

Paroxysmal Nocturnal Hemoglobinuria Panel

FOR DIAGNOSIS AND MONITORING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

Test Highlights

- This panel has two separate flow cytometric assays that detect PNH in white blood cells (WBC) and red blood cells (RBC).
- The five-color PNH WBC and the two-color PNH RBC flow assay detect the loss of glycosyl phosphatidylinositol (GPI) anchoring proteins (GPI-AP), which are diagnostic of PNH.
- This panel (testing of WBCs and RBCs simultaneously) is recommended for initial diagnosis, but each assay is available individually. Determination of PNH clone size is best made by quantifying the percentage of GPI-AP-deficient WBCs, while the type of PNH cells (i.e., type I, type II and type III) is best determined by analyzing RBCs.

Disease Overview

- PNH is a rare acquired hemolytic disorder caused by a nonmalignant clonal expansion of one or more stem cell lines.
- Clinical presentation:
 - Chronic hemolysis and hemoglobinuria
 - Symptoms of anemia (fatigue, pallor, weakness)
 - Thrombophilia (occurs in up to 40 percent of patients)
 - Thromboses at unusual sites, particularly abdominal veins, including hepatic vein thrombosis (Budd-Chiari syndrome) may complicate PNH. Cerebral vein thrombosis may also complicate PNH.
 - Thrombosis is the leading cause of death in PNH.
- Bone marrow failure (PNH may complicate aplastic anemia).
- Other signs and symptoms may include dysphagia, abdominal pain, iron deficiency, jaundice, smooth muscle dysfunction, and male impotence.

Epidemiology

- Incidence is 1.3:1,000,000.
- Median age of onset is 40 years.
- Males and females are equally affected.

Pathophysiology

- Acquired mutation of the *PIGA* gene in one or more hematopoietic stem cells results in the deficiency or absence of GPI-anchored cell membrane proteins on progeny of the affected stem cell(s).
- RBC sensitivity to complement lysis occurs due to deficiency of GPI-anchored membrane proteins that regulate complement.
- Pathophysiology of bone marrow failure and thromboses is not known.
- Approximately 60 percent of patients with acquired aplastic anemia have detectable PNH cells.
- A subclinical PNH population in aplastic anemia or other bone marrow failure disorder may correlate with a positive immunotherapeutic response.

Indications for Ordering

- Unexplained hemoglobinuria
- Coombs-negative hemolytic anemia
- Unusual presentation of thrombosis (Budd-Chiari syndrome or cerebral thrombosis)
- Coexistent thrombosis and intravascular hemolysis or cytopenias
- Aplastic or hypoplastic anemia

Interpretation

- PNH cells ≥ 1 percent in WBCs and RBCs indicates PNH.
- RBC PNH cells ≥ 0.005 percent but less than 1 percent indicate subclinical PNH, often associated with bone marrow failure syndromes.
- Assay sensitivity is 0.1 percent for WBCs and 0.005 percent for RBCs; results below these limits are normal.

Limitations

- Very neutropenic samples may not have enough cells for accurate WBC analysis.
- Gross hemolysis or samples that lack CD15, CD33, or Glycophorin A expression will compromise accuracy.
- Recent RBC transfusions may compromise accuracy.

Methodology

- WBCs and RBCs are analyzed by flow cytometry.
- WBCs are stained using a panel of five markers. CD15 and CD33 expression are used as lineage-specific markers for granulocytes and monocytes, respectively. Reductions in CD14 on monocytes and CD24 expression on granulocytes, along with low FLAER binding, are used to identify PNH cells.
- RBCs are stained with Glycophorin A as a lineage-specific marker and CD59 to identify PNH cells. If the RBC PNH cells are ≥ 1 percent, the fraction of type II and type III cells is also reported.

Related Tests

- Paroxysmal Nocturnal Hemoglobinuria Profile—Red Blood Cells ([2004366](#))
- Paroxysmal Nocturnal Hemoglobinuria Profile—White Blood Cells ([2005003](#))

References

1. Borowitz MJ, et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. *Cytometry B Clin Cytom* 2010; 78: 211–30.

2. Madkaikar M, et al. Paroxysmal nocturnal haemoglobinuria: diagnostic tests, advantages, & limitations. *Eur J Haematol* 2009;83:503–11.
3. Parker CJ. Bone marrow failure syndromes: paroxysmal nocturnal hemoglobinuria. *Hematol Oncol Clin North Am* 2009; 23:333–46.
4. Brodsky RA. Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. *Blood Rev* 2008; 22:65–74.

Test Information

2005006 Paroxysmal Nocturnal Hemoglobinuria Panel

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.