Paroxysmal Nocturnal Hemoglobinuria Testing

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hemolytic disorder caused by nonmalignant clonal expansion of one or more stem cell lines due to an acquired mutation in the *PIGA* gene. PNH is associated with intravascular hemolysis, thrombotic complications, and bone marrow failure.[1] For information about the testing strategy for PNH, refer to the ARUP Consult Paroxysmal Nocturnal Hemoglobinuria - PNH topic.

Featured ARUP Testing

Paroxysmal Nocturnal Hemoglobinuria (PNH), High Sensitivity, RBC and WBC 2005006

Method: Quantitative Flow Cytometry

- Preferred test for initial diagnosis of PNH and quantification of PNH clones
- Use to diagnose PNH in patients with unexplained hemoglobinuria, Coombs-negative hemolytic anemia, unusual thrombotic sites (eg, Budd-Chiari, cerebral), thrombosis combined with intravascular hemolysis or cytopenias, or aplastic or hypoplastic anemia
- Use to monitor individuals with confirmed PNH
- Includes high-sensitivity WBC and RBC analysis
- If >1% PNH RBCs are detected, then PNH RBC type will be added to report Type II and Type III PNH RBC's.

For additional test options for paroxysmal nocturnal hemoglobinuria, refer to the Laboratory Test Directory.

Disease Overview

Incidence

1.3/million[1]

Symptoms

- Hemolysis[1]
  - Symptoms include dysphagia, lethargy, renal failure, anemia, hemoglobinuria, male impotence, pulmonary hypertension
- Thrombophilia[1]
  - Potentially life-threatening
  - Thromboses located at unusual sites (eg, hepatic portal)
- Bone marrow (BM) failure[1]
  - May present as severe aplastic anemia

Physiology

- PNH is caused by a somatic mutation of *PIGA* gene which results in deficiency or absence of glycosylphosphatidylinositol (GPI)-anchored cell membrane proteins on progeny of affected stem cells[1]
  - Lack of CD55 and CD59 causes RBC sensitivity to complement lysis
  - Pathophysiology of thrombophilia and bone marrow failure in PNH is unknown
- Percentage of RBCs or WBCs that entirely or partially lack GPI-linked antigens is referred to as PNH clone size[1,3]
  - WBC testing is most accurate in the determination of PNH clone size
  - RBC testing is most appropriate for detection of cells only partially lacking GPI-linked antigens
    - Type I: normal levels of CD59
    - Type II: reduced levels of CD59
    - Type III: absent levels of CD59

Test Interpretation

Analytic Sensitivity

Limits of detection:

- RBCs: 0.008%
- Polymorphonuclear neutrophils (PMNs or granulocytes): 0.008%
- Monocytes: 0.2%

Limitations of quantification:

- RBCs: 0.02%
- Polymorphonuclear neutrophils (PMNs or granulocytes): 0.02%
- Monocytes: 0.5%

Results

Results	Cells Detected	Interpretation
Positive	% PNH cells: detected, ≥1% in RBCs and WBCs (PMN and monocytes)	Indicates PNH
	% PNH cells: minor population of PNH cells • % PNH RBC: 0.1% to 1% • % PNH PMN: 0.1% to 1% • % PNH monocytes: 0.5% to 1%	Indicates subclinical PNH Often associated with symptoms of bone marrow failure
	% PNH cells: rare cells with PNH phenotype • % PNH RBC: 0.02% to 0.1% • % PNH PMN: 0.02% to 0.1%	
	% PNH cells: rare cells with PNH phenotype detected below limit of quantification • % PNH RBC: 0.008% to 0.02% • % PNH PMN: 0.008% to 0.02% • % PNH monocytes: 0.2% to 0.5%	
Negative	% PNH cells: not detected • % PNH RBC: <0.008% • % PNH PMN: <0.008% • % PNH monocytes: <0.2%	Reduces, but does not eliminate the probability of PNH

Limitations

- Conditions that may compromise accuracy include significant neutropenia, gross hemolysis, and specimens that lack expression of CD15, CD64, or glycophorin A.
- WBC assay sensitivity is much lower for patients with severe pancytopenia.
- Recent RBC transfusions may decrease percentage of PNH cells measured in RBCs.

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;78(4):211-230.

2. Illingworth A, Marinov I, Sutherland DR, et al. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 3 - data analysis, reporting and case studies. *Cytometry B Clin Cytom*. 2018;94(1):49-66.

3. Sutherland DR, Acton E, Keeney M, et al. Use of CD157 in FLAER-based assays for high-sensitivity PNH granulocyte and PNH monocyte detection. *Cytometry B Clin Cytom*. 2014;86(1):44-55.

Related Information

Paroxysmal Nocturnal Hemoglobinuria - PNH

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