Overview

Useful For
Screening for and confirming the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH)
Monitoring patients with PNH

Additional Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCIMS</td>
<td>Flow Cytometry Interp, 9-15 Markers</td>
<td>No, (Bill Only)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Method Name
Immunophenotyping

NY State Available
Yes

Specimen

Specimen Type
Whole blood

Specimen Required
Specimen must arrive within 72 hours of draw.

Container/Tube:
Preferred: 2.6-mL Yellow top (ACD)
Acceptable: 7-mL ACD or lavender top (EDTA)

Specimen Volume: 2.6 mL

Collection Instructions: 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
Test Definition: PLINK
PNH, PI-Linked AG, B

Reject Due To

| Gross hemolysis | Reject |

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Ambient (preferred)</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>Refrigerated</td>
<td>72 hours</td>
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</table>

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. Mutations 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 cell-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.

Our 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**


Performance

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

RBC: CD235a, CD59

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

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, CD16 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 and 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

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<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tr>
<td>PLINK</td>
<td>PNH, PI-Linked AG, B</td>
<td>90735-2</td>
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<th>Test Result Name</th>
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<td>CK079</td>
<td>Interpretation</td>
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<td>CK080</td>
<td>PNH RBC-Partial Ag Loss</td>
<td>33662-8</td>
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<tr>
<td>CK081</td>
<td>PNH RBC-Complete Ag Loss</td>
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<tr>
<td>CK082</td>
<td>PNH Granulocytes</td>
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<tr>
<td>CK083</td>
<td>PNH Monocytes</td>
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