FLO.30790 Final Report **Phase II**

The final report includes information about the immunophenotype of the abnormal cells, if identified, and comments necessary to facilitate the interpretation.

NOTE: Clinical information and available pathologic material should be reviewed to select appropriate antibodies. In cases of suspected hematolymphoid neoplasia direct morphologic correlation of all applicable sample types should be performed when possible and clinically appropriate. In cases involving leukemia and lymphoma phenotyping, correlation should be made between the immunologic and pathologic results. The flow histograms, rather than just the percentage of positive cells, should be reviewed by the interpreting pathologist in difficult cases. The peak channel and shapes of the curves may be helpful in identifying clonal populations.

Reporting requirements for use of analyte-specific reagents and other reagents used in laboratory-developed tests are included in the All Common Checklist (COM.40850).

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
2) Nguyen AND, *et al*. A relational database for diagnosis of hematopoietic neoplasms using immunophenotyping by flow cytometry. *Am J Clin Pathol.* 2000;113:95-106
3) Wood BL, et al. 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia. Cytometry Part B (Clinical Cytometry) 2007;72B:S12-S22.
4) Sever C, Abbott CL, de Baca ME, et al. Bone marrow synoptic reporting for hematologic neoplasms: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med;* 2016;140(9):932-49.
5) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition.* CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.

RARE EVENT FLOW CYTOMETRIC ASSAYS

Inspector Instructions:

	• Sampling of rare event flow cytometric assay policies and procedures • Sampling of patient reports (to include lower limit of enumeration)
	• How does your laboratory distinguish normal populations from residual disease or abnormal PNH clones?

FLO.30800 Rare Event Flow Cytometric Assays **Phase II**

For rare event flow cytometric assays, the lower limit of enumeration has been validated.

NOTE: The detection of rare events may occur in assays, such as Paroxysmal Nocturnal Hemoglobinuria (PNH) clone testing or minimal residual disease (MRD) testing. Analytic sensitivity of the lower detection limit should be validated by performing dilutional studies using known patient or suitable reference material, such as proficiency testing material.

For high sensitivity paroxysmal nocturnal hemoglobinuria (PNH) testing (detecting and reporting clones less than or equal to 1%), validations to establish the lower limit of enumeration (quantitation) are required for red blood cells, granulocytes, and monocytes.

REFERENCES

1) Sutherland DR, Illingworth A, Marinov I, et al. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 2 - reagent selection and assay optimization for high sensitivity testing. *Clin Cytom*. 2018: 94(1):23-48.

FLO.30820 Rare Event Flow Cytometric Assays — Phase I

For rare event flow cytometric assays, the lower limit of enumeration is included in the diagnostic report.

NOTE: When performing rare event flow cytometric assays (such as minimal residual disease (MRD) and/or high sensitivity PNH testing) on low cellularity samples, the number of events needed to achieve the laboratory's validated lower limit of enumeration/sensitivity may not be able to be collected. In these cases, laboratories must clearly state in the flow cytometric assay report that the sample was paucicellular and may thus have reduced analytical sensitivity.

FLO.30830 Rare Event Flow Cytometric Assays - MRD Testing — Phase I

For minimal residual disease (MRD) testing (including B lymphoblastic leukemia, acute myeloid leukemia, plasma cell myeloma, and mature lymphoid disorders), the method appropriately separates normal populations from residual disease.

NOTE: Examples include:
- *B lymphoblastic leukemia - distinguish hematogones (normal immature B-cell precursors) from B lymphoid blasts*
- *Acute myeloid leukemia - distinguish normal myeloid blasts from disease-associated/ neoplastic blasts*
- *Plasma cell myeloma - distinguish neoplastic from non-neoplastic plasma cells*
- *Non-Hodgkin lymphoma - separate normal B cells from abnormal/neoplastic B cells (or T cells if assay targets T cell non-Hodgkin lymphoma)*

Evidence of Compliance:
- ✓ Validation data showing normal and abnormal population analysis **OR**
- ✓ Examples of methods used to distinguish normal from abnormal and sample patient data from both normal and abnormal cases

REFERENCES

1) Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia*. 2017; 31(10):2094-2103.
2) Rawstron AC, Paiva B, Stetler-Stevenson M. Assessment of minimal residual disease in myeloma and the need for a consensus approach. *Cytometry B Clin Cytom*. 2016; 90(1):21-5.
3) Oldaker TA, Wallace PK, Barnett D. Flow cytometry quality requirements for monitoring of minimal disease in plasma cell myeloma. *Cytometry B Clin Cytom*. 2016; 90(1):40-6.
4) Gupta S, Devidas M, Loh ML, et al. Flow cytometric vs morphologic assessment of remission in childhood acute lymphoblastic leukemia: A report from the Children's Oncology Group (COG). *Leukemia*. 2017; Dec 18. doi: 10.1038/leu.2017.341.
5) Keeney M, Wood BL, Hedley BD, et al. A QA program for MRD testing demonstrates that systematic education can reduce discordance among experienced interpreters. *Cytometry B Clin Cytom*. 2017; May 5. doi: 10.1002/cyto.b.21528.
6) Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: consensus document from ELN MRD Working Party. *Blood*. 2018 Jan 12. pii:blood-2017-09-801498.
7) Lacombe F, Campos L, Allou K, et al: Groupe d'Etude Immunologique des Leucemies (GEIL). Prognostic value of multicenter flow cytometry harmonized assessment of minimal residual disease in acute myeloblastic leukemia. *Hematol Oncol.* 2017; Dec 7. doi: 10.1002/hon.2488.
8) Bottcher S, Ritgen M, Kneba M. Flow cytometric MRD detection in selected mature B-cell malignancies. *Methods Mol Biol.* 2013; 971:149-74.

FLO.30840 PNH Testing — Phase I

The laboratory has a defined process to separate normal populations from abnormal PNH clones.

NOTE: For RBC analysis, the procedure must include an appropriate panel of antibodies/ reagents and methods for distinguishing normal type I RBCs from PNH type II and type III clones. This typically applies to larger RBC clones with well-defined clusters of RBC populations. Minor