Experience.
MPLN

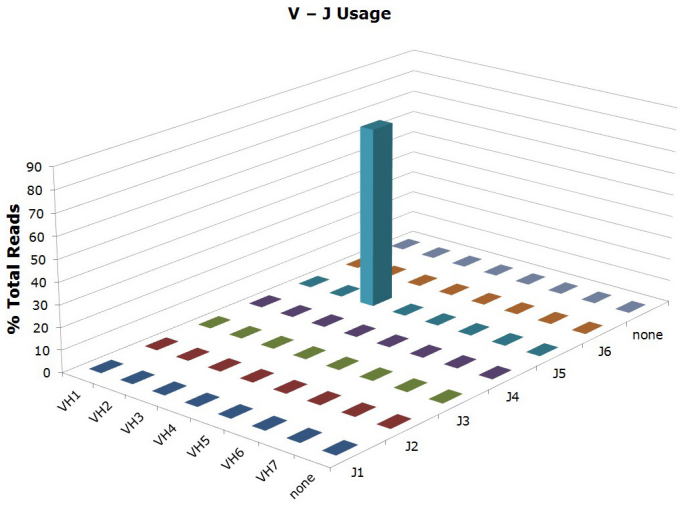
IgVH SOMATIC HYPERMUTATION BY NEXT GENERATION SEQUENCING

Molecular Pathology Laboratory Network Inc. now offers testing for IgVH somatic hypermutation by next generation sequencing in CLL/SLL.

CLL/SLL is the most common leukemia diagnosed among adults in Western countries and is associated with heterogeneous clinical outcomes. IgVH somatic hypermutation (SHM) status is a primary component of the CLL International Prognostic Index (CLL-IPI) working group formulation for disease risk stratification. Un-mutated IgVH has been established as a strong and independent predictor of adverse clinical prognosis and reduced overall survival.

MPLN approach to IgVH SHM testing

Using a next generation sequencing-based approach to IgVH SHM detection, the MPLN methodology utilizes patient DNA as a sample starting material, eliminating many of the challenges related to RNA-based testing. Laboratory workflow is streamlined and automated to result in non-subjective data output that includes percentage homology of clonal reads to germline *IGH* reference sequences, VDJ gene utilization, and frequencies of *IGH* gene sequences.



NGS data output includes relative frequencies of IGH gene sequences and VDJ gene utilization.

Limitations of Traditional Methodologies

Traditionally, IgVH SHM has been evaluated by rt-PCR followed by Sanger Sequencing using patient sample RNA. However, RNA lability places a significant burden on the submitting physician to minimize specimen transit time. The Sanger Sequencing approach is also time and labor intensive and may show limited sensitivity in detection of IgVH SHM for low abundance clones. Flow cytometric analysis of ZAP-70 expression has therefore been widely utilized as a surrogate for IgVH SHM status, however, standardization for this marker is known to be poor due to the subjective nature of its interpretation. Significant discordance between ZAP-70 expression patterns and IgVH SHM results may also be seen and has been attributed in some studies to pre-analytic sample processing factors.

NGS testing for IgVH SHM is superior to traditional methodologies:

- Utilizes patient DNA instead of RNA as sample starting material
- Minimal sample requirements: 1ml blood or bone marrow aspirate, or 0.01 µg DNA
- Non-subjective data output includes percentage homology to germline *IGH* sequences, clonal IGH sequences abundance, and VDJ gene utilization.
- Demonstrates improved sensitivity in detection of clonal populations

Ordering Requirements

Order Test Code	M IGVH
Turnaround Time	10-14 days
Specimen Requirements	Whole Blood or Bone Marrow in EDTA
Storage and Handling	Transport at ambient temperature (18-25°C)

References

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2. Wilhelm C, Neubauer A, Brendel C. 2006. **Discordant results of flow cytometric ZAP-70 expression status in B-CLL samples if different gating strategies are applied.** Cytometry B Clin Cytom. 15;70(4):242-250.
3. Sheikholeslami MR et al. **Variations in the detection of ZAP-70 in chronic lymphocytic leukemia: Comparison with IgV(H) mutation analysis.** Cytometry B Clin Cytom. 2006 Jul 15;70(4):270-275.
4. Hamblin TJ, Davis Z, Gardiner A, et al. **Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia.** Blood 1999 September 15;94(6):1848-1854.

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