

High Sensitivity Flow Cytometry for Paroxysmal Nocturnal Hemoglobinuria

Background Information

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal stem cell disorder characterized by mutations in the PIGA gene leading to loss of cell surface proteins linked to glycosylphosphatidylinositol (GPI) anchors. Patients affected by PNH display complement mediated hemolysis, thrombosis and bone marrow failure though the clinical presentation is variable. The presence of a PNH clone occurs in classical hemolytic PNH, generally at levels above 1%; however, PNH clones may also be seen in other disorders such as aplastic anemia and myelodysplastic syndrome. Flow cytometric immunophenotypic analysis is the method of choice to detect populations of GPI anchor-deficient cells used in the diagnosis of PNH and to monitor patients with an established diagnosis.¹ PNH erythrocyte clones may be divided into those with partial loss of CD59 (PNH Type II Cells) or complete loss of CD59 (PNH Type III Cells).

Cleveland Clinic Laboratories now offers high sensitivity flow cytometry testing for PNH using whole blood. This procedure labels red blood cells (RBC) and white blood cells (WBC) for the detection of GPI-linked surface antigens using monoclonal antibodies and a fluorochrome labeled bacterial aerolysin (FLAER).² Through high sensitivity flow cytometry testing, as few as 0.01% PNH cells can be detected.¹

Clinical Indications

This test may be useful in the evaluation of patients with intravascular hemolysis, unexplained hemolysis, thrombosis with unusual features, or bone marrow failure.

Results and/or Interpretation

Results are reported as:

- Negative
- Low-level PNH clone positive (.01 – <1%), with percentage
- PNH clone positive (≥1%), with percentage

Limitations of the Assay

Results of PNH flow cytometry studies must be interpreted in the context of the clinical, laboratory and histologic findings.

Blood not collected in an EDTA tube or which has been improperly stored and handled prior to receipt cannot be processed.

Blood stability for PNH testing is 48 hours. Samples greater than 48 hours will be rejected.

Methodology

High sensitivity flow cytometry for PNH is performed in accordance with Clinical Cytometry Society recommendations for high sensitivity flow cytometry testing.¹

Cells are stained directly with FITC, PE, PE/Cy7, PerCP/Cy5.5, APC and APC/Cy7-labeled monoclonal antibodies to detect antigens of interest. Antibodies used include CD15, CD24, CD33, CD45, CD59 and glycoprotein A. Additionally, FLAER is employed. For erythrocytes, antibodies to glycoprotein A are used to specifically gate red cells and PNH clones are identified by lack of CD59 expression. PNH erythrocyte clones are divided into those with partial loss of CD59 (PNH Type II Cells) or complete loss of CD59 (PNH Type III Cells). For granulocytes, CD15 and CD33 are used to specifically gate granulocytes. PNH-type granulocytes are then identified by lack of expression of CD24 and lack of reactivity to FLAER.

References

1. Borowitz MJ, Craig FE, DiGiuseppe JA *et al.* Guidelines for the Diagnosis and Monitoring of Paroxysmal Nocturnal Hemoglobinuria and Related Disorders by Flow Cytometry. *Cytometry B Clin Cytom.* 2010; 78B:211-230.
2. Sutherland DR, Kuek N, Davidson J *et al.* Diagnosing PNH with FLAER and Multiparameter Flow Cytometry. *Cytometry B Clin Cytom.* 2007; 72B: 167-177.

Test Overview

Test Name	High Sensitivity Flow Cytometry for Paroxysmal Nocturnal Hemoglobinuria
Ordering Mnemonic	<i>PNHPNL</i>
Reference Range	Negative: No PNH clone detected
Specimen Requirements	Volume/Size: 4 mL; Type, Whole Blood; Tube/Container, EDTA (Lavender); Transport Temperature, Ambient or Collect refrigerated and transport refrigerated. Frozen unacceptable. <i>Note: Peripheral blood samples to be delivered to the flow cytometry lab within 24 hours of draw time. Samples greater than 48 hours old will be rejected. Do not draw on Fridays, weekends or holidays.</i>
Test Ordering Information	Clearly indicate specimen source on sample label
CPT Codes	88184, 88185, 88187

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