Overview

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

Highlights
Although the determination of mutation 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 (e.g., using the Illumina platform) 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 mutation 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. Refrigerate specimens must arrive within 5 days of collection, and ambient specimens must arrive within 3 days (72 hours) of collection.

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

Necessary Information
The following information is required:

1. Pertinent clinical history
2. Clinical or morphologic suspicion
3. Date of collection
4. Specimen source

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Peripheral blood

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

**Preferred:** EDTA (lavender top)

**Acceptable:** ACD (yellow top)

**Specimen Volume:** 10 mL

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

**Specimen Type:** Bone marrow

**Container/Tube:**

**Preferred:** EDTA (lavender top)

**Acceptable:** ACD (yellow top)

**Specimen Volume:** 4 mL

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

**Forms**
1. Molecular Hematopathology Patient Information: B-Cell Chronic Lymphocytic Leukemia (CLL) for IGVH and/or TP53 Somatic Mutation Testing 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: 4 mL
Bone Marrow: 2 mL

**Reject Due To**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acceptance</th>
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</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>Mild OK; Gross reject</td>
</tr>
<tr>
<td>Lipemia</td>
<td>NA</td>
</tr>
<tr>
<td>Icterus</td>
<td>NA</td>
</tr>
<tr>
<td>Other</td>
<td>Moderately to severely clotted</td>
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</table>
Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Refrigerated (preferred)</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td>Ambient</td>
<td>72 hours</td>
</tr>
</tbody>
</table>

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 mutations 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 mutation predicts a poorer prognosis.

Reference Values

An interpretive report will be provided.

Interpretation

The presence or absence of somatic hypermutation in the immunoglobulin heavy chain gene (\( IGH \)) variable (V) region DNA will be reported. A mutation 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 somatic hypermutation of the \( IGHV \) region (unmutated) is associated with a significantly worse prognosis than B-CLL containing somatic hypermutation of the \( IGHV \) 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 \( IGHV \) mutation status is applicable at this time only to this subtype of B-cell malignancy and the test is not intended for use in other B-cell neoplasms or hematopoietic tumors.

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 (SHM) 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 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
RNA is extracted from B-cell chronic lymphocytic leukemia specimens and converted to cDNA using reverse transcription. PCR is then used to amplify the IGH gene rearrangements with indexed multiplex primers designed to include a portion of the leader segment, all of the variable (V) and diversity (D) segments, and a portion of the joining (J) segment. A check gel is performed to make sure that there is amplified product. If no amplified product is visible a second PCR is performed with a different multiplex primer set targeting the framework 1 (FR1) regions of the V genes. FR1 PCR rearrangements are shorter in length than leader primer products and result in slightly truncated 5' V-region sequence coverage. The amplified product is then purified and the DNA concentration measured. Pooled patient samples (identifiable by the index bar codes) are subjected to sequencing on the Illumina platform. FASTQC sequence data is subsequently analyzed using proprietary software to identify the IGHV rearrangement and the unique sequence. Results are compared to a germline IGVH sequence database by the software to calculate the percent identity of the tumor IGHV rearrangement to the closest germline sequence. Rearrangements containing a mutation frequency of greater than 2% are interpreted as mutated. Rearrangements containing a mutation 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
Up to 2 weeks

Specimen Retention Time
RNA 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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<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tbody>
<tr>
<td>BCLL</td>
<td>IGH Somatic Hypermutation in B-CLL</td>
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<table>
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<th>Result LOINC Value</th>
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<td>19674</td>
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