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Assay Summary

Factor IX Gene Mutation Analysis

Hemophilia B

Synopsis

Identification of causative mutations in the factor IX gene for families with hemophilia B can permit very accurate determination of carrier status of at-risk females in these families and provides options for prenatal diagnosis. To identify the mutation in a hemophilia B family, a blood sample is required from an affected individual (proband). Full mutation analysis of the factor IX gene is performed. After a mutation is detected in a proband, carrier testing and prenatal diagnosis may be offered to the family. In cases where a proband is not available for testing, analysis may be performed on a sample from an obligate carrier.

Indications for testing

Individuals with a diagnosis of hemophilia B, appropriate at-risk female relatives of probands with identified mutations, and hemophilia B carriers with previously identified factor IX gene mutations desiring prenatal diagnosis, with genetic counseling, are candidates for testing.

Methodology

Factor IX sequencing: All coding exons (A-H) and associated intron junctions of the Factor IX gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

Dosage analysis for known large deletions: Carrier testing in females for known large deletions is performed by Robust Dosage-PCR (RD-PCR), a quantitative method for the detection of large heterozygous deletions. A robust dosage PCR (RD-PCR) assay for exons all exons (A-H) for the Factor IX gene has been developed, optimized, and validated to screen for large deletions. This assay utilizes the natural sex chromosome ratio as an internal control for the accurate determination of copy number of the region of interest. The precision and accuracy of the method as a whole has been previously established by us at >95%, and >99%, respectively.

Performance

Factor IX sequencing: From previous experience, we have been able to detect factor IX gene mutations in about 99% of individuals with the diagnosis of hemophilia B. The specificity of mutation detection in probands is estimated to be greater than 99%. The sensitivity and specificity for carrier detection and for prenatal diagnosis for families with identified factor IX gene mutations are both estimated to be greater than 99%.

Dosage analysis for known large deletions: Large deletions, usually associated with severe hemophilia B, account for approximately 6% of alterations², giving an estimated diagnostic sensitivity of about 6%. The assay sensitivity of deletion detection is >99%.

Limitations

Factor IX sequencing: The sequence analysis will not detect mutations located in regions of the Factor IX gene that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The sequencing method also will not detect gross genetic alterations including most duplications, inversions, or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

Dosage analysis for known large deletions: The dosage analysis will not detect mutations located in regions of the Factor IX gene that are not analyzed (introns and upstream or downstream regions not analyzed). Dosage analysis will not detect sequence alterations or inversions.

Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Note: Prenatal diagnosis is available for a male fetus once positive carrier status has been established for the mother.

Specimen Requirements

(a) 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.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W.

A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

Test Request Form (TRF)

- (a) A completed MDL [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.
- (b) [Hemophilia Patient Information Form](#): Include a completed Hemophilia Patient Information Form for the proband and a complete pedigree.

Order Codes	CPT Codes	TAT
F9-SEQ (Factor IX gene, full gene sequencing)	83890, 83898(x8), 83904(x8), 83894, 83912	4 wks
F9-CAS (Factor IX gene, targeted mutationa analysis)	83890, 83898, 83904, 83894, 83912	3 wks
F9-PD (Factor IX gene, known mutation detection, prenatal)	83890, 83898, 83904, 83894, 83912	2 wks
F9-RD-SEQ (Factor IX gene, dosage analysis (full gene))	83890, 83898(x8), 83894, 83912	6 wks
F9-RD-CAS (Factor IX gene, targeted dosage analysis)	83890, 83898(x4), 83894, 83912	4 wks
F9-RD-PD (Factor IX gene, known deletion - dosage analysis, prenatal)	83890, 83898(x4), 83894, 83912	4 wks

References

1. Green P.M. et al. (2003) Haemophilia B Mutation Database, Version 13. King's College of London, University of London
2. <http://www.hgmd.cf.ac.uk/ac/gene.php?gene=F9>

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