

The purpose of the 2N-von Willebrand Disease panel is to distinguish type 2N-von Willebrand disease from mild hemophilia A and hemophilia A carriers.

BACKGROUND:

von Willebrand factor (VWF) is an important hemostatic protein¹ that functions in two ways to support normal hemostasis: it is required for platelet adhesion at sites of vascular injury; and it carries and stabilizes factor VIII coagulant (FVIII:C) in plasma.² Type 2N-von Willebrand disease (VWD) is an inherited bleeding disorder characterized by a qualitative defect in VWF in which it does not bind factor VIII (FVIII) adequately, and the plasma half life of FVIII:C is shortened.³ At least 5 coding defects of the VWF gene have been shown to be associated with the type 2N variant of VWD, and in several families studied there have been compound heterozygous members with both type 1 (quantitative deficiency of VWF) and type 2N.^{4,5,6} Clinically, patients present with a picture similar to mild hemophilia A or a hemophilia A carrier, but family studies may indicate that inheritance is autosomal, consistent with a defect of VWF (and not sex-linked as in hemophilia). Laboratory studies in patients with 2N VWD reveal a discrepancy between FVIII:C and von Willebrand factor: FVIII:C levels are disproportionately depressed compared to VWF levels (which are either normal or modestly depressed).

REASONS FOR REFERRAL:

For selection of proper therapy, it is important to distinguish between 2N VWD and hemophilic disorders. Patients with 2N VWD have a very short correction of FVIII:C levels in response to DDAVP or purified FVIII replacement therapy. Optimal therapy of patients with type 2N VWD requires replacement with VWF containing concentrates.⁷

METHOD:

In the FVIII-VWF binding assay, the ability of patient VWF to bind recombinant FVIII is tested in a microtiter plate format^{8,9} using citrated patient plasma. This is achieved by capturing patient VWF in a microtiter well coated with monoclonal antibody to VWF, clearing the well of any patient-derived FVIII:C, and then allowing the patient VWF to bind recombinant FVIII. The patient-derived VWF is quantitated by enzyme-linked immunoassay, the bound FVIII:C is quantitated by chromogenic assay, and the results are reported as the ratio of FVIII to VWF. Comparison is made to the results obtained from a patient with compound type 1/type 2N VWD and a patient with 2N carrier state. To assist in interpretation of the factor VIII/VWF binding assay, patient FVIII:C and VWF antigen levels are also determined.

LIMITATIONS:

Samples taken after transfusion therapy may reflect the characteristics of transfused VWF. If the VWF antigen level is < 10 IU/dL, then the VWD 2N Binding assay will be cancelled. This is due to the VWF level being too low to allow an adequate assessment of the ability of the patient VWF to bind the rFVIII.

NORMAL VALUES:

Normal binding: no evidence for 2N VWD.

Abnormal results will be interpreted as decreased FVIII binding, consistent with type 2N VWD.

SPECIMEN REQUIREMENTS:

Three 0.5 ml aliquots of citrated plasma frozen in plastic tubes, shipped on dry ice.

SHIPPING REQUIREMENTS:

Place the frozen specimen and the test requisition form in plastic bags, seal and surround with at least 5 pounds of dry ice in a Styrofoam container. Place the sealed Styrofoam container in a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Hemostasis Reference Laboratory
BloodCenter of Wisconsin
638 N. 18th St.
Milwaukee, WI 53233
800-245-3117, ext. 6129

TURNAROUND TIME: 7-10 days

von Willebrand Disease Type 2N Profile includes:

TEST	CPT CODES
VWD Type 2N Binding	85240,85246
VWF Antigen	85246
FVIII Activity	85240

REFERENCES:

1. Montgomery RR, Collier BS: von Willebrand Disease, in Colman RW, Hirsh J, Marder VJ, Salzman EW (eds): Hemostasis and Thrombosis: Basic Principles and Clinical Practice. Philadelphia PA, Lippincott 1994.
2. Truddenham EGD, Lane RS, Rotblat F, Johnson AJ, Snape TJ, Middleton S, Kernoff PBA: Response to infusions of polyelectrolyte fractionated human factor VIII concentrate in human haemophilia A and von Willebrand=s disease. British Journal of Haematology 1982;52:259.
3. Mazurier C, Dieval J, Joriex S, Delobel J, Goudemand M: A new von Willebrand factor (vWF) defect in a patient with factor VIII (FVIII) deficiency but with normal levels and multimeric patterns of both plasma and platelet vWF. Characterization of abnormal vWF/FVIII interaction. Blood 1990;75:20.
4. Kroner PA, Friedman KD, Fahs SA, Scott JP, Montgomery RR: Abnormal binding of factor VIII is linked with the substitution of glutamine for arginine 91 in von Willebrand factor in a variant form of von Willebrand disease. J Biological Chemistry 1991;266:19146.
5. Gaucher C, Mercier B, Jorriex S, Oufkir D, Mazurier C: Identification of two point mutations in the von Willebrand factor gene of three families with the "Normandy" variant of von Willebrand disease. British Journal of Haematology. 1991;78:506.
6. Rick ME, Krizek DM: Identification of a His54Gln substitution in von Willebrand factor from a patient with defective binding of factor VIII. American Journal of Hematology 1996;51:302.
7. Mazurier C, Gaucher C, Jorriex S, Goudemand M, et al: Biological effect of desmopressin in eight patients with type 2N ("Normandy") von Willebrand disease. British Journal of Haematology 1994;88:849.
8. Nishino M, Girma J-P, Rothchild C, Fressinaud E, Meyer D: New Variant of von Willebrand Disease with defective binding to factor VIII. Blood 1989;74:1591.
9. Kroner PA, Foster PA, Fahs SA, Montgomery RR: The defective interaction between von Willebrand factor and factor VIII in a patient with type 1 von Willebrand disease is caused by substitution of Arg 19 and His 54 in mature von Willebrand factor. Blood 1996;87:1013.