

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

BACKGROUND:

PNH is an acquired stem cell disorder that affects all hematopoietic lineages.^{1,2} Clinical manifestations include intravascular hemolysis, venous thrombosis, and diminished hematopoiesis. A transition to aplastic anemia, myelodysplasia (MDS) or leukemia occurs in some patients.

A somatic mutation in the PIG-A gene of a hematopoietic precursor clone leads to a defect in the synthesis of the glycosylphosphatidylinositol (GPI) anchor, through which a number of proteins are attached to the cell membrane³. GPI anchored proteins are deficient or completely missing rendering PNH blood cells exquisitely sensitive to hemolysis by complement. Among the proteins affected is the complement regulatory protein CD59, for which testing on erythrocytes is commonly used to diagnose the syndrome.

Sensitive flow cytometric assays that measure proteins tethered by GPI anchors to the cell membrane are the laboratory methods of choice for detection of PNH cells.⁴⁻⁶ Measuring CD59 on erythrocytes allows an estimate of the proportion of PNH I (normal), PNH II (partially deficient) and PNH III (completely deficient) erythrocytes, reflecting the proportion of abnormal hematopoietic bone marrow cells.

Testing for the presence of PNH granulocytes is performed using fluorescent aerolysin (FLAER).⁵⁻⁷ Aerolysin is a bacterial protein that directly binds the GPI anchor and thus can detect all GPI-anchored proteins on the cell. Granulocytes that are missing GPI anchors (FLAER negative) are characterized as PNH granulocytes. Testing of granulocytes with FLAER gives a better estimate of clone size and is more sensitive than detection of CD59 on erythrocytes.^{6,8} Large PNH granulocyte clones may be predictive of thrombosis.^{6,8}

REASONS FOR REFERRAL:

- Evaluation of patients with hemolysis not attributable to other causes such as red cell-specific autoantibodies, hereditary spherocytosis, or drug sensitivity.
- Occasionally useful in the diagnosis of unexplained anemia in the absence of overt hemolysis or bone marrow abnormalities. (Rule out more common causes of anemia such as blood loss and iron or vitamin deficiency before testing.)

METHOD: Flow cytometry

- Granulocytes: The percentage of GPI+ cells is determined on CD15+ leukocytes measured by cell-bound FLAER.
- Erythrocytes: The level of cell-surface CD59 on patient erythrocytes is determined using a specific monoclonal antibody.

LIMITATIONS:

Positive results may be seen in patients with anemia secondary to bone marrow aplasia or erythrodysplasia, even in the absence of clinically significant hemolysis. Accuracy of erythrocyte PNH clone size determination may be adversely affected for patients who have received a red cell transfusion within 1 month of sample collection.

REFERENCE RANGE:

- Granulocytes: < 0.5% abnormal (FLAER negative) cells
- Erythrocytes: < 2% abnormal (CD59 intermediate and CD59 negative) cells.

SPECIMEN REQUIREMENTS:

- Granulocytes only, or granulocytes and erythrocytes: 5 ml whole blood collected in a CytoChex® tube (speckled black/lavender top). Call Client Services at (800) 245-3117, ext. 6250 to request a blood collection kit containing CytoChex® tubes. Blood for granulocyte testing is accepted Monday through Friday only and must be received within 2 days of blood collection date.
- Erythrocytes only: 5 mL EDTA (lavender top) whole blood.

SHIPPING REQUIREMENTS:

- CytoChex® (speckled black/lavender top) tubes: Ship at room temperature. Place specimen and requisition into sample collection kit supplied and ship by FedEx priority overnight. *Ship samples Monday through Thursday. Do not ship the day before a holiday.*
- EDTA (lavender top) tubes: Ship refrigerated. Protect whole blood from freezing by wrapping in paper toweling. Place the specimen and the test requisition form into plastic bags and seal. Insert into a Styrofoam container; place into a sturdy cardboard box, tape securely and ship by an overnight carrier. Ship the package in compliance with your overnight carrier guidelines.

Ship to: Client Services/Platelet and Neutrophil Immunology Laboratory
BloodCenter of Wisconsin
638 N.18th St.
Milwaukee, WI 53233
800-245-3117, ext. 6250

TURNAROUND TIME: 2-4 days

CPT CODES:

- Paroxysmal Nocturnal Hemoglobinuria - Granulocytes 88184
- Paroxysmal Nocturnal Hemoglobinuria - Erythrocytes 88184

TEST FREQUENCY:

- Granulocytes: performed as needed, Monday through Friday
- Erythrocytes: performed 2 days per week.

REFERENCES:

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4. Hall SE, Rosse WF (1996) Blood 87:5332-5340
5. Brodsky RA, et al. Am J Clin Path 2000;114:459-466
6. Moyo VM, Mukina GL, Barrett ES, Brodsky RA. Br J Haematol. 2004;126:133-138
7. Sutherland R, et al. Clinical Cytometry. 2007: 72B:167-177
8. Hall C, Richards S, Hillmen P. Blood 2003;102:3587-3591

December 2009