1158/2159-8290.CD-19-0029
9. Lee J, Wang YL. Prognostic and predictive molecular biomarkers in chronic lymphocytic leukemia. J Mol Diagn. 2020;22(9):1114-1125
10. Liebers N, Roider T, Bohn JP, et al. BRAF inhibitor treatment in classic hairy cell leukemia: a long-term follow-up study of patients treated outside clinical trials. Leukemia. 2020;34(5):1454-1457

Performance

Method Description
This is a target-enriched next-generation sequencing (NGS) panel. DNA is extracted from validated specimen sources including, but not limited to, peripheral blood, bone marrow aspirate, and formalin-fixed paraffin embedded tissues. Library preparation for NGS is performed followed by probe hybridization and capture. Sequencing of the final sample library is performed on a NGS instrument. Following bioinformatic processing of the sequencing data, the sequencing results are interpreted to provide a final clinical report. Genomic alterations are called according to human genome reference build GRCh37 (hg19).(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
16 to 21 days

Specimen Retention Time
Bone marrow aspirate/Whole blood: 2 weeks; Tissue: 1 month; Extracted DNA: 3 months; FFPE tissue: Unused portions of blocks will be returned to the client. Unstained slides/body fluid: 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

81450

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NGCLN	Chronic Lymphoid Neoplasms, NGS, V	104238-1

Result ID	Test Result Name	Result LOINC® Value
MP065	Specimen Type	31208-2
MP066	Indication for Test	42349-1
618485	NGCLN Result	No LOINC Needed
618486	Pathogenic Mutations Detected	82939-0
618487	Interpretation	69047-9
618489	Variants of Unknown Significance	93367-1
618490	Additional Information	48767-8
618488	Clinical Trials	82786-5
618491	Method Summary	85069-3
618492	Disclaimer	62364-5
618493	Panel Gene List	36908-2
618494	Reviewed By	18771-6