**BACKGROUND:**
Hemophilia A is an X-linked inherited bleeding disorder caused by mutation of the F8 gene that encodes for coagulation factor VIII. The degree of plasma factor VIII deficiency correlates with both the clinical severity of disease and genetic findings. Severe hemophilia A is characterized by plasma factor VIII levels of under 1 IU/dl, with approximately 50% of cases attributable to gene inversions, 44% to point mutations and 6% to deletions and duplications. Moderate and mild hemophilia A are characterized by factor VIII levels of 1-5 IU/dl or 6 – 40 IU/dL, respectively. The majority of cases are attributable to point mutations within the F8 gene. Sequence analysis of the F8 gene is useful for identification of the underlying genetic defect in males with severe hemophilia A in whom inversion defects have been excluded, in males with moderate or mild hemophilia A, and for determination of carrier status in the female individuals within their families.

**REASONS FOR REFERRAL:**
- Diagnosis of Affected Individuals
- Female Carrier Detection
- Prenatal Diagnosis

**METHOD:**
PCR-direct DNA sequencing.

**REFERENCE INTERVAL:**
Normal - None Detected  
Abnormal - Presence of mutation or sequence variation.

**LIMITATIONS:**
Analytical sensitivity is >99% for mutations within the coding sequence and intron/exon borders. Mutations that are outside the regions sequenced will not be detected. Rare polymorphisms within primer or probe regions may interfere with detection of gene variants. Clinical sensitivity for severe hemophilia A where inversion mutations are excluded is approximately 99% for males and 95% for females. Clinical sensitivity for mild/moderate hemophilia A is 76%-98%. Deletions will be detected in males by lack of amplification of exons; deletions will not be detected in carrier females. Duplications are not detected.
SPECIMEN REQUIREMENTS:
5 ml EDTA (lavender top) whole blood, 2 ml minimum, shipped at room temperature. A sample from an affected family member is highly recommended. Testing can also be performed on 7-15 ml amniotic fluid or 2 x 10^6 cultured amniocytes. Please inquire about specific requirements for the analysis of prenatal samples by calling 1-800-245-3117, ext. 6250.

SHIPPING REQUIREMENTS:
Place the specimen and the completed test requisition in plastic bags, seal and insert in a Styrofoam container. Seal the container and place in a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. Notify the laboratory prior to shipping by calling 800-245-3117, ext. 6250. Address package to:

Client Services/Molecular Diagnostics Laboratory
BloodCenter of Wisconsin
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

TURNAROUND TIME: 21 days

CPT CODES: 81407

REFERENCES:
