CANADIAN PNH NETWORK
Partners in Care for PNH

A GUIDE TO UNDERSTANDING THE RATIONALE FOR HOW THIS PNH FLOW CYTOMETRY REPORT WAS CREATED

This note is directed to the clinician, specifically to focus on bringing the patient's clinical presentation to the laboratory finding for a definitive PNH diagnosis. This statement correlates with the 6Ps patient presentation concept and allows the clinician to make the final diagnosis based on laboratory findings and patient clinical and symptomatic presentation.

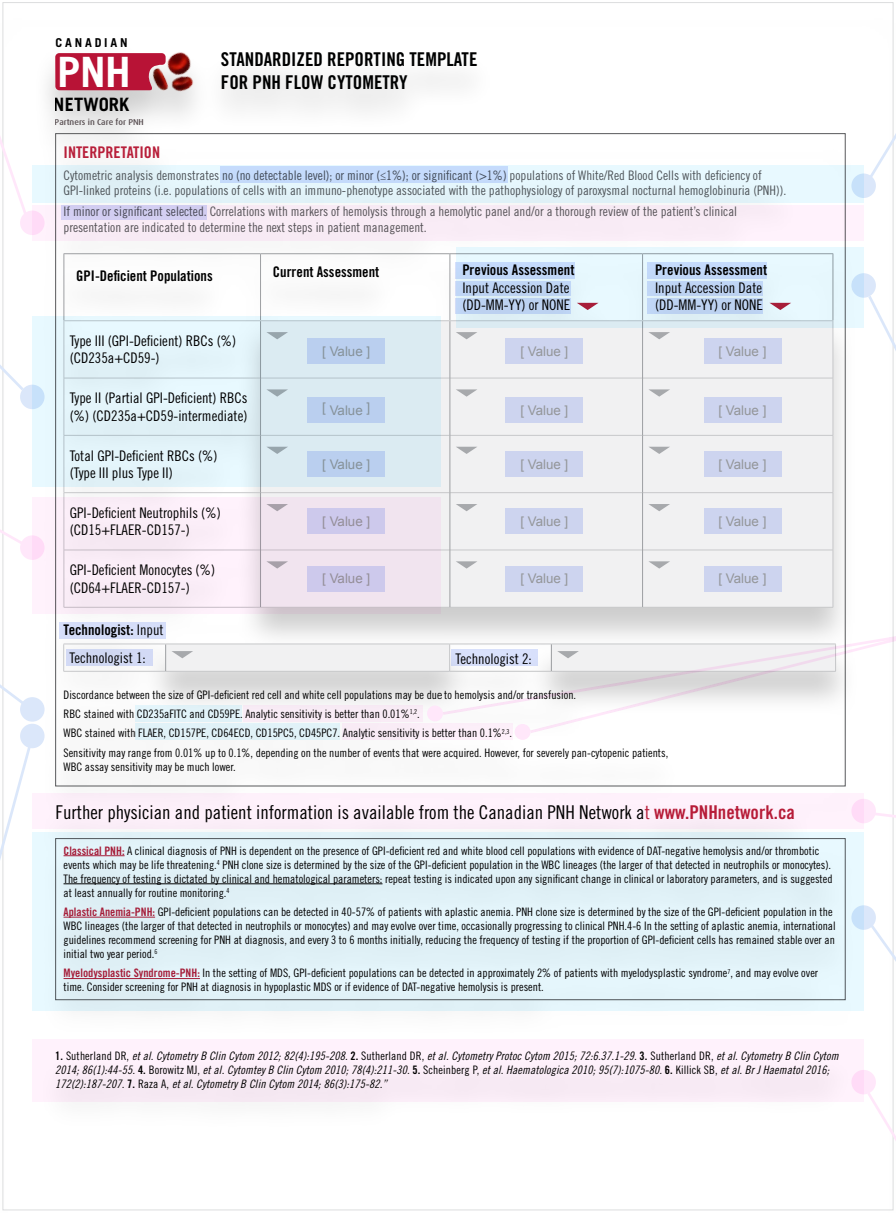
Type II (partial CD59 deficiency) and Type III (complete CD59 deficiency) PNH RBCs are reported, as well as the total (Type II + Type III). To avoid confusion, Type I cells (normal CD59 expression) are not reported. These results indicate PNH clones in both cell types.

Granulocytes/neutrophils and monocytes should both be evaluated and reported separately. These results indicate PNH clones in both cell types.

Preferred reagents for RBCs:
- CD235aFITC for gating
- CD59PE for GPI detection

Look for details of which reagents were used, to confirm that appropriate high-sensitivity methods were used. Two GPI-linked markers and one lineage-specific marker (for gating) should be used per lineage. Look for the following preferred reagents:

- For granulocytes (neutrophils):
  - CD15 for gating;
  - FLAER plus CD24 or CD157 for GPI detection
- For monocytes:
  - CD64 for gating;
  - FLAER plus CD14 or CD157 for GPI detection



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STANDARDIZED REPORTING TEMPLATE FOR PNH FLOW CYTOMETRY

INTERPRETATION

Cytometric analysis demonstrates no (no detectable level); or minor (≤1%); or significant (>1%) populations of White/Red Blood Cells with deficiency of GPI-linked proteins (i.e. populations of cells with an immuno-phenotype associated with the pathophysiology of paroxysmal nocturnal hemoglobinuria (PNH)).

If minor or significant selected: Correlations with markers of hemolysis through a hemolytic panel and/or a thorough review of the patient's clinical presentation are indicated to determine the next steps in patient management.

GPI-Deficient Populations	Current Assessment	Previous Assessment Input Accession Date (DD-MM-YY) or NONE	Previous Assessment Input Accession Date (DD-MM-YY) or NONE
Type III (GPI-Deficient) RBCs (%) (CD235a+CD59-)	[Value]	[Value]	[Value]
Type II (Partial GPI-Deficient) RBCs (%) (CD235a+CD59-intermediate)	[Value]	[Value]	[Value]
Total GPI-Deficient RBCs (%) (Type III plus Type II)	[Value]	[Value]	[Value]
GPI-Deficient Neutrophils (%) (CD15+FLAER-CD157-)	[Value]	[Value]	[Value]
GPI-Deficient Monocytes (%) (CD64+FLAER-CD157-)	[Value]	[Value]	[Value]

Technologist: Input

Technologist 1: Technologist 2:

Discordance between the size of GPI-deficient red cell and white cell populations may be due to hemolysis and/or transfusion.

RBC stained with CD235aFITC and CD59PE. Analytic sensitivity is better than 0.01%[1,2].

WBC stained with FLAER, CD157PE, CD64ECD, CD15PC5, CD45PC7. Analytic sensitivity is better than 0.1%[2,3].

Sensitivity may range from 0.01% up to 0.1%, depending on the number of events that were acquired. However, for severely pan-cytopenic patients, WBC assay sensitivity may be much lower.

Further physician and patient information is available from the Canadian PNH Network at www.PNHnetwork.ca

Classical PNH: A clinical diagnosis of PNH is dependent on the presence of GPI-deficient red and white blood cell populations with evidence of DAT-negative hemolysis and/or thrombotic events which may be life threatening.[4] PNH clone size is determined by the size of the GPI-deficient population in the WBC lineages (the larger of that detected in neutrophils or monocytes). The frequency of testing is dictated by clinical and hematological parameters: repeat testing is indicated upon any significant change in clinical or laboratory parameters, and is suggested at least annually for routine monitoring.[4]

Aplastic Anemia-PNH: GPI-deficient populations can be detected in 40-57% of patients with aplastic anemia. PNH clone size is determined by the size of the GPI-deficient population in the WBC lineages (the larger of that detected in neutrophils or monocytes) and may evolve over time, occasionally progressing to clinical PNH.4-6 In the setting of aplastic anemia, international guidelines recommend screening for PNH at diagnosis, and every 3 to 6 months initially, reducing the frequency of testing if the proportion of GPI-deficient cells has remained stable over an initial two year period.[5]

Myelodysplastic Syndrome-PNH: In the setting of MDS, GPI-deficient populations can be detected in approximately 2% of patients with myelodysplastic syndrome[7], and may evolve over time. Consider screening for PNH at diagnosis in hypoplastic MDS or if evidence of DAT-negative hemolysis is present.

1. Sutherland DR, *et al. Cytometry B Clin Cytom 2012; 82(4):195-208.* 2. Sutherland DR, *et al. Cytometry Protoc Cytom 2015; 72:6.37.1-29.* 3. Sutherland DR, *et al. Cytometry B Clin Cytom 2014; 86(1):44-55.* 4. Borowitz MJ, *et al. Cytomtey B Clin Cytom 2010; 78(4):211-30.* 5. Scheinberg P, *et al. Haematologica 2010; 95(7):1075-80.* 6. Killick SB, *et al. Br J Haematol 2016; 172(2):187-207.* 7. Raza A, *et al. Cytometry B Clin Cytom 2014; 86(3):175-82."*

Look for unambiguous language describing the result. "Deficiency of GPI-linked proteins" is appropriate wording because lab results should be combined with the clinical picture to determine or confirm the clinical diagnosis. Classification of GPI-deficient cells has clear classification and is adapted from: Davis BH, et al. CLSI H52-A2 Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. 2014.

Results - reporting results of current AND previous assessments in order to allow the clinician to see trends in change of clone size over time in a single view.

Assay sensitivity describes the lower limit of detection – how many GPI-deficient cells do there need to be for them to be detected, compared to background levels in normal samples? Sensitivity should be reported separately for WBCs and RBCs.

For any further clinical or lab-related questions, please contact the Canadian PNH Network.

Consensus statements on re-testing based on patient clinical presentation. These are included to help in highlighting appropriate testing and to minimize non-necessary and inappropriate re-testing.

Look to the supporting references if you need details of how the assays were developed and validated.