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Laboratory Bulletin

DATE:	2021 November 1
TO:	Hematology, Hematopathology, Molecular Pathology
FROM:	Molecular Pathology Laboratory South
RE:	**Updated Reporting for IGHV Somatic Hypermutation (SHM) Testing**

PLEASE POST OR DISTRIBUTE AS WIDELY AS APPROPRIATE

Key Message

- Molecular pathology reporting of IGHV somatic hypermutation analysis will be updated to a new format so as to reflect reporting recommendations from the European Research Initiative on CLL. Please familiarize yourself with the new formatting. There is no change to the assay itself or process of test ordering.

Background

- Assessment of B cell immunoglobulin heavy chain V segment for somatic hypermutation (IGHV-SHM) is a key prognostic and predictive assay used in the management and treatment of chronic lymphocytic leukemia (CLL). Reporting of key elements of IGHV-SHM testing have been established by the European Research Initiative on CLL (ERIC) reporting guidelines. We has updated IGHV-SHM reporting templates to be more in line with these guidelines.

How this will impact you

- Clinicians who order and/or interpret these tests should review the changes to the report format.

Action Required

- Please review the attached mock test report to familiarize yourself with the data layout of new report.

Effective

- October 20, 2021

Questions/Concerns

- Please contact:
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  - Dr. Adrian Box, Medical Lead, Molecular Pathology Lab South, 403-944-6686, Adrian.Box@APLabs.ca

Approved by

- Dr. Adrian Box, Medical Lead, Molecular Pathology Lab South, Calgary
- Dr. Imran Mirza, Provincial Medical Lead – Molecular Pathology.

Clinical History:
Chronic Lymphocytic Leukemia (CLL)

Result:
MUTATED productive clonal IGH rearrangement identified.
% IGHV mutations: XX.X%
Stereotyped Subset: Unassigned
Most homologous germline V sequence: ____
Most homologous germline D sequence: ____
Most homologous germline J sequence: ____

Interpretation:
Examination of the productive clonal IGH rearrangement in comparison to the most homologous germline sequence shows less than 98% nucleotide identity (greater than 2% mutation rate).

The identified productive rearrangement is considered MUTATED. Mutated CLL is associated with a better prognosis when compared to unmutated CLL.

Assay Description:
Genomic DNA is extracted from peripheral blood, fresh tissue or cells suspended in flow cytometry media. Bidirectional leader (VHL) sequence PCR is performed using the IGH Hypermutation Assay 2.0 (Invivoscribe) and the resulting monoclonal product (if present) is sequenced via Sanger sequencing using BigDye terminator kit v3.1 (Lifetech). Determination of the most homologous germline IGH sequence and mutational burden is performed using the International Immunogenetics Information System (IMGT/V-Quest) (http://www.imgt.org/IMGT_vquest/input) and examination for possible stereotyped subsets is performed via the ARResT/AsignSubsets bioinformatics website (http://tools.bat.infspire.org/arrest/assignsubsets/).

Test Limitation:
Due to the random nature of somatic hypermutation within the IGH locus, there is the possibility of a false negative result in true IGH monoclonal due to mutation of PCR primer sites in the IGH loci.
This Sanger sequencing assay cannot separate biallelic IGH clones and is unable to provide SHM status in these instances (Indeterminant).
This assay is not validated on formalin fixed paraffin embedded tissue (FFPE) and FFPE samples will not be accepted for testing.

Clinical Disclaimer:
Disclaimer: Interpretation of the above results must occur within the context of other clinical data.

General Disclaimer: This assay was validated and its performance characteristics determined by the Molecular Pathology Laboratory South, Alberta Precision Labs, Foothills Medical Center. This test is used for clinical purposes at the Foothills Medical Center. It should not be regarded as investigational or for research.

References:

1) IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. Brochet, X. et al., Nucl. Acids Res. 36, W503-508 (2008).

2) IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide sequences. Giudicelli, V., Brochet, X., Lefranc, M.-P., Cold Spring Harb Protoc. 2011 Jun 1;2011(6).

3) ARResT/AssignSubsets: a novel application for robust subclassification of chronic lymphocytic leukemia based on B cell receptor IG stereotypy. Bystry V. *et.al.* Bioinformatics, Volume 31, Issue 23, 1 December 2015, Pages 3844–384.

4) ten Hacken E, Gounari M, Ghia P, Burger JA. The importance of B cell receptor isotypes and stereotypes in chronic lymphocytic leukemia. Leukemia 2019;33:287–98.

5) Rosenquist R, Ghia P, Hadzidimitriou A, Sutton LA, Agathangelidis A, Baliakas P, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: Updated ERIC recommendations. Leukemia 2017;31:1477–81.