Test Definition: BCLL

IGH Somatic Hypermutation Analysis, B-Cell Chronic Lymphocytic Leukemia (B-CLL), Varies

Overview

Useful For

Providing prognostic information in patients with newly diagnosed B-cell chronic lymphocytic leukemia

This test is **not intended for use** in providing prognostic information for patient with other B-cell neoplasms or hematopoietic tumors.

Special Instructions

- Molecular Hematopathology Patient Information

Highlights

Although the determination of variant status can be accomplished by polymerase chain reaction followed by Sanger sequencing, that approach only allows for analysis of single samples at a time. Next-generation sequencing technology represents a significant improvement over existing Sanger assays by allowing for batch sample analysis and simultaneous identification of clonal *IGH* rearrangement, the tumor-specific rearrangement sequence, and determination of somatic variant percent.

Method Name

Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

1. Both refrigerated and ambient specimens must arrive within 7 days of collection.

2. Collect and package specimen as close to shipping time as possible.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)

Specimen Volume: 4 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 whole blood.
Specimen Stability: Refrigerated (preferred) 7 days/Ambient 7 days
Additional Information: To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Bone marrow
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: Ambient (preferred) 7 days/Refrigerate 7 days
Additional Information: To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Extracted DNA from whole blood or bone marrow
Container/Tube: 1.5- to 2-mL screw-top tube
Specimen Volume: Entire specimen
Collection Instructions:
1. DNA must be extracted from blood or bone marrow within 7 days of collection.
2. Label specimen as extracted DNA and source of specimen
3. Provide volume and concentration of the DNA
Specimen Stability Information: Frozen (preferred)/Refrigerate/Ambient
Additional Information:
1. Specimens are preferred to be received within 1 year after DNA extraction.
2. 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. **Molecular Hematopathology Patient Information** (T711).
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: 1 mL; Extracted DNA: 50 mcL at a concentration of 20 ng/mcL.

Reject Due To

Gross hemolysis	Reject
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

During early B-cell development, *IGH* genes are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating variable diversity joining (VDJ) combinations that are unique in both length and sequence for each B cell. In addition, newly acquired (somatic) point variations are introduced into the variable (V) regions of mature B cells during the germinal center reaction in lymph nodes in a process called somatic hypermutation (SHM). Since chronic lymphocytic leukemia (CLL) originates from the malignant transformation of single lymphoid cells, each daughter cell shares one or, sometimes, more unique "clonal" antigen receptor gene rearrangements, which are cell and tumor specific (ie, a tumor cell "fingerprint"). Clonal *IGHV* gene hypermutation status provides important prognostic information for patients with CLL and small lymphocytic lymphoma (SLL). The presence of *IGH* SHM is defined as greater than 2% difference from the germline VH gene sequence identity (mutated), whereas less than or equal to 2% difference is considered no SHM (unmutated). The status of SHM has clear influence on the median survival of CLL patients. Hypermutation of the *IGH* variable region is strongly predictive of a good prognosis, while lack of variants predicts a poorer prognosis.

Reference Values

An interpretive report will be provided.

Interpretation

The presence or absence of somatic hypermutation (SHM) in the immunoglobulin heavy chain gene (*IGH*) variable (V) region DNA will be reported. A variation frequency of greater than 2% will be reported as mutated. Both the percent mutation and the V region allele identified in the rearrangement will be included in the report.

B-cell chronic lymphocytic leukemia (B-CLL) lacking SHM of the *IGH* V region (unmutated) is associated with a significantly worse prognosis than B-CLL containing SHM of the *IGH* V region (mutated).

Cautions

This test is useful for patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) with blood or bone marrow involvement. The prognostic value of somatic *IGH* V *(IGHV)* region mutation status is applicable only for

this subtype of B-cell malignancy.

This test requires a minimum monoclonal CLL B-cell percentage in order to amplify the clonal *IGH* gene rearrangement. This level has been established at 5% of lymphocytes (eg, as determined by flow cytometric immunophenotyping). A CLL population below 5% will not have a reliable or reproducible clonal gene rearrangement and sequencing by next-generation sequencing to determine somatic mutation status will typically produce no results, or possibly a false-positive finding. Therefore, submitted CLL samples must have a minimum CLL monoclonal B-cell population of 5% of total lymphocytes.

The prognostic significance of somatic hypermutation status is only known when a single functional *IGH* rearrangement is identified (ie, in frame junctional coding region with no predicted premature protein truncation). However, a variety of situations can occur, for which the clinical significance is unknown at this time. These can broadly be grouped into the following:
1. Greater than 1 functional rearrangement is identified, with discordant mutation status
2. Only nonfunctional rearrangements are identified

Rearrangements with mutation status at or near the 2% cutoff should be interpreted with caution for the purposes of prognosis, particularly if the entire *IGH*V sequence could not be sequenced due to the use of framework region 1 V region primers. If such results are identified, an appropriate comment will be provided in the report.

Clinical Reference

1. Davi F, Langerak AW, de Septenville AL, et al. Immunoglobulin gene analysis in chronic lymphocytic leukemia in the era of next generation sequencing. Leukemia. 2020;34(10):2545-2551. doi:10.1038/s41375-020-0923-9
2. Agathangelidis A, Sutton LA, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: From patient material to sequence interpretation. J Vis Exp. 2018;(141):10.3791/57787. Published 2018 Nov 26. doi:10.3791/57787
3. Rosenquist R, Ghia P, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. Leukemia. 2017;31(7):1477-1481. doi:10.1038/leu.2017.125

Performance

Method Description

DNA is extracted from whole blood or bone marrow specimens, and *IGH* gene rearrangements are amplified by polymerase chain reaction using indexed leader and/or framework region 1 forward primers. The amplified product is then purified, and the DNA concentration measured. Pooled patient samples (identifiable by the index bar codes) are subjected to next-generation sequencing. FASTQC sequence data is subsequently analyzed using proprietary software to identify the *IGH* V rearrangement and the unique sequence. Results are compared to a germline IGHV sequence database by the software to calculate the percent identity of the tumor *IGH* V rearrangement to the closest germline sequence. Rearrangements containing a variation frequency of greater than 2% are interpreted as mutated. Rearrangements containing a variation frequency less than or equal to 2% are interpreted as unmutated.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
14 days

Specimen Retention Time
Whole blood/Bone marrow: 2 weeks; Extracted DNA: 3 months

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
81263-*IGH* (immunoglobulin heavy chain locus) (eg, leukemia and lymphoma, B-cell), variable region somatic mutation analysis

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BCLL	IGH Somatic Hypermutation in B-CLL	50627-9

Result ID	Test Result Name	Result LOINC® Value
MP005	Specimen Type	31208-2
19674	Final Diagnosis	50398-7
39465	BCLL Result	No LOINC Needed
622044	Final Interpretation	No LOINC Needed
622055	Primer Set	104271-2
622045	Primary V-gene	104269-6
623097	Primary J-gene	104270-4

622046	Primary Mutation Status %	107552-2
622047	Primary Mutation Status	107551-4
622048	Primary Rearrangement Status	21747-1
622053	Primary Subset	107550-6
622049	Secondary V-gene	104269-6
623098	Secondary J-gene	104270-4
622050	Secondary Mutation Status %	107552-2
622051	Secondary Mutation Status	107551-4
622052	Secondary Rearrangement Status	21747-1
622054	Secondary Subset	107550-6