IGHV Mutation Analysis

Overview
There are two types of Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma (SLL) - indolent and aggressive. Indolent CLL/SLL indicates the disease is slow-growing. In these cases, doctors typically will not start treatment but rather continue to follow-up with the patient to monitor if the disease has become aggressive. Aggressive cases of CLL/SLL require testing for prognosis and clinical management of the disease. Studies have shown that CLL/SLL patients with a mutated Immunoglobulin Heavy Chain Variable (IGHV) gene have a very favorable outcome and a low probability of developing progressive disease, whereas those with unmutated IGHV gene were much more likely to develop progressive disease and had a shorter survival.

Clinical Indications
Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma (SLL).

Clinical Utility
Aids in determining prognosis and clinical management of CLL/SLL. Predicts overall survival and time to treatment.

Methodology and Interpretation
PCR amplification and sequencing analysis of the IGHV region. The test is performed on genomic DNA rather than RNA, the stability of DNA increases the success rate of the test. The use of 5 sets of IGHV primers in PCR increases the efficiency of detection of IGHV clonal rearrangement, which is required to determine the IGHV mutation status. Independent primer sets are also used to broaden the coverage of IGHV clonal rearrangements and to confirm negative clonal rearrangements. Isolation of overlapping DNA sequences by subcloning increases the success rate for the determination of IGHV mutation status.

Assay Specifications
Sensitivity
The sensitivity of this assay is 10%.

Specimen Requirements
• 2-3 ml peripheral blood or bone marrow in an EDTA tube (Lavender-top).
• Specimen should be stored, transported at room temperature or 2-8°C, and received within 48 hours of collection.

Reporting
Mutated: > 2% deviation from the germline sequence.
Unmutated: < 2% deviation from the germline sequence.

Licensure
CAP (Laboratory #: 7191582, AU-ID: 1434060), CLIA (Certificate #: 31D1038733), New Jersey (CLIS ID #: 0002299), New York State (PFI: 8192), Pennsylvania (031978), Florida (800018142), Maryland (1395)

CPT Codes
81263
**IGHV MUTATION ASSAY SAMPLE REPORT**

<table>
<thead>
<tr>
<th>Results:</th>
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<tbody>
<tr>
<td><strong>IGHV Family:</strong></td>
<td>V3-74</td>
</tr>
<tr>
<td><strong>Functionality:</strong></td>
<td>Productive</td>
</tr>
<tr>
<td><strong>Mutation Frequency:</strong></td>
<td>7.3%</td>
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**Interpretation:** Mutated

**Description:**

The mutation status of the unique immunoglobulin gene (*IGHV*) rearrangement in the monoclonal proliferation of B-cells in chronic lymphocytic leukemia (CLL) is considered to have prognostic value. If mutations are detected at a level of 2% or higher in the sequenced V region of the clonal rearrangement, then the result is interpreted as "mutated". If mutations are detected at a level below 2% in the sequenced V region of the clonal rearrangement, then the results is interpreted as "unmutated". Those patients with a mutated *IGHV* gene usually have a less aggressive and more indolent disease, with longer overall survival. Those patients with an unmutated *IGHV* gene usually have a more aggressive disease and shorter overall survival.

This assay utilizes PCR to detect a monoclonal *IGHV* rearrangement followed by sequence analysis to determine the specific *IGHV* family and mutation frequency. The sensitivity of the assay is 10%. Samples in which the monoclonal B-cells are present at less than 10%, a specific *IGHV* rearrangement will not be detected and will be reported as failures.