



## HEMOSTASIS GENETIC DIAGNOSTIC LABORATORY

NEIL C. JOSEPHSON, MD – DIRECTOR

### HEMOPHILIA A AND B DNA TESTING

#### HEMOPHILIA A

We currently perform mutation screening and linkage analysis for hemophilia A families from the Pacific Northwest only. In *severe hemophilia A*, a FVIII gene inversion accounts for 45% of cases. This test is run approximately once a month. Severe hemophilia A patients negative for the inversion, can be screened for other mutations but this screening usually takes several months and only picks up another ~35%, leaving ~20% in whom we will not be able to identify a specific mutation. In *moderate and mild hemophilia A*, the mutation can be identified in ~80% of families. If the specific mutation cannot be identified, intragenic linkage is often useful. Linkage studies also help identify the *de novo* origin of mutations in families with an isolated occurrence (e.g. which maternal grandparent). Intragenic linkage may be useful in 80-90% of families where key family members (patient and both parents of potential carrier) are available.

#### HEMOPHILIA B

We screen PCR-amplified exons of the factor IX gene by direct sequence analysis and usually detect a mutation. We have been successful in 189 of 190 families to date (see Weinmann et al, Brit J Haematol 100:58, 1998). If no recurrent mutation is found, we study intragenic linkage with 90% of Caucasian, 95% of Black and 65% Asian/Native American informative. Intragenic linkage, when informative and assuming accurate relationships and diagnosis, is 99.9% accurate. Intragenic linkage can help assign origin of *de novo* mutations (nearly half of families have no previously affected member).

#### TURN AROUND TIME

FVIII inversion testing takes 2-4 weeks. Other mutation screening takes several months. Once a mutation is known, screening for family members takes 1-3 weeks; linkage studies take 2-4 weeks. For special situations such as prenatal diagnosis, speak directly to Dr. Josephson about turn around time, as high priority will be assigned for a pregnant potential carrier.

#### SAMPLE REQUIREMENTS

5-10 ml EDTA (purple-topped tube) whole blood to arrive at the Blood Center on Monday–Friday within 36 hours of being drawn. For samples from amniotic fluid or chorionic villi sampling send 2 T-25 or 1 T-75 flask of cultured cells grown to confluence (keeping a cell line culture for “back-up”). Always call 1-206-292-6570 to notify us that samples are on the way. A completed Puget Sound Blood Center laboratory request form must accompany each sample. (If you don’t have one, call our office and one will be faxed.) *Samples cannot be processed without a Social Security number and birth date. A name and phone number for a contact person plus a billing address and P.O.# are also essential.* We also appreciate knowing the affected member’s baseline factor VIII clotting activity level, if he has been infused within the past two weeks and pedigree information. We assume you obtain informed consent; a copy of our recommended form can be provided as an example if needed.

#### FOR QUESTIONS

Neil C. Josephson, MD  
Phone: (206) 292-6570  
Fax: (206) 292-8030  
E-mail: [neiljo@psbc.org](mailto:neiljo@psbc.org)

Renée Killian, RN, MPH  
Phone: (206) 292-6597  
Fax: (206) 292-8030  
E-mail: [reneek@psbc.org](mailto:reneek@psbc.org)