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.

Highlights
Although the determination of variant status can be accomplished by PCR followed by Sanger sequencing, this approach only allows for analysis of single samples at a time. Next-generation sequencing (NGS) 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.

Special Instructions
- **Molecular Hematopathology Patient Information**

Method Name
Polymerase Chain Reaction (PCR) and Next-Generation Sequencing

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.

Necessary Information

1. **Molecular Hematopathology Patient Information**: B-Cell Chronic Lymphocytic Leukemia (CLL) for IGVH and/or **TP53 Somatic Mutation Testing (T711) is required**, see Special Instructions. Testing may proceed without the patient information, however, it aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.

2. If form is not provided, include the following information with the test request: specimen source, pertinent clinical history (ie, CBC results and relevant clinical notes), and clinical or morphologic suspicion.

Specimen Required

Submit only 1 of the following specimens:

**Specimen Type**: Peripheral blood

**Container/Tube**: 
Test Definition: BCLL
IGH Somatic Hypermutation in B-CLL

**Preferred:** Lavender top (EDTA)

**Acceptable:** Yellow top (ACD)

**Specimen Volume:** 4 mL

**Collection Instructions:**
1. Invert several times to mix blood.
2. Send specimen in original tube.
3. Label specimen as blood.

**Specimen Stability:** Refrigerated/ Ambient

**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 specimen in original tube.
3. Label specimen as bone marrow.

**Specimen Stability:** Refrigerated/ Ambient

**Specimen Type:** Extracted DNA from blood or bone marrow

**Container/Tube:** 1.5- to 2-mL screw-top tube

**Specimen Volume:** Entire specimen

**Collection Instructions:**
1. Label specimen as extracted DNA and indicate specimen source (blood or bone marrow).
2. The required volume of DNA is 50 mcL at a concentration of 20 ng/mcL
3. Include volume and concentration on tube.

**Specimen Stability:** Frozen (preferred)/Refrigerated
Forms
1. Molecular Hematopathology Patient Information: B-Cell Chronic Lymphocytic Leukemia (CLL) for IGVH and/or TP53 Somatic Mutation Testing (T711) is required, see Special Instructions

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

Specimen Minimum Volume
Blood: 1 mL
Bone Marrow: 1 mL
Extracted DNA: see Specimen Required

Reject Due To

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tr>
<td>Gross hemolysis</td>
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<tr>
<td>Moderately to severely clotted</td>
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Specimen Stability Information

Clinical and 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, new acquired (somatic) point variations are introduced into the variable (V) regions of mature B cells during the germinal center reaction in lymph nodes, and this process is called somatic hypermutation (SHM). Since chronic lymphocytic leukemia (CLL) originates from the malignant transformation of single lymphoid cells, each daughter cell shares 1 or (sometimes) more unique “clonal” antigen receptor gene rearrangements, which are cell and, therefore, 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 \textit{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 \textit{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 \textit{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 (SHM) status is only known when a single functional \textit{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 \textit{IGHV} sequence could not be sequenced due to the use of framework region 1 (FR1) V region primers. If such results are identified, an appropriate comment will be provided in the report.

**Clinical Reference**


**Performance**

**Method Description**

DNA is extracted from whole blood or bone marrow specimens and \textit{IGH} gene rearrangements are amplified by polymerase chain reaction (PCR) using indexed leader and/or FR1 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 by proprietary software to identify the \textit{IGHV} 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 \textit{IGHV} 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) and Time(s) Test Performed
Monday, Wednesday, Friday

Analytic Time
2 weeks

Specimen Retention Time
DNA 3 months

Performing Laboratory Location
Rochester

Fees and 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 U.S. 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

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