Paroxysmal Nocturnal Hemoglobinuria (PNH)

Versiti offers flow cytometric evaluation of erythrocytes and leukocytes in evaluation for paroxysmal nocturnal hemoglobinuria (PNH).

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 membrane.[3] 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.

High sensitivity 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] High sensitivity flow cytometry assays are particularly useful for detecting the small PNH populations associated with bone marrow failure disorders like aplastic anemia and myelodysplastic syndrome.[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 leukocytes (granulocytes and monocytes) is performed using fluorescent aerolysin (FLAER).[4-6] 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.[4,7] Large PNH granulocyte clones may be predictive of thrombosis.[4,7] Testing monocytes along with granulocytes offers an additional level of confidence when testing for a PNH clone using leukocytes.

Indications for testing:

- 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).
- Unprovoked venous thrombosis, particularly in abdominal or cerebral circulation or in the presence of hemolysis.

Test method:

Immunophenotyping by Flow Cytometry

- Erythrocytes: The level of cell-surface CD59 on patient erythrocytes is determined on CD235a+ erythrocytes using a specific monoclonal antibody.
- Granulocytes: The percentage of GPI-deficient cells is determined on CD45+/CD15+ leukocytes measured by cell-bound FLAER and CD24.
- Monocytes: The percentage of GPI-deficient cells is determined on CD45+/CD33+ leukocytes measured by cell-bound FLAER and CD14. Monocyte testing is only performed and reported in support of findings for granulocytes.

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Assay sensitivity and 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, and in those experiencing hemolysis.

Reporting of results:

- Erythrocytes: < 0.01% abnormal (CD59 intermediate plus CD59 negative) cells
- Granulocytes: < 0.01% abnormal (FLAER negative) cells
- Monocytes: NA

Specimen requirements:

Leukocytes only, or leukocytes and erythrocytes: 5 ml whole blood collected in EDTA whole blood (lavender top). Minimum volume of 2 ml.

SHIP

Shipping requirements:

Samples must be received within 2 days from the time of blood collection. Protect whole blood from freezing by wrapping in paper toweling. Ship refrigerated. 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 in compliance with your overnight carrier guidelines, Monday - Thursday.

Send to:

Versiti Client Services
Platelet and Neutrophil Immunology Laboratory
638 N. 18th St.
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

Please complete all pages of the requisition form.

CPT Codes/Billing/Turnaround time:

Test code: 5549 (Leukocytes), 5550 (Erythrocytes and Leukocytes)

CPT code: For recommended CPT codes, visit the versiti.org/test-catalog

Turnaround time: 2-4 days

References:

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4. Borowitz MJ. et al. Clinical Cytometry. 2010. 78B: 211-230.
5. Moyo VM, Mukina GL, Barrett ES, Brodsky RA. Br J Haematol. 2004;126:133-138
6. Sutherland R, et al. Clinical Cytometry. 2007: 72B:167-177
7. Hall C, Richards S, Hillmen P. Blood 2003;102:3587-3591

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