

Identifying Paroxysmal Nocturnal Hemoglobinuria With Flow Cytometry

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA OVERVIEW

PNH is a rare hematologic disorder with variable clinical presentation^{1,2}



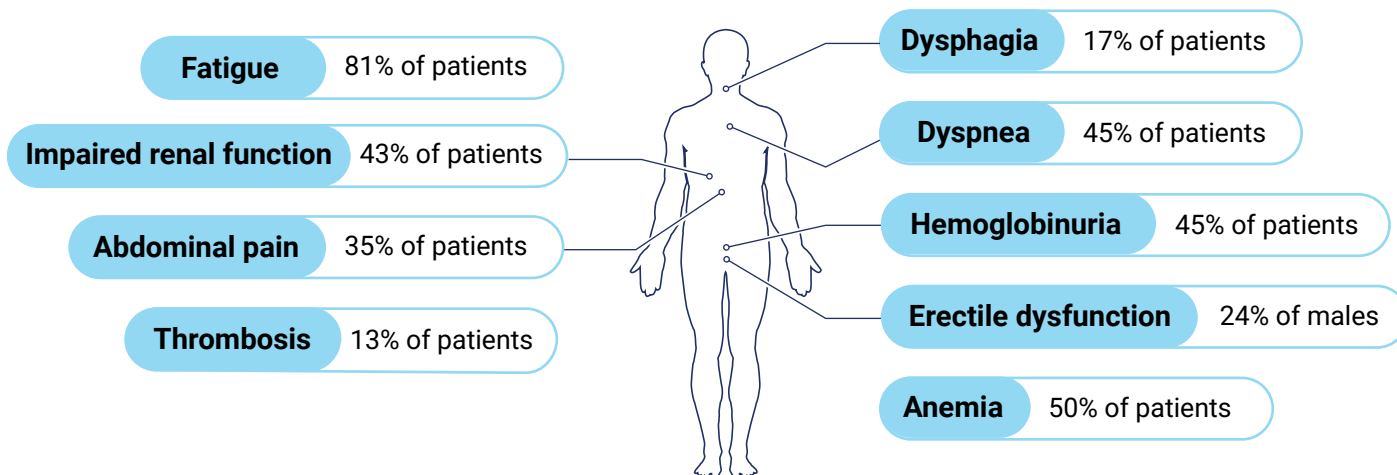
The estimated global prevalence of PNH is

10 to 20
per million¹

The estimated global incidence of PNH is

≈1 to 2
per million¹

Common signs and symptoms for PNH include²:



Diversity of symptoms and patient presentation contributes to diagnostic delays^{3,4}



In one study, which surveyed 163 patients with PNH^{4,5}:

<40%

of patients were diagnosed within 12 months of symptom onset

≈38%

of patients saw ≥5 HCPs prior to diagnosis

24%

of patients were diagnosed after >5 years

IMPORTANCE OF A TIMELY DIAGNOSIS



Patients with PNH, before diagnosis, can experience an extensive history of complications including²:

- **61%** history of RBC transfusions²
- **19%** thrombosis and other major vascular events²

A CORRECT DIAGNOSIS IS FUNDAMENTAL TO MAKING APPROPRIATE TREATMENT DECISIONS IN PNH



Many hematologic diseases have similar symptoms but vastly different treatments, making an accurate and timely diagnosis essential^{3,6,7}

- PNH symptoms often overlap those of AA and myelodysplastic syndrome, as they can be associated with each other
- PNH is not mutually exclusive with BMF disorders

PNH can affect both males and females equally, with a median age of 36 years at disease onset^{1,2}

PNH is a rare hematologic disorder with variable signs and symptoms^{1,2}

Category	Observed hemolysis	Treatment strategy	Typical clone size*
Classic PNH	High	PNH-specific therapy	Large (>50%)
PNH in the setting of another specified bone marrow disorder ^a	Low	Treat underlying disease; patients with large clones may benefit from complement inhibitors	Variable (usually relatively small, <50%)
Subclinical PNH	Absent	Treat underlying disease (MDS, AA)	Small (<10%)



Biomarker testing is a fundamental component of the diagnostic workup for hematologic diseases^{3,6-8}

PNH IS A RARE BUT TREATABLE DISEASE

Make Sure Your Patients Don't Wait Years for a Diagnosis

For all your patients, remember to^{1,2,b}:

- Coombs-negative hemolytic anemia
- Hemoglobinuria
- Erectile dysfunction
- Cytopenias
- Kidney disease
- Thrombosis at unusual sites
- Anemia
- Tiredness

If your patient has some or all of these symptoms, consider biomarker testing for PNH with flow cytometry

*PMN clone size.

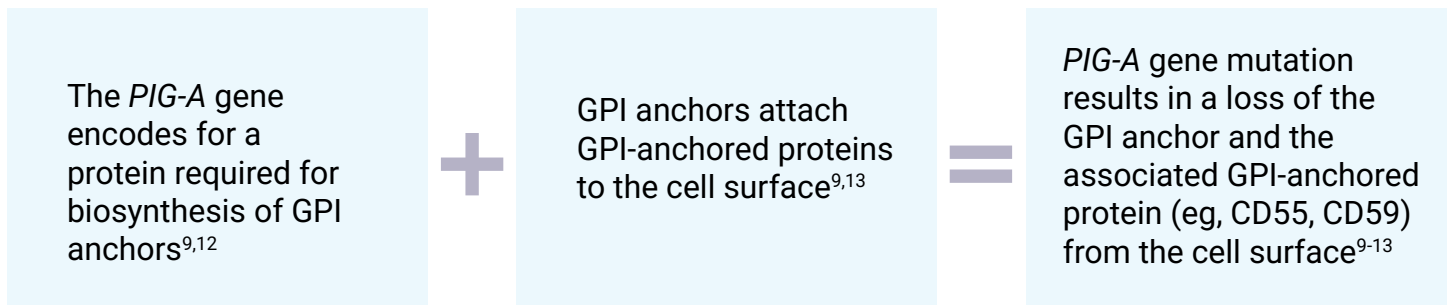
^aAA or low-risk myelodysplastic syndrome.


^bThis list does not include all possible symptoms associated with PNH.

AA, aplastic anemia; BMF, bone marrow failure; MDS, myelodysplastic syndrome.

FLOW CYTOMETRY ASSAYS CAN DETECT THE ABSENCE OF GPI-ANCHORED PROTEINS, INCLUDING CD55 AND CD59

PNH is caused by acquired *PIG-A* gene mutations, resulting in the loss of GPI-anchored complement regulatory proteins⁹⁻¹¹

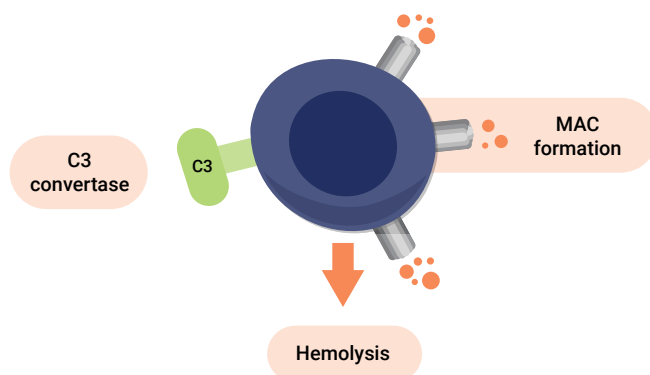
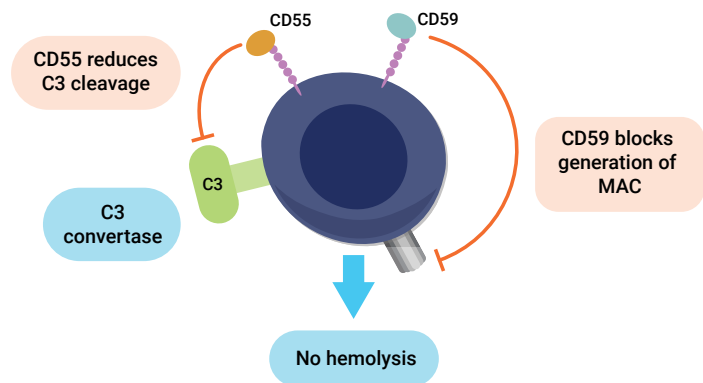


 Because the *PIG-A* gene mutation occurs in a hematopoietic stem cell, blood cells derived from that mutant stem cell also lack GPI-anchored proteins^{9,10,14}

 Presence of a *PIG-A* gene mutation does not confirm PNH; high-sensitivity flow cytometry is the gold standard test for a definitive diagnosis of PNH^{3,9}

Without PNH

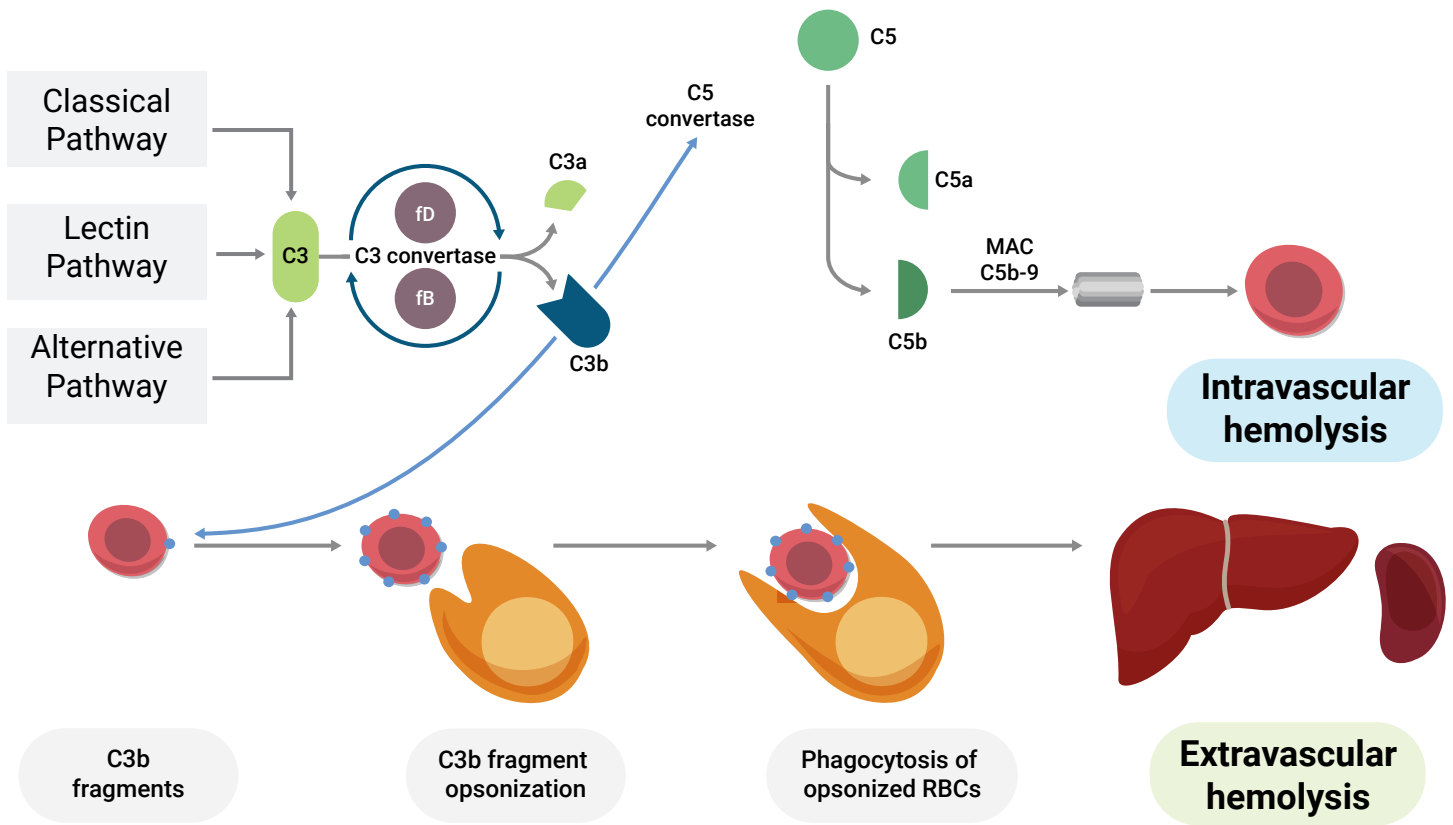
In normal RBCs, the GPI-anchored complement regulatory proteins CD55 and CD59 protect the RBC from complement-mediated hemolysis^{9,15-18}



With PNH

In PNH, the acquired *PIG-A* gene mutation leads to a lack of CD55 and CD59 on the RBC surface, exposing the RBCs to complement-mediated hemolysis^{9-11,15-18}

Complement-mediated hemolysis occurs via 2 mechanisms^{9,19,20}



IVH^{9,19}

PNH RBCs are destroyed within the blood vessels by the formation of the MAC

EVH^{9,19,20}

Interaction between the proximal pathway proteins such as C3b, fB, and fD drives EVH

Once suspected based on clinical and laboratory evaluation, PNH can be definitively diagnosed using flow cytometry²¹

DIAGNOSING PNH WITH FLOW CYTOMETRY



PNH should be diagnosed based on the absence or severe deficiency of GPI-anchored proteins on ≥ 2 peripheral blood cell lineages^{1,9,22}



Flow cytometry estimates the PNH clone size—the percentage of cells that lack GPI-anchored proteins^{22,23}

- PNH clone size at diagnosis positively correlates with symptom burden^{24,25}
- Patients with classic PNH typically have clone sizes of $>50\%$; patients with PNH with other BMF disorder, $<50\%$; and patients with subclinical PNH, $<10\%$ ²¹



Proteins that can be assayed include^{1,23}:

- GPI-anchored proteins (CD59, CD55, CD14, and CD24)
- Cell lineage markers (CD15, CD16, CD45, and CD64)



Both RBCs and WBCs should be analyzed^{21,22}

- WBC analysis (eg, FLAER) more accurately determines clone size
- Hemolysis and transfusion can lead to underestimation with RBC analysis alone
- RBC analysis is necessary to determine the percentage of type I (normal expression of GPI-anchored proteins), type II (partial deficiency), and type III (complete deficiency) RBCs



Depending on the markers assayed, flow cytometry for PNH can provide standard sensitivity (SS) or high sensitivity (HS)^{3,22,23}

- SS assays are adequate for diagnosis of classic PNH but may miss patients with clone sizes $<4\%$
- HS panels include ≥ 2 GPI-linked antibodies per lineage, one of which must be FLAER for WBC analysis and CD59 for RBC analysis

Consider testing for PNH in patients with BMF disorders and persistent and unexplained cytopenia^{8,26}

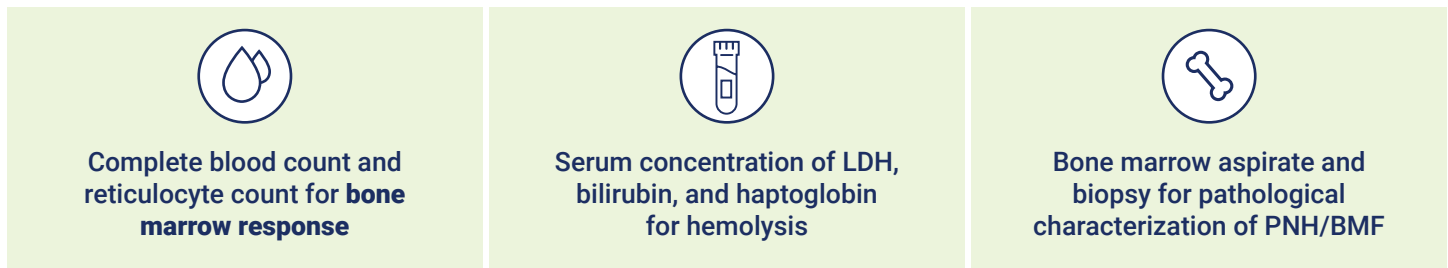
ASSESSMENT OF CLONE SIZE WITH FLOW CYTOMETRY IS IMPORTANT FOR PNH CLASSIFICATION^{1,21,27}

Category	Observed hemolysis	Treatment strategy	Typical clone size*
Classic PNH	High	PNH-specific therapy	Large (>50%)
PNH in the setting of another specified bone marrow disorder ^a	Low	Treat underlying disease; patients with large clones may benefit from complement inhibitors	Variable (usually relatively small, <50%)
Subclinical PNH	Absent	Treat underlying disease (MDS, AA)	Small (<10%)

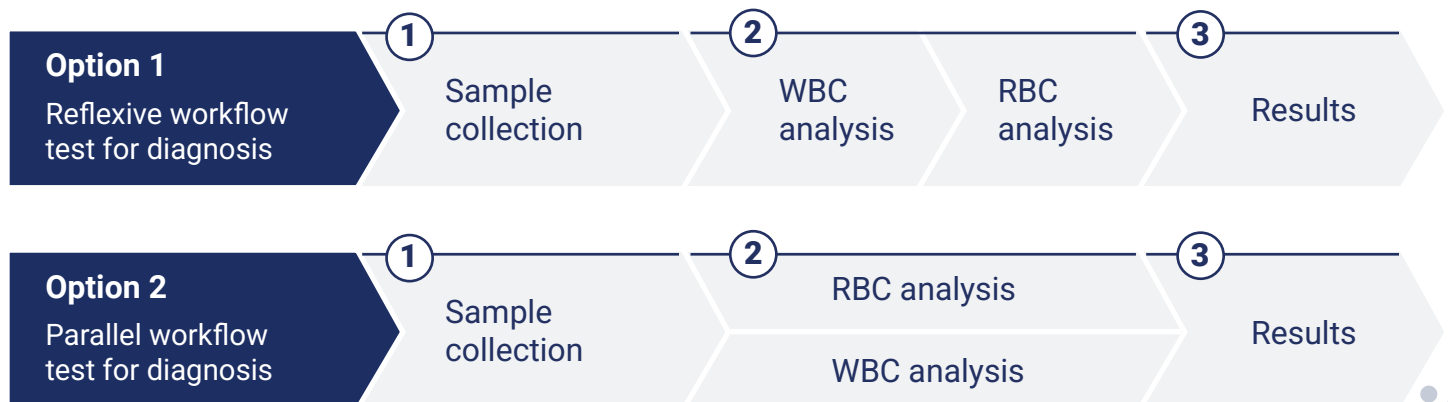
*PMN clone size.

TESTS TO DIFFERENTIATE CLASSIC PNH FROM PNH/BMF^{21,28}

The following tests are performed to differentiate classic PNH from PNH associated with BMF:







DETECTING GPI-DEFICIENT CELLS IN PNH^{22,23}



^aAA or low-risk MDS.
LDH, lactate dehydrogenase.

① SAMPLE REQUIREMENTS^{23,29-31}

 Draw 2-5 mL of peripheral blood	 Use EDTA, heparin, or ACD tubes
 Refrigerate after 24 hours	 Test within 24-48 hours

② GUIDELINES FOR TESTING^{22,23,29}

		Standard sensitivity	High sensitivity
Gating	RBCs	Forward scatter vs side scatter (log mode)	CD235a-labeled cells
	WBCs	Forward scatter vs side scatter (linear mode)	<ul style="list-style-type: none"> Neutrophils: CD15-labeled cells Monocytes: CD64-labeled cells
Cell markers	RBCs	CD55 ^a and/or CD59 ^a	CD235a and CD59
	WBCs	CD55 ^a and/or CD59 ^a	<ul style="list-style-type: none"> Neutrophils: CD15, CD45, CD24,^a and FLAER^b Monocytes: CD64, CD45, CD14,^a and FLAER^b
Limit of detection^c		4%	0.05%

MULTIPLE REFERENCE LABS OFFER FLOW CYTOMETRY TESTING TO CONFIRM PNH DIAGNOSES³⁰⁻⁵²

Laboratory	Sensitivity	Sample information	Contact information
ARUP PNH, High Sensitivity, RBC and WBC ³⁰	High	https://ltd.aruplab.com/Tests/Pub/2005006	1-800-522-2787 clientservices@aruplab.com
BioReference Laboratories PNH by Flow Cytometry ³¹	NR	https://www.bioreference.com/physicians/resources/test-directory/?tc=5564	1-800-229-5227 https://www.bioreference.com/contact/
Cleveland Clinic Laboratories High-Sensitivity Flow Cytometry for PNH ³²	High	https://clevelandcliniclabs.com/high-sensitivity-flow-cytometry-for-paroxysmal-nocturnal-hemoglobinuria/	1-800-628-6816/1-216-444-5755 https://clevelandcliniclabs.com/contact-us/
CSI Laboratories PNH High-Sensitivity ³³	High	https://www.csilaboratories.com/flow/pnh-high-sensitivity/	1-800-459-1185 clientservice@csilaboratories.com

^aGPI-anchored protein.

^bLabeling method that detects all GPI-anchored proteins.

^cBased on the smallest PNH clone that can be reliably detected.

ACD, acid citrate dextrose; EDTA, ethylenediaminetetraacetic acid; NR, not reported.

Laboratory	Sensitivity	Sample information	Contact information
Dahl-Chase Diagnostic Services ^{34,35} PNH Analysis	High	http://dahlchase.host4kb.com/article/AA-00231/15/	1-207-941-8200/1-800-660-1626 cservice@dahlchase.com
Hematogenix ³⁶	High	https://hematogenix.com/technologies/flow-cytometry	1-708-444-0444 ClientServices@hematogenix.com
Inform Diagnostics ³⁷	High	https://www.informdx.com/wp-content/uploads/MLS-20-0100_4-Client-Resource-Guide.pdf	1-888-354-8168 https://www.informdx.com/contact-us/
Labcorp PNH Evaluation ³⁸	High	https://oncology.labcorp.com/tests/zzl0-295/pnh-evaluation	1-800-447-5816 https://oncology.labcorp.com/contact-us
Mayo Clinic Laboratories PLINK ^{39,40}	High	https://www.mayocliniclabs.com/test-catalog/overview/62139#Specimen	1-800-533-1710/1-507-266-5700 mcl@mayo.edu
Michigan Medicine Laboratories Flow Cytometric Immunophenotyping ⁴¹	NR	https://mlabs.umich.edu/tests/pnh-marker-panel	1-800-862-7284 https://mlabs.umich.edu/form/contact
Molecular Pathology Lab Network PNH–High Sensitivity by Flow ^{42,43}	High	https://mplnet.com/test-menu	1-865-380-9746 services@mplnet.com
NeoGenomics High Sensitivity PNH Evaluation ⁴⁴	High	https://neogenomics.com/test-menu/high-sensitivity-pnh-evaluation	1-866-776-5907, option 3 Client.Services@neogenomics.com
Oregon Health & Science University Lab Services PNH Test (High Sensitivity) ⁴⁵	High	https://www.ohsu.edu/lab-services/pnh-test-high-sensitivity	1-503-494-7383 1-888-375-4636 https://www.ohsu.edu/about/contact-us
PathGroup PNH Analysis (Flow Cytometry) ⁴⁶	High	https://pathconnect.pathgroup.com/testmenu/#/testinfo/UE5IRQ%3D%3D	1-615-562-9300/1-888-474-5227 http://www.pathgroup.com/company/contact/
Quest Diagnostics PNH With FLAER (High Sensitivity) ⁴⁷	High	https://testdirectory.questdiagnostics.com/test/test-detail/94148/paroxysmal-nocturnal-hemoglobinuria-pnh-with-flaer-high-sensitivity?cc=MASTER	1-866-697-8378 https://www.questdiagnostics.com/contact-us
UF Pathology Laboratories PNH–FLAER, Granulocytes/ Monocytes ⁴⁸	NR	https://pathlabs.ufl.edu/tests/test-directory-p/paroxysmal-nocturnal-hemoglobinuria-pnh-cd55-59-erythrocytes-flaer-granulocytes-monocytes/	Main: 1-352-265-9900 Jacksonville: 1-904-427-0865 Toll-Free: 1-888-375-5227 https://pathlabs.ufl.edu/contact-us/
University of Iowa Diagnostic Laboratories PNH Screening ⁴⁹	NR	https://www.healthcare.uiowa.edu/path_handbook/rhandbook/test1123.html	Local: 1-319-384-7212 Toll-Free: 1-866-844-2522 https://medicine.uiowa.edu/uidl/about-us
University of Pittsburgh Department of Pathology Clinical Flow Cytometry–PNH Evaluation ⁵⁰	NR	https://www.path.pitt.edu/divisions/section-laboratory-medicine/division-clinical-hematopathology/clinical-flow-cytometry-0	1-412-864-6173 https://www.path.pitt.edu/contact-us
University of Texas Medical Branch PNH, High Sensitivity, RBC and WBC ⁵¹	High	https://www.utmb.edu/lsg2/Home/Details?id=1366	1-409-772-2222 1-800-917-8906 https://www.utmb.edu/contact
UW Medicine Laboratory Medicine and Pathology PNH by Flow Cytometry ⁵²	NR	https://dlmp.uw.edu/test-guide/view/PNHFLO	1-206-520-4600 1-800-713-5198 commserv@uw.edu

The laboratories listed in the table above offer tests to identify patients with PNH. Novartis does not endorse the use of any specific laboratory or test. This information is intended for reference only. There may be additional laboratories that conduct PNH testing. Conduct your own research and verify the capabilities and services of laboratories for your specific needs.



Expected TATs for the above tests range from 1 to 7 days.^a

^aHematogenix, BioReference Laboratories, and PathGroup do not report the TAT of their PNH testing. TAT, turnaround time.

3 GUIDELINES FOR REPORTING OF RESULTS²²



PNH reports should include:

Clear, final interpretation

- “PNH clone [present, absent] in [WBC, RBC, WBC and RBC]”
- Avoid the use of the terms “positive” or “negative”

Reported clone sizes

- WBC: total (neutrophils + monocytes) percentage clone size
- RBC: clone size; total percentage, type II percentage, and type III percentage

Appropriate clone size terminology (WBC and RBC)

- >1%: “PNH clone”
- 0.1% to 1%: “minor PNH clone”
- <0.1%: “rare cells with PNH phenotype”

All diagnostic and gating antibodies listed

Lower limit of quantification (%) indicated

Both WBC and RBC assays

Histograms or dot plots if possible

Retesting recommendations

When “minor” or “rare” clone populations are detected

RECOMMENDATIONS FOR RETESTING AND MONITORING



Consider monitoring PNH clone size at regular intervals using high-sensitivity flow cytometry^{3,22,29}

Changes in clone size may reflect a changing clinical picture and/or progression from subclinical to hemolytic PNH²⁹

Consider analysis of PNH clone size using high-sensitivity flow cytometry at least every 6 months for the first 2 years, and then once a year thereafter if the disease is being treated and is stable; any change in clinical or hematologic parameters may require more frequent monitoring^{3,22,29,53}

Testing for clone size expansion in patients with AA and/or “minor” PNH clones can be conducted every 3 to 6 months for the first 2 years, and then annually thereafter if the clone size remains stable²²

REFERENCES

1. Cançado RD et al. *Hematol Transfus Cell Ther*. 2021;43(3):341-348. doi:10.1016/j.htct.2020.06.006
2. Schrezenmeier H et al. *Ann Hematol*. 2020;99(7):1505-1514. doi:10.1007/s00277-020-04052-z
3. Shah N, Bhatt H. Paroxysmal nocturnal hemoglobinuria. In: *StatPearls*. StatPearls Publishing; 2023. Accessed July 31, 2023. <https://www.ncbi.nlm.nih.gov/books/NBK562292/>
4. Bektas M et al. *J Manag Care Spec Pharm*. 2020;26(12)(suppl b):S8-S14.
5. Mitchell R et al. *SM Clin Med Oncol*. 2017;1(1):1001.
6. Moore CA, Krishnan K. Aplastic anemia. In: *StatPearls*. StatPearls Publishing; 2023. Accessed July 17, 2023. <https://www.ncbi.nlm.nih.gov/books/NBK534212/>
7. Dotson JL, Lebowicz Y. Myelodysplastic syndrome. In: *StatPearls*. StatPearls Publishing; 2023. Accessed July 18, 2022. <https://www.ncbi.nlm.nih.gov/books/NBK534126/>
8. Bonadies N et al. *J Clin Med*. 2021;10(5):1026. doi:10.3390/jcm10051026
9. Brodsky RA. *Blood*. 2014;124(18):2804-2811. doi:10.1182/blood-2014-02-522128
10. Miyata T et al. *N Engl J Med*. 1994;330(4):249-255.
11. Bessler M et al. *EMBO J*. 1994;13(1):110-117.
12. Miyata T et al. *Science*. 1993;259(5099):1318-1320.
13. Mahoney JF et al. *Blood*. 1992;79(6):1400-1403.
14. Takeda J et al. *Cell*. 1993;73(4):703-711.
15. Wilcox LA et al. *Blood*. 1991;78(3):820-829.
16. Medof ME et al. *Proc Natl Acad Sci U S A*. 1985;82(9):2980-2984.
17. Holguin MH et al. *J Clin Invest*. 1989;84(5):1387-1394.
18. Davies A et al. *J Exp Med*. 1989;170(3):637-654.
19. Bektas M et al. *J Manag Care Spec Pharm*. 2020;26(12)(suppl b):S3-S8.
20. Kelly RJ et al. *touchREV Oncol Haematol*. 2021;17(2):84-89. doi:10.17925/OHR.2021.17.2.84
21. Parker CJ. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):208-216.
22. Illingworth A et al. *Cytometry B Clin Cytom*. 2018;94(1):49-66. doi:10.1002/cyto.b.21609
23. Sutherland DR et al. *Cytometry B Clin Cytom*. 2018;94(1):23-48. doi:10.1002/cyto.b.21610
24. Fattizzo B et al. *Leukemia*. 2021;35(11):3223-3231. doi:10.1038/s41375-021-01190-9
25. Dingli D et al. *Ann Hematol*. 2023;102(7):1637-1644. doi:10.1007/s00277-023-05269-4
26. Manivannan P et al. *Indian J Hematol Blood Transfus*. 2017;33(4):453-462. doi:10.1007/s12288-017-0868-y
27. Parker C et al. *Blood*. 2005;106(12):3699-3709. doi:10.1182/blood-2005-04-1717
28. van Bijnen ST et al. *Thromb Res*. 2015;136(2):274-281.
29. Borowitz MJ et al. *Cytometry B Clin Cytom*. 2010;78(4):211-230. doi:10.1002/cyto.b.20525
30. ARUP Laboratories. Accessed June 5, 2024. <https://ltd.aruplab.com/Tests/Pub/2005006>
31. BioReference. Accessed June 5, 2024. <https://www.bioreference.com/physicians/resources/test-directory/?tc=5564>
32. Cleveland Clinic Laboratories. Accessed June 5, 2024. <https://clevelandcliniclabs.com/high-sensitivity-flow-cytometry-for-paroxysmal-nocturnal-hemoglobinuria/>
33. CSI Laboratories. Accessed June 5, 2024. <https://www.csilaboratories.com/flow/pnh-high-sensitivity/>
34. Dahl-Chase Diagnostic Services. Accessed June 5, 2024. <http://dahlchase.host4kb.com/article/AA-00231/15/>
35. Dahl-Chase Diagnostic Services. Accessed June 5, 2024. <http://dahlchase.host4kb.com/article/AA-00231/15/Dahl-Chase-Directory-of-Services/Flow-Cytometry-Testing/Paroxysmal-Nocturnal-Hemoglobinuria-Analysis.html>
36. Hematogenix. Accessed June 5, 2024. <https://hematogenix.com/technologies/flow-cytometry>
37. Inform Diagnostics. Accessed June 5, 2024. <https://www.informdx.com/wp-content/uploads/MLS-20-0100.4-Client-Resource-Guide.pdf>
38. Labcorp. Accessed June 5, 2024. <https://oncology.labcorp.com/tests/zzIO-295/pnh-evaluation>
39. Mayo Clinic Laboratories. Accessed June 5, 2024. <https://www.mayocliniclabs.com/test-catalog/overview/62139#Specimen>
40. Mayo Clinic Laboratories. Accessed June 5, 2024. <https://www.mayocliniclabs.com/test-catalog/overview/62139#Fees-and-Codes>
41. Michigan Medicine Laboratories. Accessed June 5, 2024. <https://mlabs.umich.edu/tests/pnh-marker-panel>
42. Molecular Pathology Laboratory Network. Accessed June 5, 2024. <https://mplnet.com/test-menu/>
43. Molecular Pathology Laboratory Network. Accessed June 5, 2024. <https://www.mplnet.com/cellular-immunology/>
44. NeoGenomics. Accessed June 5, 2024. <https://neogenomics.com/test-menu/high-sensitivity-pnh-evaluation>
45. Oregon Health & Science University Lab Services. Accessed June 5, 2024. <https://www.ohsu.edu/lab-services/pnh-test-high-sensitivity>
46. PathGroup. Accessed June 5, 2024. <https://pathconnect.pathgroup.com/testmenu/#/testinfo/UE5IRQ%3D%3D>
47. Quest Diagnostics. Accessed June 5, 2024. <https://testdirectory.questdiagnostics.com/test/test-detail/94148/paroxysmal-nocturnal-hemoglobinuria-pnh-with-flaer-high-sensitivity?cc=MASTER>
48. UF Pathology Laboratories. Accessed June 5, 2024. <https://pathlabs.ufl.edu/tests/test-directory-p/paroxysmal-nocturnal-hemoglobinuria-pnh-cd55-59-erythrocytes-flaer-granulocytes-monocytes/>
49. University of Iowa Diagnostic Laboratories. Accessed June 5, 2024. https://www.healthcare.uiowa.edu/path_handbook/rhandbook/test1123.html
50. University of Pittsburgh. Accessed June 5, 2024. <https://www.path.pitt.edu/divisions/section-laboratory-medicine/division-clinical-hematopathology/clinical-flow-cytometry-0>
51. University of Texas Medical Branch. Accessed June 5, 2024. <https://www.utmb.edu/lsg2/Home/Details?id=1366>
52. UW Medicine Laboratory Medicine and Pathology. Accessed June 5, 2024. <https://dlmp.uw.edu/test-guide/view/PNHFLO>
53. Kulasekararaj AG et al. *Blood Rev*. 2023;59:101041. doi:10.1016/j.blre.2023.101041

SUMMARY.

Routine Laboratory Testing Along With High-Sensitivity Flow Cytometry Is Essential to Guiding Therapeutic Decisions^{3,6-8,21,27}



The diverse presentation of PNH contributes to diagnostic delays^{3,4}



Timely and accurate diagnoses are critical for making appropriate treatment decisions for patients with PNH^{3,7,8,21,27}



Biomarker testing is a fundamental component of the diagnostic workup^{3,6-8}



Once suspected, PNH can be definitively diagnosed using flow cytometry²¹



Multiple reference labs offer flow cytometry testing for PNH³⁰⁻⁵²



For all of your patients, remember to **CHECK ThAT** and consider biomarker testing for PNH with flow cytometry



Are you interested in learning more about **Precision Medicine?**



Visit our website and you will find a digital copy of this brochure, additional Precision Medicine resources, and more.

www.novartis.com/us-en/healthcare-professionals/personalized-and-precision-medicine

