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Assay Summary

von Willebrand Disease 2N Gene Mutation Analysis

von Willebrand Factor Normandy

Synopsis

Factor VIII is stabilized in plasma by complexing with von Willebrand factor. Specific mutations in the factor VIII binding site of von Willebrand factor result in destabilization of this complex causing rapid removal of factor VIII from the circulation[1]. Three mutations (T791M in exon 18[2], R816W in exon 19[3], and R854Q in exon 20[3]) in the von Willebrand factor gene may account for a majority of the Normandy mutations. However, other mutations have been described[4]. The phenotype of these von Willebrand factor Normandy mutations resembles mild hemophilia A showing low factor VIII levels and normal von Willebrand factor levels and multimers. The inheritance of this “pseudo” hemophilia A is autosomal recessive rather than X-linked as is the case for hemophilia A. Mutations altering factor VIII binding are detected by direct DNA sequence analysis of exons 18-20 of the von Willebrand factor gene.

Note on amino acid numbering convention for the von Wi l lebrand factor: Amino acid number 1 corresponds to the first amino acid (met) of the von Willebrand factor propeptide. Removal of the first 763 amino acids of the propeptide resul ts in mature von Willebrand factor. Previous nomenclature designated the first amino acid of the mature protein (ser) as number 1. The f irst amino acid of the mature pept ide corresponds to amino acid 764 by the current nomenclature. Thus, T791M, R816W and R854Q have previously been designated T28M, R53T and R91Q, respectively.

Indications for testing

Individuals diagnosed with hemophilia A with no detectable mutation in the factor VIII gene may be tested for the von Willebrand Normandy mutations. We routinely test for the Normandy mutations in all hemophilia A patient samples for which we have not been able to detect a mutation in the factor VIII gene.

Methodology

Exons 18, 19 and 20 of the von Willebrand factor gene is analyzed by automated DNA sequencing in both directions.

Performance

The sensitivity of DNA sequence analysis for detection of heterozygous point mutations is estimated to be greater than 95%. The specificity of the analysis is estimated to be greater than 98%.

Limitations

This assay analyzes only exons 18, 19 and 20 of the von Willebrand factor gene. Although the vast majority of von Willebrand factor Normandy mutations lie within these three exons, at least one has been reported outside of exons 18–20. This mutation, and any others lying outside of exons 18–20, would not be detectable by this assay. Some sequence alterations that may be detected (such as those causing missense or

synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes.
Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:

i) for infants, a minimum of 3 ccs is sufficient.
ii) we accept DNA; at least 10 micrograms is required.

Test Request Form (TRF)

A completed CMDL TRF is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

Order Codes	***CPT Codes***	***TAT***
VWFNa (von Willebrand Disease, Normandy (2N) subtype)	81405, G0452	4 wks

References

1. Mazurier, C. (1992). Thrombosis and Haemostasis 67:391-396.
2. Gaucher, C. et al. (1991). Blood 77:1937-1941.
3. Gaucher, C. et al. (1991). Br.J.Haematol. 78:506-514.
4. Meyer, D. et al. (1997). Thrombosis and Haemostasis 78:451-456.

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc