Test Definition: PLINK

PNH, PI-Linked AG, B

Overview

Useful For

Screening for and confirming the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH)

Monitoring patients with PNH

Additional Tests

Test ID	Reporting Name	Available Separately	Always Performed
FCIMS	Flow Cytometry Interp, 9-15 Markers	No, (Bill Only)	Yes

Method Name

Immunophenotyping

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Specimen Required

Specimen must arrive within72 hours of draw.

Container/Tube:

Preferred: Yellow top (ACD)

Acceptable: Lavender top (EDTA)

Specimen Volume:2.6 mL

Collection Instructions: Send specimen in original tube. Do **not** transfer blood to other containers.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-Hematopathology/Cytogenetics Test Request Form (T726)

-Benign Hematology Test Request Form (T755)

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	72 hours	
	Refrigerated	72 hours	

Clinical and Interpretive

Clinical Information

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematologic disorder characterized by nocturnal hemoglobinuria, chronic hemolytic anemia, thrombosis, pancytopenia, and, in some patients, acute or chronic myeloid malignancies.

PNH appears to be a hematopoietic stem cell disorder that affects erythroid, granulocytic, and megakaryocytic cell lines. The abnormal cells in PNH have been shown to lack glycosylphosphatidylinositol (GPI)-linked proteins in erythroid, granulocytic, megakaryocytic, and, in some instances, lymphoid cells. Variants in the phosphatidylinositol glycan A gene, *PIGA*, have been identified consistently in patients with PNH, thus confirming the biological defect in this disorder.

A flow cytometric-based assay can detect the presence or absence of these GPI-linked proteins in granulocytes, monocytes, erythrocytes, and lymphocytes, thus avoiding the problems associated with red blood cell (RBC)-based diagnostic methods (Ham test) in which recent hemolytic episodes or recent transfusions can give false-negative results. A partial list of known GPI-linked proteins include CD14, CD16, CD24, CD55, CD56, CD58, CD59, C8-binding protein, alkaline phosphatase, acetylcholine esterase, and a variety of high frequency human blood antigens. In addition, fluorescent aerolysin (FLAER) binds directly to the GPI anchor and can be used to evaluate the expression of the GPI linkage.

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In-house studies, as well as others in the literature, have shown that flow cytometry-based assays will detect all Ham-positive PNH cases, as well as some Ham-negative PNH cases. This assay replaces the sugar water test and the Ham test for the evaluation of patients with possible PNH.

Patients with PNH should be transfused with ABO-specific RBCs, which do not need to be washed. If, for some reason, they need to receive non-ABO type-specific (type O) cells, these RBC units should be washed. Since recipient antibodies to granulocyte antigens can trigger hemolytic episodes in PNH, if they have such antibodies these patients should receive leukoreduced RBCs and platelets.

Reference Values

An interpretive report will be provided.

RED BLOOD CELLS:

PNH RBC-Partial Antigen loss: 0.00-0.99%

PNH RBC-Complete Antigen loss: 0.00-0.01%

PNH Granulocytes: 0.00-0.01%

PNH Monocytes: 0.00-0.05%

Interpretation

Individuals with paroxysmal nocturnal hemoglobinuria (PNH) have absent or decreased expression of all the glycosylphosphatidylinositol (GPI)-linked antigens and fluorescent aerolysin (FLAER) on peripheral blood cells derived from the PNH clone.

Recent data showed that small PNH clones can be detected in a relatively high percentage of cases of aplastic anemia and myelodysplastic syndrome. While the significance of this finding is still uncertain, it appears that these patients may benefit from immunosuppressive therapy.

This test incorporates a sophisticated technique of separating different cell populations using gating on antigen-positive cells, as well as the sensitivity to enable detection of small PNH clones. In addition, this test detects a partial loss of CD59 on RBCs (type II RBC). Patients with large proportion of type II RBC are unlikely to show high levels of hemolysis, unlike patients with complete loss of GPI-linked proteins (predominantly type III cells). While PNH is a disorder of hematopoietic stem cells and all lineages are affected, the percentage of affected cells can differ between lineages, most commonly due to RBC hemolysis and/or transfusion.

Individuals without PNH have normal expression of FLAER (neutrophils and monocytes) and normal expression of all GPI-linked antigens-CD14 (monocytes), CD16 (neutrophils and NK cells), CD24 (neutrophils), and CD59 (RBCs).

Cautions

The sugar water test and the Ham test are no longer recommended for the evaluation of patients with possible paroxysmal nocturnal hemoglobinuria.

Recent transfusion can decrease the sensitivity of this test and interfere with accuracy.

Clinical Reference

1. Miyata T, Yamada N, Iida Y, et al: Abnormalities of PIG-A transcripts in granulocytes from patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1994;330:249-255

2. Brodsky RA: Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. Blood Rev. 2008 Mar;22(2):65-74

3. Richards SJ, Barnett D: The role of flow cytometry in the diagnosis of paroxysmal nocturnal hemoglobinuria in the clinical laboratory. Clin Lab Med. 2007 Sep;27(3):577-590

4. Parker C, Omine M, Richards S, et al: Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005 Dec 1;106(12):3699-3709

5. Richards SJ, Hill A, Hillman P: Recent advances in the diagnosis, monitoring and management of patients with paroxysmal nocturnal hemoglobinuria. Cytometry B Clin Cytom. 2007 Sep;72(5):291-298

6. Shichishima T, Terasawa T, Hashimoto C, et al: Discordant and heterogeneous expression of GPI-anchored

membrane proteins on leukemic cells in a patient with paroxysmal nocturnal hemoglobinuria. Blood. 1993;81:1855-1862

7. Brodsky RA, Mukhina GL, Li S, et al: Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. Am J Clin Pathol. 2000;114:459-466

8. Rosse WF: Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. Blood. 1990;75:1595-1601

9. 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

10. van der Schoot CE, Huizinga TW, van't Veer-Korthof ET, Wijmans R, Pinkster J, von dem Born AEe: Deficiency of glycosyl-phosphatidylinositol-linked membrane glycoproteins of leukocytes in paroxysmal nocturnal hemoglobinuria, description of a new diagnostic cytofluorometric assay. Blood. 1990;76:1853-1859

Performance

Method Description

Flow cytometric immunophenotyping of peripheral blood (WBC and RBC) is performed using the following antibodies;

RBC: CD235a, CD59Â

WBC: CD14, CD15, CD16, CD24, CD33, CD45, and FLAERÂ

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This assay evaluates the presence or absence of glycosylphosphatidylinositol (GPI)-linked proteins using monoclonal antibodies directed against CD235, CD33, and CD15 to isolate different cell lineages. GPI-linked proteins that are checked within different lineages include CD14 for monocytes, CD's 16 and 24 for granulocytes, and CD59 for RBCs. Fluorescent aerolysin, a fluorescently labeled inactive variant of the protein aerolysin binds selectively to GPI anchors and is also evaluated for presence or absence of expression on WBCs. In addition, this test will detect a partial loss of CD59 on RBCs (type II RBCs).

Individuals without paroxysmal nocturnal hemoglobinuria have normal expression of all GPI-linked antigens on peripheral blood and leukocytes and erythrocytes.(Richards SJ, Hill A, Hillman P: Recent advances in the diagnosis, monitoring and management of patients with paroxysmal nocturnal hemoglobinuria. Cytometry B Clin Cytom 2007 Sep;72[5]:291-298)

PDF Report

No

Day(s) and Time(s) Test Performed

Specimens are processed Monday through Sunday.

Results reported Monday through Friday.

Analytic Time

1 day

Maximum Laboratory Time

2 days

Specimen Retention Time

14 days-any remaining

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

88184-Flow cytometry, RBC x 1

88184-Flow cytometry, WBC x 1

88185-Flow cytometry, additional marker (each), RBC x 1

88185-Flow cytometry, additional marker (each), WBC x 6

88188-Flow Cytometry Interpretation, 9-15 Markers x 1

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
PLINK	PNH, PI-Linked AG, B	90735-2

Result ID	Test Result Name	Result LOINC Value
CK079	Interpretation	90739-4
CK080	PNH RBC-Partial Ag Loss	33662-8
CK081	PNH RBC-Complete Ag Loss	90738-6
CK082	PNH Granulocytes	90737-8
CK083	PNH Monocytes	90736-0