CLL MRD Assay

Multiparametric Flow Cytometry

CAP/CLIA accredited (US)
ISO certified (EU and Asia)

Clinical Information

Globally, the age-standardized incidence rate of chronic lymphocytic leukemia (CLL) has increased over the past 30 years, with a higher incidence among men and adults over 65.[1,2] CLL is currently the most prevalent type of leukemia, comprising 25% - 30% of all leukemias in Western populations.[3] While clinical remission (CR) has been the standard goal of end-of-treatment response for decades, updated understanding of prognosis as well as novel treatment strategies that can achieve deeper levels of remission now necessitate newer, more sensitive, and standardized assessments of treatment response, such as measurable (or minimal) residual disease (MRD) assessments.[4,5]

MRD is a highly sensitive indicator of disease burden, both during and after therapy, and has been correlated with progression free survival (PFS) and overall survival (OS)[6], while also serving as an independent prognostic indicator in patients with CLL. Flow cytometry has long been an important part of the standard of care for the diagnosis and ongoing management of many blood cancers, including CLL. However, achieving MRD-level sensitivity for detection of disease burden, especially with limited samples, requires new and specifically designed assays. Multiparameter flow cytometry (MFC) MRD assays are state-of-the-art and widely-used methods for MRD detection and quantification in a variety of hematologic malignancies, achieving high sensitivity, high specificity, short turn-around-time, and cost-effectiveness compared to some alternatives.

Our CLL MRD MFC assay is a streamlined and targeted 11-color panel designed to characterize potential CLL cells with clear separation from other B-lineage cells.[4,7,8] The assay is validated on bone marrow and peripheral blood samples to detect the presence or absence of CLL MRD in patients at any stage of treatment, including targeted immunotherapies. The technical component of the assay will be performed in our clinical laboratories, and the interpretation will be performed by our team of expert hematopathologists.

If available, information from diagnosis will be used to assist with the assessment, although the assay can identify aberrant cells that have diverged from normal populations without previous patient history or baseline assessment.

Indications for Testing

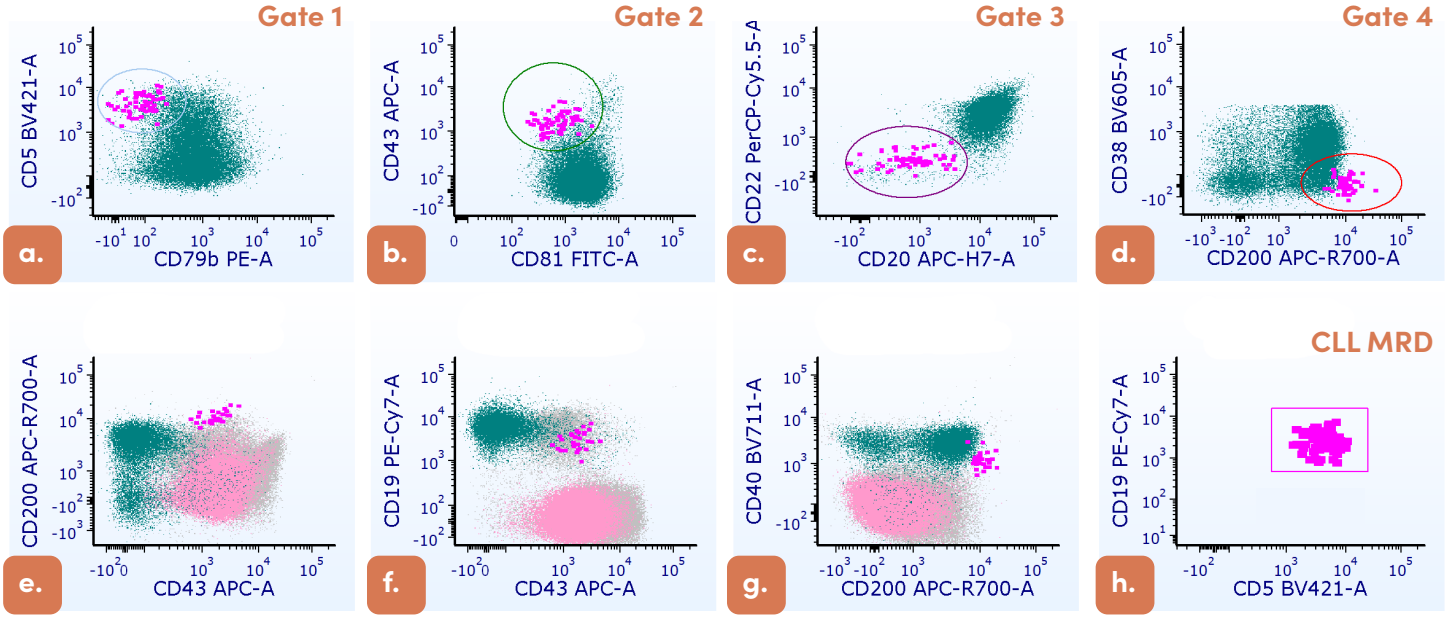
- Identify tumor-specific immunophenotypes for post treatment monitoring and evaluation for relapse
- Stratify patient risk and streamline clinical trial enrollment
- Use as a potential surrogate endpoint in clinical trials
- Assess response to therapy

Biomarkers in the CLL MRD Assay		
CD3	CD22	CD79b
CD5	CD38	CD81
CD19	CD40	CD200
CD20	CD43	7AAD

Key Points

- Single Tube 11-Color MRD Assay
- Using a standardized panel across all time points, MRD populations can be characterized and tracked to 0.005% sensitivity
- Alignment with International Harmonized Approach[7,8,10] and ERIC protocol[7,8]
- Internationally standardized testing through LabPMM Network
- Optimize shared/limited samples by co-testing with MFC and NGS
- Enhanced analyses using artificial intelligence (AI) and machine learning (ML)

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Sample Stability
An interpretive report will indicate the presence/absence of CLL MRD in relation to the assay level of detection and clinical cut-off value.	24 hours	2 mL of bone marrow in EDTA or Sodium Heparin 2-4 mL of peripheral blood in EDTA or Sodium Heparin	Ambient or Cool; Do not freeze	Store specimens at 2-8°C; must be received by lab within 72 hours after draw



After initial quality assessment of stable flow of acquisition, exclusion of doublets, non-viable cells, RBCs, and debris, mononuclear cells are gated based on CD43 vs. side scatter (SSC). A cleaned singlet B-cells population is delineated (after exclusion of hematogones, plasma cells with combination of markers such as CD5, CD3, CD81, CD40, not shown here). A combination gate (created by Boolean logic intersecting of Gate 1, Gate 2, Gate 3 and Gate 4) populates plot h where clustered aberrant events can be gated to calculate CLL MRD (bright pink colored population depicted here in panels a, b, c, d, e, f, g, h) within CD19 positive singlet B-cells by the aberrant expression of CD5 and CD43, and weak expression of CD79b, CD81, CD20, CD22 and strong CD200. Alternative gating strategies will also be concurrently assessed to evaluate for CLL populations that may lack standard biomarker expression secondary to targeted immunotherapy. MRD is calculated as number of CLL events as a percentage of total Nucleated Cells.

References

1. Y Dong et al. *Exp Hematol Oncol* 9, 14 (2020). https://doi.org/10.1186/s40164-020-00170-6 2. Y Ou et al. *Frontiers in Oncology* https://doi.org/10.3389/fonc.2022.840616. 3. Batista, JL et al. (2017). *Pathology and Epidemiology of Cancer.* Springer, Cham. https://doi.org/10.1007/978-3-319-35153-7_29 4. WG Wierda et al. *Leukemia.* 2021; 35: 3059–3072. https://doi.org/10.1038/s41375-021-01241-1 5. M Hallek and O Al-Sawaf. *Am J Hematol.* 2021; 96(12):1679- 1705. doi:10.1002/ajh.26367 6. A Rawstron A et al. *Blood.* 2018;132(Suppl 1):S181. 7. AC Rawstron et al. *Leukemia.* 2016; Apr;30(4):929-36. doi: 10.1038/leu.2015.313. Epub 2015 Dec 7. PMID: 26639181; PMCID: PMC4832072. 8. AC Rawstron et al. *Leukemia.* 2013 Jan;27(1):142-9. doi: 10.1038/leu.2012.216. Epub 2012 Jul 31. PMID: 23041722. 9. MM Sartor et al. *Cytometry* Part B. 2013; 84B:96–103.