



TECHNICAL NOTICE

SOUTH BEND MEDICAL FOUNDATION

March 2010

Paroxysmal Nocturnal Hemoglobinuria (PNH)

Effective Date: April 1, 2010

Performing Department: Flow Cytometry

Method: Flow cytometric evaluation of hematologic cells provides a rapid, sensitive and specific test for identification of PNH cell clones. This test replaces the outdated Ham's Acidified Serum Test which was not sensitive for PNH. In the flow cytometry procedure two at least glycosyl phosphatidylinositol (GPI)-linked antigens are assessed to confirm dual negativity and the cell clone can be correlated with the presence or absence of hemolysis.

A PNH clone size of more than 3% for RBCs and/or more than 10% for granulocytes indicates classic PNH.

Use: Screening for or confirming a diagnosis of PNH. Evaluation of patients with hemolytic anemia or pancytopenia of undetermined cause.

Clinical Significance: Paroxysmal Nocturnal Hemoglobinuria (PNH) is an uncommon acquired hematologic disorder characterized by nocturnal hemoglobinuria, chronic hemolytic anemia with acute episodes, pancytopenia, thrombosis and, in some patients, acute or chronic myeloid malignancies. It is a disorder of hematopoietic stem cells and affects erythroid, myeloid and megakaryocytic cell lines. It is caused by mutations in the phosphatidylinositol glycan A (PIG-A) gene of the stem cell. This results in disruption of the synthesis of GPI and a deficiency in all GPI-anchored proteins in the affected cells. A consequential absence of the GPI linked complement inhibitors CD55 and CD59 causes the blood cells to be susceptible to complement mediated hemolysis.

Reference Range: A PNH clone size of more than 3% for RBCs and/or more than 10% for granulocytes indicates classic PNH, whereas smaller cell clone sizes suggest hypoplastic PNH. Normal individuals (without PNH) have normal expression of all GPI-linked antigens on peripheral blood erythrocytes, granulocytes and monocytes.

SPECIMEN REQUIREMENTS AND COLLECTION:

Specimen Type: Whole Blood

Container: One lavender (EDTA) tube

Preferred Volume: 5.0 mL

Minimum Volume: 2.5 mL

Collection: Routine Venipuncture

Processing: Do not centrifuge • Do not remove Plasma from cells

Stability:

- Erythrocyte: Ambient: 3 days; Refrigerated: 4 days; Frozen: Unacceptable.
- Granulocyte: Ambient: 24 hours; Refrigerated: 2 days; Frozen: Unacceptable.
- **NOTE:** Testing for the absence of GPI- linked antigens on both red cells and granulocytes is recommended for the diagnosis of PNH. However, only erythrocyte markers will be performed and reported if the sample is received more than 48 hours from collection. In that event, a second sample will be requested for granulocyte analysis.

Storage/ Transport: Two days at room temperature (20-30°C) • Frozen (-20°C) is Unacceptable.

Testing Schedule: Collect Monday through Thursday only and not the day before a holiday.

Order: Paroxysmal Nocturnal Hemoglobinuria (PNH)....Test #: 36161.....CPT: 88184, 88185x3, 88187

Please direct any questions, or comments regarding this notice to William Kaliney, M.D. (wkaliney@sbfm.org), Deborah H. Sun, Ph.D. (dsun@sbfm.org), or Sally Cornwall (scornwall@sbfm.org) or call South Bend Medical Foundation, (574) 234-4176 or (800) 544-0925.

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