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

fluorescent microscopic examination of the stained nuclear suspension may provide additional documentation of cellular aggregates.

FLO.31020 DNA Content Linearity **Phase II**

Criteria are established for determining acceptable linearity for DNA content measurement using cells or particles of known relative fluorescence.

FLO.31050 Staining Methodology **Phase II**

The staining and analytical processes described in the procedure manual are based upon established methodology (reference cited).

NOTE: Many different variables need to be controlled to ensure proper stoichiometry of dye binding to DNA. Therefore, it is essential that procedures adopted by a laboratory are based on published work.

FLO.31100 Specimen Treatment **Phase II**

Specimen treatment with nucleic acid dye includes treatment with RNAse if the dye is not specific for DNA.

NOTE: Certain dyes used to stain fixed cells, (eg, ethidium and propidium iodide) bind to RNA. Prior treatment with RNAse eliminates artifactual broadening of the DNA content distributions that would result from fluorescence of complexes of the dye with RNA.

REFERENCES
1) Shapiro HA. Practical flow cytometry. New York, NY: Alan R. Liss, 1985

FLO.31150 Neoplasm DNA Analysis Criteria **Phase I**

The laboratory uses defined criteria for the type of neoplasms acceptable for DNA analysis.

NOTE: The laboratory must show evidence that it restricts analysis to those neoplasms for which the literature supports significant independent prognostic significance for DNA ploidy and/or S-phase analysis.

REFERENCES
1) DNA cytometry consensus conference. *Cytometry.* 1993;14:471-500
2) Henson D, *et al*. College of American Pathologists Conference XXVI on clinical relevance of prognostic markers in solid tumors. Summary. *Arch Pathol Lab Med.* 1995;119:1109-1112

FLO.31200 Histogram Acceptability Criteria **Phase II**

The laboratory uses defined criteria for acceptability of histograms for interpretation.

FLO.31300 Nucleic Acid-Specific Dye Concentration **Phase II**

The concentration of nucleic acid-specific dye has been determined to be a saturating concentration.

NOTE: Standard techniques use an excess concentration of fluorochrome since concentrations below saturation will make the cells appear hypoploid.

REFERENCES
1) Shapiro HA. Practical flow cytometry. New York, NY: Alan R. Liss, 1985