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

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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.
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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

clones (equal or less than 1%) may not show distinct separation between the type II and type III populations and one gate for the combined RBC PNH clone is sufficient.

Assay specific PMT settings must be established (see FLO.30255). For the red cell assay, unstained red blood cells are appropriate. For white cell assays, the method should include an appropriate panel of antibodies/reagents to distinguish normal from abnormal PNH clones in the populations of interest (granulocytes and/or monocytes). For PMT settings, it is important to ensure the unstained lymphocyte population is clearly on scale. Some WBC samples may show type II populations; current consensus is that these cells should be included in total "PNH neutrophils".

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.

DNA CONTENT AND CELL CYCLE ANALYSIS

Inspector Instructions:

READ	• Sampling of DNA analysis policies and procedures (includes reference to established methodology and list of acceptable neoplasms for DNA analysis) • Sampling of specimen evaluation records • Sampling of DNA analysis linearity and QC records • Sampling of sub-optimal/specimen rejection records/log
ASK	• What is your laboratory's course of action when unacceptable or sub-optimal specimens are received? • How does your laboratory ensure debris and aggregates are excluded from consideration? • How does your laboratory ensure that the analysis contains neoplastic cells of interest? • How does your laboratory ensure detection of DNA aneuploidy?

FLO.31000 Neoplastic Cell Content **Phase II**

The laboratory ensures that specimens processed for DNA content and cell cycle analysis contain neoplastic cells of interest.

NOTE: It is critical that specimens submitted for flow cytometric analysis are representative samples of the neoplastic disorder being characterized. In specimens in which no population of abnormal DNA content is detected, it is especially important to demonstrate that neoplastic cells are present in the sample run through the flow cytometer. This generally requires microscopic evaluation of the specimen by a qualified pathologist.

Evidence of Compliance:
✓ Records of specimen evaluation

FLO.31010 Cellular Debris **Phase II**

The laboratory accounts for cellular debris and aggregates.

NOTE: Cellular debris can affect measurements of S-phase fraction, and aggregates can alter ploidy assessments; these need to be excluded from consideration. DNA analysis software programs generally provide options for debris subtraction and doublet discrimination. Each laboratory must incorporate such methods into their procedures. Confirmation with