UNIVERSITY OF WASHINGTON
DEPARTMENT OF LABORATORY MEDICINE
COAGULATION LABORATORY

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           hours, weekends, and holidays.
Fax: (206) 548-6189
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Consultation Service: Consultation on bleeding patients, thrombosis, fibrinolysis, and test interpretation is
available from Dr. Schmer, Dr. Chandler or Dr. La Spada during regular hours Monday through Friday at
(206) 548-6131. For assistance after regular hours, weekends, or holidays, call University of Washington
Medical Center paging at (206) 548-6190 and request Laboratory Medicine Resident on call.

Venipuncture service: If requested, specimens will be drawn in the outpatient blood draw laboratory at
University of Washington Medical Center or Harborview Medical Center. This service is provided even if
the patient is not a registered patient at either hospital. Please call Community Services at (206) 548-6066 or
800 713-5198 to make the necessary arrangements.

Research Service: The laboratory provides specialized research tests to clinical investigators for evaluation
of platelets, coagulation, thrombosis, and fibrinolysis. Expert consultation is available for research protocol
design and data interpretation. Please call Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131
during regular laboratory hours.

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INTRODUCTION

The Coagulation Laboratory offers a wide variety of testing to aid in the diagnosis, management, and monitoring of patients with bleeding disorders, thrombosis, and fibrinolysis. The laboratory is involved in research and development of diagnostic tests and their clinical application in providing cost effective assays that enhance and expedite the diagnostic process.

STAFF:
The technical staff in the Clinical Coagulation Laboratory are certified medical technologists with years of experience in clinical coagulation, research, and development. This expertise is complemented by Dr. Schmer, Dr. Chandler and Dr. La Spada, who provide clinical interpretation of laboratory results and consult with individual clinicians and researchers.

ACCREDITATION:
Departmental laboratories are accredited by the College of American Pathologists (CAP), thereby meeting all requirements of CLIA '88, and participate in formal proficiency programs of CAP.

LABORATORY RESULTS:
All laboratory results are mailed, transmitted to our remote on-site printers, or delivered by our courier depending on the client location. If requested, we will fax test results. To expedite faxing test results, please indicate fax number on requisition form.

SPECIMEN REQUIREMENTS:
The quality of test samples is very important in providing reliable test results. Please refer to pages 4 to 5 in this brochure for general guidelines on sample collection, processing, and storage.

SPECIMEN TRANSPORT SERVICE:
Courier service is provided in the greater Seattle, Everett, Bellingham, Bellevue, Tacoma and Olympia areas including Sea-Tac Airport and the Greyhound Bus Terminal on week days. Please contact Community Services at (206) 548-6066 or 800 713-5198 for more information and assistance in determining the best means of transportation.

SUPPLIES:
Community Services provides mailing containers and supplies at no charge. Our courier carries dry ice for transporting frozen samples.

PATIENT BILLING:
We will bill the patients directly if so requested. There is no additional charge for this service.

PLEASE CONTACT THE COMMUNITY SERVICES OFFICE FOR A COMPLETE DIRECTORY OF LABORATORY SERVICES:

Community Services
Department of Laboratory Medicine
University of Washington
P.O. Box 15246
Seattle, Washington 98115
(206) 548-6066 or 800 713-5198
Since the establishment of the Clinical Coagulation Laboratory in 1969, the laboratory has steadily expanded its service to keep pace with new technological advancements and the increased significance of testing to evaluate bleeding disorders, thrombosis, and fibrinolysis. The laboratory offers the latest diagnostic tests to assist in the diagnosis of these disorders.

The laboratory has expanded its research and development division. This expanded service represents a commitment of quality service in a cost effective manner in the development and critical evaluation of clinical diagnostic and research assays. A range of specialized research assays are made available to the medical community for investigational purposes. The availability and cost of these research assays are dependent upon the number of tests, frequency of testing, cost of reagents, etc. In addition to the availability of research assays the laboratory offers expert consultation for research protocol design and data interpretation. Please call Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131 for more information.

This handbook provides information on the clinical diagnostic assays and research assays available at the present time. Clinical Coagulation is a rapidly changing and expanding area in Laboratory Medicine. Periodically, new or updated diagnostic tests and research assays will be offered. Community Services will notify the medical community of these changes. In addition, current information on available clinical tests can be obtained by calling Community Services at (206) 548-6066 or 800 713-5198. For information and consultation on research assays not listed in this manual please call Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131.
SPECIMEN COLLECTION, PROCESSING, AND STORAGE

Proper sample collection is of utmost importance for reliable test results to evaluate the bleeding patient, thrombosis, or fibrinolysis. All these tests are influenced by sample collection, sample processing, and sample storage. The test results generated are a direct reflection of the sample integrity. The laboratory will not evaluate samples that are hemolyzed, clotted, contain fibrin strands, or improperly stored. Community Services will immediately notify the client of any problems with the sample.

An evacuated test tube system or two syringe technique is acceptable. This laboratory has standardized coagulation tests based on blood collected into buffered 3.2% sodium citrate. Therefore, clients are encouraged to use this specific anticoagulant. The blood sample should be obtained from a peripheral vein away from an intravenous line and be obtained without trauma. In some patients it may be necessary to draw through a catheter because a venipuncture site is not available. When drawing through a catheter, withdraw and discard 20 mL of whole blood prior to collection of blood for coagulation studies. If heparin is present, it will be necessary to withdraw and discard 30 mL whole blood. Depending upon the concentration of heparin, this amount of discard may not be sufficient to remove all the heparin. Please note on the requisition form that the sample was drawn through a line.

GUIDELINES FOR THE COLLECTION, PROCESSING, AND STORAGE OF SAMPLES:

CITRATED PLASMA

1. To avoid tissue fluid contamination draw 1-2 mL whole blood into a tube for discard.
2. Draw blood into buffered citrate collection tube (blue top) filled to the proper level. It is recommended that 0.109 M (3.2 %) buffered citrate tube be used.
3. Invert tube gently 5- 6 times. Process immediately.
4. Centrifuge whole blood at 3000 rpm for ten minutes.
5. Transfer plasma with plastic transfer pipette to a plastic centrifuge tube. Centrifuge plasma at 3000 rpm for 10 minutes. The second centrifuge step is important to remove all platelets in the sample.
6. Aliquot plasma into several polypropylene tubes not just one tube. Label tubes appropriately.
7. Freeze plasma immediately. Send samples on dry ice. Samples must remain frozen during transport. Allowing the plasma to thaw completely or partially will have an adverse affect on some coagulation test results.
8. Contact Community Services (206) 548-6066 or 800 713-5198 for complete instruction for transporting sample.
**ACID CITRATE PLASMA**

1. To avoid tissue fluid contamination, draw 1-2 mL whole blood into a tube for discard.

2. Draw blood into a special black top tube containing acidic buffered citrate (available from Community Services at (206) 548-6066 or 800 713-5198 American Diagnostica Product number 452 TUB). Fill the tube to the proper level.

3. Invert tube gently 5-6 times. Process immediately.

4. Centrifuge whole blood at 3000 rpm for ten minutes.

5. Transfer plasma with a plastic transfer pipette into a plastic centrifuge tube. Centrifuge plasma at 3000 rpm for ten minutes.

6. Dispense plasma into a polypropylene tube. Label appropriately. Make certain to comment Acid citrate on the label.

7. Freeze plasma immediately. Send samples on dry ice. Samples must remain frozen during transport.

8. Contact Community Services at (206) 548-6066 or 800 713-5198 for complete instructions for transporting sample.

**SERUM**

1. Draw blood into plain red top tube.

2. Allow blood to clot for 30 minutes.

3. Centrifuge at 3000 rpm for ten minutes.

4. Dispense serum into plastic tubes.

5. Label appropriately.

6. Freeze serum immediately. Send sample on dry ice. Serum must remain frozen during transport.

7. Contact Community Services at (206) 548-6066 or 800 713-5198 for complete instructions for transporting sample.

**EDTA WHOLE BLOOD SAMPLE**

1. Draw blood into lavender topped EDTA tube.

2. **Do not centrifuge sample.**

3. Send sample at room temperature. Contact Community Services at (206) 548-6066 or 800 713-5198 for complete instructions for transporting sample.
HEMOSTASIS OVERVIEW

Hemostasis is a complex interaction between vessels, platelets, and coagulation proteins that is regulated to stop bleeding while maintaining the integrity of the vascular system. Hemostasis involves four distinct but at the same time interrelated functions: vessel wall function, platelet function, coagulation and fibrinolysis. Specific tests are available to evaluate platelet function, coagulation proteins, natural occurring inhibitors, and fibrinolysis. Research assays are available to evaluate vessel wall function and damage.

Platelets play an immediate and central role in hemostasis. Qualitative and/or quantitative defects may exist which lead to excessive bleeding. When vascular injury occurs the subendothelium is exposed and in the presence of von Willebrand factor, platelets adhere to collagen. Stimulated platelets release ADP which potentiates platelet aggregation and expose anionic phospholipid and release factor V and fibrinogen which promotes coagulation. The fragile primary platelet clot is quickly stabilized by fibrin formation via the coagulation cascade.

For many years it was felt that coagulation proceeded under two distinct pathways, intrinsic and extrinsic, with the difference between the two being the method of activation. Today, we understand the two pathways are not separate entities but are intertwined in a complex coagulation mechanism. In vivo almost all coagulation reactions are initiated by exposure of tissue factor and platelet activation. TF autocatalyzes factor VII to VIIa. Factor VIIa in turn activates factor IX, which activates factor X, which converts prothrombin to thrombin, finally resulting in conversion of fibrinogen to fibrin.

In the activated partial thromboplastin time (APTT) test, the contact system is activated which in turn initiates the old intrinsic coagulation pathway through factor XII. Contact phase factors include prekallikrein, high molecular weight kininogen, and factor XII, which when activated converts factor XI to Xla. Factor XIa converts IX to IXa which then follows the common pathway through factor X. During the prothrombin time (PT) test, a massive amount of tissue factor and phospholipid are added to plasma. Under these conditions factor VIIa that is formed preferentially activates factor X, bypassing factor IX.

In vivo there are a variety of control mechanisms to limit thrombus formation through the natural occurring inhibitors protein C, protein S, and antithrombin III. Thrombomodulin present on the endothelial surface binds thrombin, “modulating” its specificity and turning thrombin into an activator of protein C. Activated protein C with its cofactor protein S proteolytically degrade factors Va and VIIIa. Thrombin in plasma in inhibited by antithrombin. This reaction is accelerated by heparinoids on the endothelial surface and heparin given therapeutically. Activated protein C is regulated by activated protein C inhibitor (APCI). Protein S exists in two forms in plasma: free and bound to C4b-binding protein. Only the free form of protein S is active.

The fibrinolytic system plays an important role in regulating the formation and removal of thrombi. Fibrinolysis is initiated by the release of active tissue plasminogen activator (TPA) from vascular endothelial cells. TPA, in the presence of fibrin, converts plasminogen to plasmin which in turn lyses fibrin in the thrombus. The concentration of active TPA in circulating blood is regulated by the secretion of TPA by the vascular endothelium, clearance of TPA by the liver, and the inhibition of TPA by plasminogen activator inhibitor type 1 (PAI-I). The concentration of plasmin in the blood is regulated by antiplasmin (aka plasmin inhibitor). Increased levels of fibrinolytic activity in blood are associated with bleeding, while decreased levels are associated with thrombosis.
CLINICAL SYNDROMES AND SUGGESTED DIAGNOSTIC TESTS

BLEEDING DISORDERS

Bleeding disorders may be due to abnormalities of the coagulation system, platelets, vascular system, or fibrinolytic system. Hereditary disorders are usually due to an abnormality of a single system, whereas acquired abnormalities may involve two or all of the systems listed above. Clinical laboratory tests are available to evaluate platelets, the coagulation system and fibrinolysis.

EVALUATION OF A BLEEDING DISORDER

Coagulation Screen (includes PT, APTT, Thrombin time, fibrinogen, qualitative D-Dimer and platelet count).

Appropriate factor assays as indicated by screening test results and/or clinical history of the patient.

COAGULATION DISORDERS

Bleeding associated with coagulation abnormalities is characterized by the formation of large hematomas, hemarthrosis, large single ecchymosis (either spontaneous or following minor trauma), or delayed bleeding following trauma, surgical or dental procedures. Petechia and mucosal hemorrhage are rare unless there is an associated platelet disorder. Coagulation disorders may be congenital or acquired.

Congenital bleeding disorders are often associated with a positive family history. A moderate to severe deficiency usually presents in early infancy through adolescence. A mild deficiency may not be detected until the patient is challenged with surgery or trauma. The most common of the congenital disorders is von Willebrand disease. It is a platelet-like bleeding disorder with a quantitative and/or qualitative defect of von Willebrand factor and a borderline to decreased factor VIII activity. Von Willebrand disease is further described on pages 10 to 12. Hemophilia A (VIII deficiency) is the next most common, followed by hemophilia B (IX deficiency). Both are inherited as sex-linked recessive disorders. Hemophilia C (XI deficiency) is inherited as an autosomal recessive trait. Factor XII deficiency is not associated with a bleeding disorder but is often associated with thrombosis. All of the mentioned disorders will present with a mild to marked prolongation of the APTT with all other screen procedures normal. The other congenital coagulation factor deficiencies (II, V, VII, and X) are extremely rare. Factor II, V, or X deficiency will have a moderate to marked prolongation of the prothrombin time and mild to moderate prolongation of the APTT. An isolated Factor VII deficiency has a prolonged prothrombin time with all other screen assays normal.

Acquired bleeding disorders are more common in hospitalized patients and can be life threatening. Acquired disorders are associated with acute and chronic disseminated intravascular coagulation (DIC), liver disease, vitamin K deficiency (dietary, wide spectrum antibiotic therapy, and/or oral anticoagulant therapy), heparin therapy, and the use of fluid for volume replacement as in trauma patients or dilutional as in massive blood transfusion. The patients may have multiple abnormalities including variable prolongation of the prothrombin time, APTT, thrombin time, and decreased fibrinogen and platelets.

Bleeding can be associated with acquired factor inhibitors. Inhibitors have been described for all coagulation proteins. Although inhibitors are rare, the most common have been described against factor VIII and factor V. A prolonged APTT which does not correct on a 1:1 mix in a bleeding patient is suggestive of a factor VIII inhibitor. A patient previously exposed to topical thrombin with a prolonged prothrombin time which does not correct on a 1:1 mix should be evaluated for a possible inhibitor against factor V. These same patients may
demonstrate a prolonged thrombin time when bovine thrombin is used in the test procedure. The patient thrombin time is normal if human thrombin is used.

**PLATELET DISORDERS**

Platelets play a central role in maintaining hemostasis and must be present in adequate number and have normal function. Platelets undergo a complex series of morphological and biochemical changes when activated. Platelets have the ability to bind to non-endothelial surfaces (adhesion), bind to other platelets (aggregation), and secrete substances that are stored in internal granules (secretion).

**EVALUATION OF QUALITATIVE PLATELET DISORDERS**

- Platelet count and smear evaluation
- Platelet aggregation studies
  - Platelet count must be greater than 200,000/uL.
  - Patient must not be taking aspirin or NSAID containing medication.
  - Test requires patient to come to laboratory site for blood collection.
  - Please call Community Services at (206) 548-6066 or 800 713-5198 for complete instructions and help in scheduling the test with the Coagulation Laboratory.

Platelet and vascular disorders are characterized by petechiae and/or small superficial ecchymosis, mucosal hemorrhage (e.g. gingival bleeding, GI bleeding, menorrhagia), and immediate profuse bleeding from small cuts. This immediate bleeding distinguishes platelet disorders from a coagulation protein deficiency where the bleeding is typically delayed. Platelet disorders can be quantitative or qualitative.

Qualitative platelet disorders can be inherited or acquired and are further classified into disorders of adhesion, aggregation or secretion. The inherited disorders usually have normal platelet counts. The most common adhesion disorder is von Willebrand disease, a quantitative and/or qualitative defect of von Willebrand factor. vWF serves as a bridge between platelets and collagen, permitting adhesion of platelets to injured vessels. Bernard Soulier is a rare adhesive disorder where the patient’s platelets lack the membrane receptor GPIb that is required for platelets to bind to von Willebrand factor. This group of patients may present with mild to moderate thrombocytopenia and abnormally large platelets on smear evaluation. The rare inherited aggregation disorders include Glanzmann's Thrombasthenia (missing or defective fibrinogen receptor, GPIIbIIIa) and afibrinogenemia. Defective secretion disorders include storage pool disease, aspirin like defect, Wiskott-Aldrich syndrome, Hermansky-Pudlak syndrome and Chediak-Higashi. Of this group, storage pool is the most common.

Acquired platelet function abnormalities are more common than inherited defects and can be associated with decreased number and/or abnormal function. Drugs such as aspirin, penicillin and alcohol affect platelet function. Uremia, disseminated intravascular coagulation and myeloproliferative disorders are associated with abnormal platelet function as well. Thrombocytopenia can be a result of a production defect, non-immune destruction, immune platelet destruction, or splenic sequestration. The severity of bleeding is usually related to the degree of thrombocytopenia and may be more severe when there is a rapid loss of platelets. Some drugs may cause thrombocytopenia through a variety of mechanisms. Platelet counts usually return to normal within 7-10 days once the drug is discontinued. Heparin-induced thrombocytopenia is a potentially life threatening form of acquire immune thrombocytopenia, caused by development of an antibody to the complex of PF4 and heparin on the platelet surface. At the same time, the PF4-heparin complex also binds to the endothelial surface where it is thought to promote potentially life threatening arterial and venous thrombosis. The first indication of the development of this antibody is a rapid unexplained drop in the platelet count after the administration of heparin.
Thrombocytosis (marked increase in platelet count) maybe primary or secondary. Primary thrombocytosis is observed in myeloproliferative disorders such as polycythemia vera, essential thrombocythemia, and chronic granulocytic leukemia. Polycythemia vera and essential thrombocythemia are associated with prolonged elevations of the platelet count and thrombosis due to abnormal platelet number and function. In secondary or reactive thrombocytosis the platelets have normal function and the elevated platelet count is usually transient.

VON WILLEBRAND DISEASE

Von Willebrand disease (vWD) is considered one of the most common of the inherited bleeding disorders, occurring in 1-3% of the population. Most cases are inherited as a heterozygous autosomal dominant trait with a mild to moderate bleeding tendency. A rare form (<5% of vWD) is inherited as an autosomal recessive disorder with a severe bleeding tendency. The clinical presentation of vWD is similar to qualitative platelet disorders. Patients may present with easy bruising, menorrhagia, epistaxis and mucosal membrane bleeding. One of the most common presentations is immediate bleeding post trauma, surgery, or dental extraction which is in contrast to the delayed bleeding characteristic of inherited clotting factor deficiencies.

Classification of vWD is based on quantitative and/or qualitative abnormalities of von Willebrand factor (vWF). vWF is a glycoprotein that is synthesized, stored, and released by endothelial cells and megakaryocytes. In vivo, vWF has two important functions. First, it mediates the adhesion of platelets to the injured vessel wall which in turn promotes the formation of thrombin at the site of injury. Second, it is the carrier protein for factor VIII stabilizing its activity and reducing the rate of factor VIII clearance from blood. To be fully functional, von Willebrand factor must polymerize into large multimers (over 2,000,000 MW). Several qualitative defects have been described due to abnormalities of vWF multimer structure.

Three principal tests are used in the evaluation of von Willebrand disease: von Willebrand factor antigen, Factor VIII activity and von Willebrand factor multimer analysis. The vWF antigen assay measures the total amount of vWF in plasma. It is typically reduced to less than 50% of normal in patients with vW disease. Factor VIII activity measures coagulant activity of the factor VIII molecule, which binds to and is stabilized by vW factor. Typically in vW disease, factor VIII activity is reduced to approximately the same level as vWF antigen. vWF multimer analysis is used to determine the subtype of von Willebrand disease. An older assay, the vWF ristocetin cofactor activity tests the ability of patient plasma to aggregate formalin fixed platelets. Ristocetin cofactor activity is proportional to functional vWF in plasma. This assay is less precise than either vWF antigen or factor VIII activity, and cannot accurately differentiate the different subtypes of vW disease. While available, it is not recommended.

EVALUATION OF VON WILLEBRAND DISEASE

Von Willebrand Disease Panel (Panel includes the following tests)

- von Willebrand factor antigen
- Factor VIII activity
- von Willebrand factor multimer analysis
- Interpretation of test results
TYPE 1 VON WILLEBRAND DISEASE

Type 1 vWD is due to a reduction in the level of von Willebrand factor in plasma; vWF function is normal. Approximately 70% of vWD is due to Type 1. Typically patients present with a mild platelet-like bleeding disorder. Patients have a normal prothrombin time, thrombin time, and fibrinogen. The APTT can range from normal to moderate prolongation. The factor VIII activity level, von Willebrand factor and ristocetin cofactor activity are typically reduced to less than 50% of normal. All multimers are present but will be reduced in quantity. Factor VIII activity, von Willebrand factor antigen and ristocetin cofactor activity may be increased during an acute phase response or pregnancy, leading to a false negative result. If this is suspected in a patient, these assays should be repeated when the patient has recovered from pregnancy or the cause of the acute phase response has resolved.

It is important to take into consideration the patient’s ABO blood group when considering the diagnosis of von Willebrand disease, as each blood type group has a different reference ranges for von Willebrand factor antigen.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Mean vWF:Ag</th>
<th>Range vWF:Ag (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>75</td>
<td>36-157</td>
</tr>
<tr>
<td>A</td>
<td>106</td>
<td>48-234</td>
</tr>
<tr>
<td>B</td>
<td>117</td>
<td>57-241</td>
</tr>
<tr>
<td>AB</td>
<td>123</td>
<td>64-238</td>
</tr>
</tbody>
</table>

TYPE 2 VON WILLEBRAND DISEASE

Type 2 vWD is due to a qualitative abnormality of von Willebrand factor. Multimer analysis of patients with Type 2 vWD shows the lack of large multimers, and in some subgroups lack of the intermediate weight multimers as well. This group has been further divided into four subgroups, Type 2A, Type 2B, Type 2M, and Type 2N.

Type 2A is the most common form of Type 2 vWD, accounting for approximately 10-15% all patients with vWD. Characteristic of this group is the lack of intermediate and high molecular weight multimers and a more prominent fast-moving low molecular weight multimer band. Other laboratory results include a normal prothrombin time, fibrinogen and thrombin time. The APTT is normal to borderline prolonged. The factor VIII activity and vWF antigen are borderline normal to slightly decreased. Ristocetin cofactor activity is typically less than 50% of normal. Factor VIII activity is often higher than von Willebrand factor antigen.

Type 2B vWD is characterized by a qualitative defect of von Willebrand factor antigen causing an increased affinity for GP1b. Characteristic of this group is the absence of only the high molecular weight multimers and enhanced aggregation to ristocetin. Other laboratory results include a normal prothrombin time, fibrinogen and thrombin time. The APTT is normal to borderline prolonged. The factor VIII activity and vWF antigen are borderline normal to slightly decreased. Factor VIII activity is often higher than von Willebrand factor antigen. Ristocetin cofactor activity is typically less than 50% of normal. These patients often present with mild to moderate thrombocytopenia.

The ristocetin-induced platelet aggregation on the patients platelets will differentiate Type 2B vWD from Type 2A vWD and from platelet pseudo-vWD. In Type 2B vWD there is an increased aggregation response to low concentrations of ristocetin compared to normal subjects in patient platelet rich plasma and when patient plasma is mixed with normal platelets. In Type 2A vWD there is a decreased response to ristocetin as compared to a normal subjects. In platelet pseudo von Willebrand disease, the vWF levels are normal but the ristocetin response is increased with the patients platelets but not when the patient’s plasma is mixed with normal platelets.
**TYPE 2M** vWD is a rare qualitative disorder with reduced platelet function, normal levels of intermediate and high MW multimers and the presence of ultra high molecular weight multimers. At the present time this disorder cannot be reliably diagnosed with current assays. For more information regarding this disorder, please contact Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131.

**TYPE 2N** is also known as vWD Normandy. This classification refers to a qualitative abnormality with markedly decreased affinity of vWF for factor VIII. The pattern is similar to hemophilia A in that the von Willebrand factor antigen level is much higher than the factor VIII activity. All multimers are present. The disorder is inherited as autosomal dominant. At the present time this disorder cannot be reliably distinguished from hemophilia A with current assays. For more information regarding this disorder, please contact Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131.

**TYPE 3 VON WILLEBRAND DISEASE**

This is the rare form of vWD inherited as autosomal recessive. These patients present with a severe bleeding disorder similar to hemophilia A or B. The factor VIII activity is usually less than 5%, unmeasureable von Willebrand factor antigen, with no multimers detected. This is the only congenital vWD that has been associated with the development of antibodies.

**PLATELET PSEUDO VON WILLEBRAND DISEASE**

This disorder mimics the Type 2B von Willebrand disease patient. On multimer analysis there is the lack of high molecular weight multimers. The patient is differentiated from the Type 2B by the ristocetin-induced aggregation test on the patient's plasma and platelets. The patient's platelets but not the patient's plasma will support enhanced ristocetin induced aggregation indicating there is an abnormality in patient's platelets and not a qualitative defect of von Willebrand factor.

Acquired von Willebrand disease syndromes are often associated with angioblastoma, lymphoproliferative disorders or monoclonal gammopathies. Other diseases associated with this disorder are other malignancies, autoimmune disease, hypothyroidism and some drugs. In acquired vW disease the patient past history does not support hereditary von Willebrand disease. Most of the acquired abnormalities are associated with the loss of high molecular weight multimers. Most cases of acquired vWD are due to a circulating antibody which combines with the high molecular weight multimers. This vWF multimer-antibody complex is cleared from the circulation or adsorbed onto the tumor cells.

**FIBRINOLYTIC DISORDERS**

Increased fibrinolytic activity can lead to an increased risk of hemorrhage while decreased fibrinolytic activity is associated with an increased risk of arterial and venous thrombosis. The most common causes of increased fibrinolytic activity are:

- Increased TPA secretion during cardiopulmonary bypass.
- Reduced TPA clearance in patients with cirrhosis and other forms of liver disease.
- Congenital deficiency of PAI-1 or antiplasmin.

The most common cause of reduced fibrinolytic activity is a hereditary or acquired elevation of PAI-1 activity.

For consultation on the diagnosis of potential fibrinolytic abnormalities please contact Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131. For after-hours consultations please contact the Laboratory Medicine Resident on call at (206) 548-6190.
THROMBOSIS

Inherited bleeding disorders have been known for centuries. We have been aware of inherited abnormalities leading to thrombosis for only the past two decades. Since 1980, major advances have been made in research leading to a better understanding of the clinical syndromes and clinical tests available to evaluate these patients. First, it must be determined whether arterial or venous thrombosis exists, as the approach to laboratory evaluation differs.

ARTERIAL THROMBOSIS

Causes of arterial thrombosis include arteriosclerosis, impaired fibrinolysis, antiphospholipid syndrome and malignancy. Arterial occlusion often results from formation of a fresh thrombus overlying a ruptured atherosclerotic plaque. Arteriosclerosis is a complex process that includes vascular injury, lipid deposition and activation of macrophages, platelets and smooth muscle cells. The arteriosclerotic vessel is morphologically abnormal and has a functional abnormality that predisposes to thrombosis or excessive vasoconstriction. The endothelium overlying the arteriosclerotic lesion synthesizes reduced amounts of prostacyclin and TPA. Blood passing over the lesion is exposed to increased shear stress, enhancing platelet activation.

A myocardial infarction or other arterial thrombosis in a patient younger than 55 years who is otherwise healthy may indicate an abnormality of the fibrinolytic system, hemostatic system or increased homocysteine levels. Some of these patients will have an inherited abnormality and associated family history of arterial thrombosis.

We recommend limiting arterial thrombosis workups to patients with first episode of arterial thrombosis before age 55 and no known lipid abnormalities. Patients should be studied at least 2 months after their last episode of arterial thrombosis, as acute phase changes associated with thrombosis and infarction can alter results.

EVALUATION OF ARTERIAL THROMBOSIS

Arterial Thrombosis Panel (panel includes)

- PAI-1 activity
- Total TPA antigen
- C-reactive Protein

The fibrinolytic system follows a circadian rhythm. Draw samples between 0700 and 1000 (optimum 0800 to 0900). Indicate time on blood collection on label.

Lupus Anticoagulant Panel (panel includes)

- Lupus inhibitor assay
- Anticardiolipin antibodies

Plasma Homocysteine

Patients with arterial thrombosis associated with fibrinolytic abnormalities typically present with myocardial infarctions at a young age (20-55 years old), clinically insignificant atherosclerosis, increased levels of PAI-1 activity and increased total TPA antigen, but decreased TPA activity. Fibrinolytic abnormalities are unlikely in patients presenting with the first episode of cardiac symptoms after the age of 60, further laboratory workup is not considered useful in these patients.
Elevated homocysteine levels in young adults are associated with an increased risk of arterial and venous thrombosis. These patients also have accelerated atherosclerosis.

Antiphospholipid syndrome is also associated with both arterial and venous thrombosis. Characteristic of this disorder is a persistently elevated lupus inhibitor (lupus anticoagulant) and/or anticardiolipin antibodies. Auto immune diseases may be present, and there usually is no family history of arterial thrombosis.

Research studies have indicated that increased levels of fibrinogen and factor VII may be associated with an increased risk of arterial thrombosis. Presently though, screening of coronary artery disease patients for high fibrinogen or factor VII is not recommended.

VENOUS THROMBOSIS

Venous thromboembolism may occur for a variety of reasons including stasis (post-operative or immobilization), vascular disorders (infection or inflammation), and quantitative and/or qualitative abnormalities of the coagulation and fibrinolytic systems. A predisposition to forming venous thrombi is known as thrombophilia and may be inherited or acquired. Thrombi occur most often in the veins of the pelvis or lower extremities, but may also occur in the mesenteric veins or superior sagittal sinus. Thrombosis occurring in an otherwise healthy young or middle aged patient is often associated with an inherited disorder. A first-time thrombotic event occurring in an older patient is more often due to stasis, malignancy or anti-phospholipid syndrome.

To evaluate a patient for venous thrombophilia it is optimum to wait two months or longer after the last episode of thrombosis until the patient is recovered and off anticoagulant therapy for at least 7 to 10 days prior to testing. The protein C and protein S activity assays cannot be done on patients taking oral anticoagulants. Heparin therapy interferes with measurement of baseline antithrombin activity. Activated protein C resistance (APCR) and lupus inhibitor assays are the only testing that should be done when the patient is on oral anticoagulants or heparin.

EVALUATION OF VENOUS THROMBOSIS

Venous Thrombosis Panel (panel includes)
- Activated Protein C Resistance (APCR)
- Protein C activity
- Protein S activity
- Antithrombin activity
- Lupus Inhibitor assay

If all other assays are negative order
- Prothrombin (Factor II) DNA screen

If APCR is positive, for confirmation order
- Factor V DNA screen

Thrombophilia DNA Screen (panel includes)
- Factor V DNA screen
- Prothrombin (Factor II) DNA screen

We recommend starting with the Venous Thrombosis Panel, if this panel is negative follow-up with a Prothrombin (Factor II) DNA screen. If the Activated Protein C Resistance is positive it can be confirmed with a Factor V DNA screen.
The primary proteins associated with thrombosis are quantitative and/or qualitative deficiencies of the naturally occurring inhibitors, protein C, protein S, or antithrombin III. In 1993 Activated Protein C Resistance (APCR) was described. The defect is a single point mutation in the Factor V gene that results in the substitution of arginine at the amino acid residue 506 with glutamine (FVR506 Q). This mutation prevents APC from cleaving a peptide bond at ARG-506 that is required to inactivate Factor Va. This mutation is also known as Factor V Leiden. Recently another defect has been described, a polymorphism of the prothrombin (factor II) gene (base 20210G->A) which leads to elevated levels of prothrombin in plasma. Evaluation of the prothrombin gene requires DNA testing.

The APCR defect is the most common genetic defect associated with thrombosis, occurring in approximately 5% of the normal population and in approximately 20-40% of patients with documented thrombophilia. Approximately 2% of the normal population and 10% of thrombophilia patients have the prothrombin polymorphism. Together, hereditary protein S, protein C, and anti-thrombin III account for approximately 12-15% of documented cases of thrombophilia.

Clinically, most of the inherited disorders present with venous thrombosis after puberty, and may be associated with minor trauma, surgery or pregnancy. Approximately 50% of the patients with hereditary thrombophilia present with a thrombotic event before age 35. Recently, it has been recognized that many patients with thrombophilia have multiple genetic defects that combine to increase their thrombotic risk. Protein C, protein S, or antithrombin III deficiency may be combined with each other, but the most common combination is with the R506Q mutation of factor V or the prothrombin mutation. While a single factor deficiency may be associated with a 2-5 fold increased risk for thrombosis, patient with a combined deficiency may have a ten-fold or greater increase in the risk of venous thrombosis. The finding of combined deficiencies has led to a reevaluation of laboratory tests used to screen for thrombophilia. It is no longer recommended to run a single test or a reflexive panel, instead a complete panel of thrombophilia assays should be run to evaluate the patient.

One cause of acquired venous and arterial thrombosis is the anti-phospholipid syndrome. This disorder is associated with persistently elevated anticardiolipin antibodies and/or lupus inhibitor (also known as lupus anticoagulant). A woman presenting with multiple spontaneous abortions should also be suspect for the antiphospholipid syndrome. Other acquired causes of venous thrombosis include trauma, nephrotic syndrome, liver disease, venous insufficiency, and malignancy. When a venous thrombosis occurs in an unusual location such as a portal axillary or intracranial vein, there is a high index of suspicion for an antiphospholipid syndrome, antithrombin III deficiency, or malignancy.
REFERENCE CHART OF DIAGNOSTIC TEST/PANELS AVAILABLE

For more detailed description of tests, please see page 19. Please refer to pages 4 to 5 for instructions on the collection, processing, and storage of samples.

<table>
<thead>
<tr>
<th>TEST/PANEL</th>
<th>SAMPLE</th>
<th>REFERENCE RANGE</th>
<th>FREQUENCY OF TESTING</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Partial Thromboplastin Time (APTT)</td>
<td>1.0 mL frozen plasma</td>
<td>call 548-6066 or 800 713-5198</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Anti-beta 2 glycoprotein I</td>
<td>1.0 mL frozen serum</td>
<td>&lt; 10 units</td>
<td>T, F</td>
<td>Part of antiphospholipid panel</td>
</tr>
<tr>
<td>Anticardiolipin IgA 1.0 mL frozen serum</td>
<td>&lt; 12 APL</td>
<td>T, F</td>
<td></td>
<td>Part of antiphospholipid panel</td>
</tr>
<tr>
<td>Anticardiolipin IgG 1.0 mL frozen serum</td>
<td>&lt; 12 GPL</td>
<td>T, F</td>
<td></td>
<td>Part of antiphospholipid panel</td>
</tr>
<tr>
<td>Anticardiolipin IgM 1.0 mL frozen serum</td>
<td>&lt; 15 MPL</td>
<td>T, F</td>
<td></td>
<td>Part of antiphospholipid panel</td>
</tr>
<tr>
<td>Antiphospholipid Panel 1.0 mL frozen serum</td>
<td>see individual tests</td>
<td></td>
<td></td>
<td>Includes anticardiolipin IgG, IgM &amp; IgA plus anti-beta 2 glycoprotein I</td>
</tr>
<tr>
<td>APTT (1:1 mix)</td>
<td>1.0 mL frozen plasma</td>
<td>call 548-6066 or 800 713-5198</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Activated Protein C Resistance (APCR)</td>
<td>1.0 mL frozen plasma</td>
<td>negative T, Th</td>
<td></td>
<td>Modified Factor V method. Test not affected by oral anticoagulant therapy, heparin or lupus anticoagulant. Part of Venous thrombosis panel</td>
</tr>
<tr>
<td>Anticardiolipin IgG/IgM 0.5 mL serum</td>
<td></td>
<td></td>
<td></td>
<td>Test performed in Immunology Division. Call 548-6066 or 800 713-5198</td>
</tr>
<tr>
<td>Anti-thrombin III Activity</td>
<td>1.0 mL frozen plasma</td>
<td>75-125 %</td>
<td>T,Th</td>
<td>Chromogenic method. Part of Venous thrombosis panel. Patient should be off heparin for at least 3 days prior to testing.</td>
</tr>
<tr>
<td>Arterial Thrombosis Panel</td>
<td>3.0 mL frozen plasma; 0.5 mL frozen serum</td>
<td>see individual tests</td>
<td>weekly</td>
<td>Panel includes PAI-1, tPA antigen, lupus inhibitor, and c-reactive protein. Collect blood between 0700 and 1000. The patient should not have an ongoing thrombosis and 2 months should have elapsed since the thrombotic event.</td>
</tr>
<tr>
<td>C-Reactive Protein 0.5 mL frozen serum</td>
<td></td>
<td></td>
<td></td>
<td>Test performed in Immunology Division. Call 548-6066 or 800 713-5198</td>
</tr>
<tr>
<td>D-Dimer (semiquantitative) 0.5 mL frozen plasma</td>
<td>&lt;250 ng/mL</td>
<td>daily</td>
<td></td>
<td>latex method</td>
</tr>
<tr>
<td>D-Dimer (quantitative) 1.0 mL frozen plasma</td>
<td>&lt;400 ng/mL</td>
<td>weekly</td>
<td></td>
<td>ELISA method</td>
</tr>
<tr>
<td>Factor II Activity 1.0 mL frozen plasma</td>
<td>50-150 %</td>
<td>M-F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V Activity 1.0 mL frozen plasma</td>
<td>50-150 %</td>
<td>M-F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEST/PANEL</td>
<td>SAMPLE</td>
<td>REFERENCE RANGE</td>
<td>FREQUENCY OF TESTING</td>
<td>COMMENT</td>
</tr>
<tr>
<td>-----------</td>
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<td>----------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Factor V DNA Screen (factor V Leiden)</td>
<td>EDTA Whole Blood</td>
<td></td>
<td></td>
<td>Test performed in Genetic Division Call 548-6066 or 800 713-5198</td>
</tr>
<tr>
<td>Factor VII Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-150 %</td>
<td>M-F</td>
<td></td>
</tr>
<tr>
<td>Factor VIII Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-180 %</td>
<td>M-F</td>
<td></td>
</tr>
<tr>
<td>Factor IX Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>M-F</td>
<td></td>
</tr>
<tr>
<td>Factor X Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>M-F</td>
<td></td>
</tr>
<tr>
<td>Factor XI Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>M-F</td>
<td></td>
</tr>
<tr>
<td>Factor XII Activity</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>M-F</td>
<td></td>
</tr>
</tbody>
</table>
| Factor XIII Assay (urea solubility) | 0.5 mL frozen plasma | negative M-F | | Semiquantitative assay. Test positive if factor XIII levels less than 2%.
<p>| Factor Inhibitor Assay | 2-3 mL frozen plasma | 0 units/mL | M-F | Bethesda assay. Please indicate the specific factor on the requisition form. |
| Porcine Factor VIII Inhibitor Assay | 2 mL frozen plasma | 0 units/mL | M-F | Bethesda assay. Assay determines cross reactivity to Porcine VIII in a patient with factor VIII inhibitor. |
| Fibrinogen | 1.0 mL frozen plasma | 150-400 Daily mg/dL | | Includes total TPA, antigen, PAI-1 activity and C-reactive protein. Draw between 0700 and 1000. Note collection time on requisition form. |
| Fibrinolysis Screen | 3 mL frozen plasma | See individual tests weekly | | |
| Heparin activity (unfractionated) | 0.5 mL frozen plasma | 0.2-0.4 units/mL | M-F | Sample must be processed within one hour of collection. Please note type of heparin on requisition form. |
| Heparin Activity (low molecular weight) | 0.5 mL frozen plasma | | M-F | Sample must be process within one hour of collection. Please note type of heparin on requisition form. |
| Homocysteine | 0.5 mL frozen EDTA plasma | | | Test performed in Chemistry Division. Please call 548-6066. |
| Lupus Anticoagulant Panel | 1.0 mL frozen plasma; 0.5 mL serum | See individual tests | T, TH | Includes anticardiolipin IgG/IgM anti-beta-2 glycoprotein I, and Lupus inhibitor assay. |
| Lupus Inhibitor Assay (Lupus anticoagulant) | 1.0 mL frozen plasma | negative T,Th | | Critical the plasma is centrifuged twice to remove all platelets. Hexagonal Phospholipid method. Positive patient reported as weak positive, positive or strong positive. |
| Plasminogen Activator Inhibitorplasma type 1 (PAI-1) | 3.0 mL frozen | 2400-1000 weekly | 2.0-15 IU/mL; 1000-1600 1.7-11 IU/mL 1600-2400 | Note collection time on tube |</p>
<table>
<thead>
<tr>
<th>TEST/PANEL</th>
<th>SAMPLE</th>
<th>REFERENCE RANGE</th>
<th>FREQUENCY OF TESTING</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Aggregation</td>
<td>Citrate whole blood</td>
<td>call 548-6066 or 800 713-5198</td>
<td>by appointment only</td>
<td>Patient must come to the Laboratory to be drawn. Call 548-6066 or 800 713-5198 for instructions.</td>
</tr>
<tr>
<td>Protein C Activity</td>
<td>1.0 mL frozen plasma</td>
<td>65-150 %</td>
<td>T, Th</td>
<td>Clot based assay. Screen test for protein C deficiency. Part of venous thrombosis panel. Patient should not have ongoing thrombosis. Patient should be off oral anticoagulant therapy 7 to 10 days.</td>
</tr>
<tr>
<td>Protein C Antigen</td>
<td>1.0 mL frozen plasma</td>
<td>65-145 %</td>
<td>Th</td>
<td>ELISA assay. See Protein C activity.</td>
</tr>
<tr>
<td>Protein S Activity</td>
<td>1.0 mL frozen plasma</td>
<td>65-150 %</td>
<td>T, Th</td>
<td>Screen test for protein S deficiency. Part of venous thrombosis panel. Patient should not have ongoing thrombosis and be off oral anticoagulant therapy 7 to 10 days.</td>
</tr>
<tr>
<td>Protein S Antigen Free</td>
<td>0.5 mL frozen plasma</td>
<td>65-135 %</td>
<td>Th</td>
<td>ELISA assay. See Protein S activity.</td>
</tr>
<tr>
<td>Protein S Antigen Total</td>
<td>0.5 mL frozen plasma</td>
<td>75-140%</td>
<td>Th</td>
<td>ELISA assay. See Protein S activity.</td>
</tr>
<tr>
<td>Prothrombin DNA Screen</td>
<td>EDTA whole blood</td>
<td></td>
<td></td>
<td>Test performed in Genetic Division Call 548-6066 or 800 713-5198</td>
</tr>
<tr>
<td>Prothrombin Time (PT)</td>
<td>0.5 mL frozen plasma</td>
<td>call 548-6066 or 800 713-5198</td>
<td>daily</td>
<td></td>
</tr>
<tr>
<td>Prothrombin time 1:1 Mix</td>
<td>1.0 mL frozen plasma</td>
<td>call 548-6066 or 800 713-5198</td>
<td>daily</td>
<td></td>
</tr>
<tr>
<td>Prothrombin Fragment 1.2</td>
<td>1.0 mL frozen plasma</td>
<td>0.4-1.1 nmol/L</td>
<td>weekly</td>
<td>ELISA method</td>
</tr>
<tr>
<td>TPA Activity</td>
<td>1.0 mL acid citrate plasma frozen</td>
<td>0000-1000</td>
<td>weekly</td>
<td>Sample must be drawn in acid citrate. Label tube acid citrate. Blood should be collected between 0700-1000. Note collection time on requisition form.</td>
</tr>
<tr>
<td>tPA antigen</td>
<td>1.0 mL frozen plasma</td>
<td>3 - 12 ng/mL</td>
<td>weekly</td>
<td></td>
</tr>
<tr>
<td>Thrombin Time</td>
<td>1.0 mL frozen plasma</td>
<td>16-28 seconds</td>
<td>daily</td>
<td></td>
</tr>
<tr>
<td>Thrombin Time 1:1 mix</td>
<td>1.0 mL frozen plasma</td>
<td>call 548-6066 or 800 713-5198</td>
<td>daily</td>
<td></td>
</tr>
<tr>
<td>Thromboelastograph</td>
<td>5.0 mL whole citrate blood</td>
<td>r.3-22 mm k.2-8 mm r+k: 7-30 mm angle: 54-80 mm MA: 48-80 mm A60: 45-80 mm</td>
<td>daily</td>
<td>Sample must be received within 4 hours of the time it is collected.</td>
</tr>
<tr>
<td>TEST/PANEL</td>
<td>SAMPLE</td>
<td>REFERENCE RANGE</td>
<td>FREQUENCY OF TESTING</td>
<td>COMMENT</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Venous Thrombosis 1.0 mL</td>
<td>frozen plasma</td>
<td>see individual tests</td>
<td>T,Th</td>
<td>Panel includes protein C, activity, protein S activity, antithrombin III, APCR and lupus inhibitor. Patient should not have an ongoing thrombosis and should be off oral anticoagulant therapy 7-10 days prior to testing.</td>
</tr>
<tr>
<td>von Willebrand Disease Panel</td>
<td>1.0 mL frozen plasma</td>
<td>see individual tests</td>
<td>set up W</td>
<td>Panel includes factor VIII activity, vWF results Fantigen and multimer analysis.</td>
</tr>
<tr>
<td>von Willebrand Factor</td>
<td>1.0 mL frozen plasma</td>
<td></td>
<td>W</td>
<td></td>
</tr>
<tr>
<td>(Ristocetin cofactor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>von Willebrand Factor Antigen</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>W</td>
<td></td>
</tr>
<tr>
<td>von Willebrand Factor</td>
<td>1.0 mL frozen plasma</td>
<td>50-150%</td>
<td>W</td>
<td></td>
</tr>
<tr>
<td>Multimer Analysis</td>
<td>1.0 mL frozen plasma</td>
<td>all multimers present</td>
<td>set up W</td>
<td></td>
</tr>
</tbody>
</table>
DESCRIPTION OF CLINICAL DIAGNOSTIC TESTS

Diagnostic Panels

ANTI PHOSPHOLIPID PANEL

Useful in the diagnosis of patients with unexplained arterial or venous thrombosis, or recurrent spontaneous abortion. Panel consists of anti-cardiolipin IgG, IgM and IgA plus anti-beta 2 glycoprotein I.

ARTERIAL THROMBOSIS PANEL

Decreased fibrinolytic activity has been associated with an increased risk of arterial thrombosis, particularly myocardial infarction in men and women less than 50 years old. Tests included in the panel are plasminogen activator inhibitor type 1 (PAI-1) activity, tissue plasminogen activator (tPA) antigen and c-reactive protein. PAI-1 activity and TPA antigen can be transiently elevated by the acute phase response. Samples should be drawn at least 2 months after the thrombotic event when the patient has recovered. The fibrinolytic system has a circadian rhythm with peak levels of PAI-1 activity and TPA antigen in the morning. If possible, samples should be drawn between 0800 and 0900. It is acceptable to draw samples between 0700 and 1000. The time of collection should be noted on the requisition form. The panel is considered positive when both PAI-1 activity and TPA antigen are elevated but CRP is normal (indicating no acute phase response).

If you have any questions regarding the use or interpretation of this panel to screen for potential causes of arterial thrombosis, please contact Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131.

LUPUS ANTICOAGULANT PANEL

Anti-phospholipid antibodies are associated with an increased risk of venous and arterial thrombosis and recurrent spontaneous abortion. Comprehensive panel of assays related to the anti-phospholipid antibody syndrome including Lupus Inhibitor Assay, Anticardiolipin IgG, Anticardiolipin IgM, and Anti-beta 2 glycoprotein I.

PLASMINOGEN ACTIVATOR INHIBITOR PANEL

Patients with a deficiency of plasminogen activator inhibitor type 1 (PAI-1) may have a bleeding syndrome typically characterized by delayed bleeding after surgery or trauma. This panel includes a PAI-1 activity and PAI-1 antigen level. Typically PAI-1 activity is less than 1 U/mL and PAI-1 antigen is less than 2 ng/mL. In some cases the PAI-1 activity may be reduced while the PAI-1 antigen level is normal.

PLATELET AGGREGATION PANEL

Method: Platelet aggregometry on platelet rich plasma.

The patient should be off aspirin and other NSAID drugs for ten days prior to testing.

Platelet aggregation studies are used to assist in the diagnosis of hereditary and acquired qualitative platelet disorders. Platelet aggregation studies are most useful in patients with a platelet-like bleeding syndrome, often characterized by immediate bleeding after trauma or surgery and spontaneous mucosal bleeding with a normal platelet count. Platelet aggregation studies are not useful in the evaluation of patients with low platelet counts or in patients with thrombotic syndromes.
Platelet aggregation is tested using arachidonic acid, ADP, epinephrine, collagen, and ristocetin. Both the total amount of aggregation and the rate and type of aggregation are evaluated. In normal platelets, a low dose of aggregating reagent stimulates the platelets to release ADP from their granules, which stimulates further aggregation leading to a biphasic curve or secondary aggregation. The secondary phase is seen with low dose ADP and epinephrine. Only the primary phase is noted with arachidonic acid, collagen, and ristocetin.

Arachidonic acid aggregation requires normal activity of the cyclooxygenase enzyme in platelets, aspirin (acetylsalicylic acid) and other cyclooxygenase inhibitors such as NSAID's block platelet aggregation using arachidonic acid up to 8-10 days. Primary aggregation with ADP, epinephrine and collagen are absent in patients with homozygous thrombasthenia and diminished in heterozygous thrombasthenia. Variable abnormalities in the secondary phase of aggregation are seen in storage pool disease, aspirin-like defect, and in patients who have taken aspirin or related drugs. Failure to respond to epinephrine is present in myeloproliferative disorders. However, it must be noted that approximately 25% of the normal population does not respond to epinephrine. Decreased response to ristocetin is suggestive of von Willebrand disease, Bernard Soulier disease, or platelet pseudo-von Willebrand disease.

**THROMBOELASTOGRAPH PANEL**

The thromboelastograph is a rapid empirical screen for overall hemostatic function. Whole citrated blood is recalcified in the instrument which then measures the increase in the elastic shear modulus of the clot as it forms over the period of an hour. The width of the graph at any point is the clot strength or elasticity. Five graph parameters are measured when the TEG is complete. The "r" value is a measure of the time it takes for clotting to begin, it is similar to a whole blood clotting time. If the "r" value is prolonged, it indicates a possible coagulation deficiency that could be followed up with PT, PTT and factor assays. The "k" value and angle (α) indicate how fast clot strength is increasing once clotting starts. Reduced "k" or angle may be due to any combination of coagulation deficiencies, reduced platelet function and reduced platelet count. The maximum amplitude (MA) or maximum width of the graph is an indication of the maximum attainable clot strength. Decreases in MA are associated with reduced platelet function or number and with reduced fibrinogen levels. The amplitude after 60 minutes (A60) is a measure of fibrinolysis, if the A60 is significantly less than the MA it indicates in vivo clot lysis may be occurring.

**THROMBOPHILIA DNA SCREEN**

The panel consists of two DNA based tests for inherited venous thrombotic disorders, the Factor V DNA Screen (1691G->A, R506Q, factor V Leiden) and the Prothrombin (Factor II) DNA Screen (base 20210 G->).

**VENOUS THROMBOSIS PANEL**

Tests included in the panel are APCR, protein C activity, protein S activity, anti-thrombin III and lupus inhibitor. The tests should not be performed during an ongoing thrombosis. The only test that can be run during this period is the APCR assay. The patient should be off oral anticoagulant therapy for a minimum of 7 days. See page 13 for a detailed description of venous thrombosis.

**VON WILLEBRAND DISEASE PANEL**

Comprehensive panel for the diagnosis of von Willebrand disease including most common subtypes (type 1, type 2 and type 3). Panel consists of von Willebrand factor antigen, factor VIII activity, von Willebrand factor multimer analysis and interpretive report. See page 9 for detailed description of von Willebrand disease and different subtypes.
Individual Tests

**ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)**

*Method:* clot based assay.

The activated partial thromboplastin time (APTT) is a screening assay used to detect abnormalities in the intrinsic pathway of coagulation with specific sensitivity to factors VIII, IX, XI and XII.

**ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) 1:1 MIX**

*Method:* clot based assay.

The APTT may be prolonged due to a factor(s) deficiency or an inhibitor. A 1:1 mix is used to differentiate between the two. Equal parts of patient plasma and normal plasma pool are combined and an APTT is performed on the mixture. Correction of the prolonged APTT on the mixture indicates a factor deficiency. No correction or partial correction indicates an inhibitor.

**ACTIVATED PROTEIN C RESISTANCE (APCR) (also known as Factor V resistance to APC)**

*Method:* clot based factor V degradation assay.

This method is a modification of the assay described by Le, et al. It specifically measures the rate of factor Va degradation in patient plasma by APC. The assay has shown close correlation with the factor V DNA screen. In contrast to other APCR methods, this assay is not affected by oral anticoagulant therapy, heparin therapy, or the presence of a lupus inhibitor. The assay is not valid on a patient with an initial factor V level less than 20%. To differentiate a heterozygote from homozygote, it is recommended that the factor V DNA screen be run (available through the Genetics Division). Activated protein C resistance is inherited as an autosomal dominant. The abnormality is a point mutation in the gene encoding for coagulation factor V and is commonly called factor V Leiden mutation. This mutation prevents APC from cleaving a peptide bond at ARG-506 that is required to inactivate factor Va. This abnormality occurs in 5% of the normal population and may occur in 20-40% of patients with thrombophilia. A patient with this mutation has a lifelong increased risk of thrombosis. If the disorder is combined with another genetic defect such as a deficiency of protein C, protein S, or anti-thrombin III, the risk is increased.

**ANTITHROMBIN III ACTIVITY**

*Method:* chromogenic substrate, back titration method.

Samples for antithrombin III levels should be obtained at least a week after resolution of the acute thrombotic event and a minimum of 3-4 days post heparin therapy. Congenital antithrombin III deficiency is inherited as autosomal dominant. Patients usually have less than 60% antithrombin III activity. The disorder occurs in 2-3% of patients presenting with venous thrombophilia. Acquired antithrombin III deficiency occurs in acute thrombosis, liver disease, kidney disease, nephrotic syndrome, sepsis, DIC, post-operatively and during asparaginase or heparin therapy. Once heparin therapy is stopped the antithrombin III level should return to baseline levels within 72 hours. Elevated antithrombin III levels have no known clinical significance.
D-DIMER ASSAY - QUANTITATIVE  (cross-linked fibrin degradation products)

Method:  ELISA

Cross-linked fibrin degradation products are formed by enzymatic degradation of factor XIII cross-linked fibrin. D-dimers are elevated in pulmonary embolus, deep venous thrombosis, arterial thrombosis, malignancy, sepsis, cirrhosis, post-operatively and during disseminated intravascular coagulation. The level of D-dimer is influenced by the severity of the thrombotic episode, rate of D-dimer production, and the time the specimen was drawn after the thrombotic episode. A normal level of D-dimer by this method helps rule out the presence of evolving deep venous thrombosis or pulmonary embolus and has been used as a screening test when deep venous thrombosis has been suspected. Some use the assay to monitor the prethrombotic states of patients with protein C, protein S, or anti-thrombin III deficiency. In these patients the D-dimer levels become normal during oral anticoagulant therapy and then rise when the therapy is discontinued. The assay has to be used to assist in the decision to initiate and/or continue a long term therapy on such patients.

D-DIMER ASSAY (SEMIQUANTITATIVE)  (fibrin degradation products)

Method:  latex agglutination assay.

Rapid semi-quantitative measurement of cross linked fibrin degradation products in human plasma using a monoclonal antibody directed against cross linked D fragments.

FACTOR V DNA SCREEN (FACTOR V LEIDEN)

Method:  DNA based PCR assay.

The Gln-506 (1691G->A, R506Q, factor V Leiden) polymorphism in the factor V gene is present in approximately 3-5% of the general population, and in about 20-50% of patients with a history of unexplained recurrent venous thrombosis. The presence of a glutamine instead of an arginine residue removes a site in factor V that is normally cleaved by activated protein C, and is associated with in vitro resistance to activated protein C. Presence of this polymorphism substantially increases the lifetime risk of venous thrombosis. This DNA-based test detects the underlying defect, but does not evaluate the current state of the patient's blood coagulation system.

FACTOR ASSAYS (II, V, VII, VIII, IX, X, XI, or XII)

Method:  one-stage clotting based tests.

These assays are based on the ability of dilute patient plasma to correct the clotting time of a specific factor deficient plasma as measured by the prothrombin time (II, V, VII, or X) or APTT (VIII, IX, XI, or XII). Congenital deficiencies of factors II, V, VII, or X are rare and all are inherited as autosomal recessives. All will be decreased in liver disease. Factor II, VII, IX, and X are decreased in vitamin K deficiency and during oral anticoagulant therapy. In these situations the factor VII is decreased first and then followed in order by IX, X, and II. The rate of decrease is determined by the in vivo half life of the proteins. Factor VIII, IX, XI, and XII are known as the intrinsic group of factors. Isolated factor deficiencies in this group will present with a borderline to marked prolongation of the APTT and all other screen procedures are normal.
FACTOR XIII ASSAY

**Method:** semiquantitative urea solubility test.
This screening assay is used to detect factor XIII levels greater than 2%. If the concentration of factor XIII is greater than 2%, in the presence of calcium factor XIII will crosslink the clot making it insoluble in 6 M urea. A clot that dissolves in urea indicates the patient is homozygous for factor XIII deficiency. The assay will not detect a mild deficiency. The homozygous patient presents with spontaneous bleeding and poor wound healing with unusual scar formation. A patient that has a normal prothrombin time, APTT, thrombin time, fibrinogen and platelet count in the presence of excessive bleeding should be screened for factor XIII deficiency.

FACTOR INHIBITOR ASSAY

**Method:** Bethesda assay.
Acquired factor inhibitors circulate as antibodies that neutralize the procoagulant activity of a specific coagulation factor. This results in a acquired deficiency state with an associated risk of bleeding. Inhibitors have been described against all the clotting factor proteins. A factor inhibitor should be suspected when a prolonged prothrombin time and/or APTT does not correct on a 1:1 mix and a lupus inhibitor has been excluded. In the inhibitor assay, dilutions of patient plasma are incubated with pooled normal plasma for two hours at 37°C. At the end of the incubation period the factor activity in the mixture is measured. One inhibitor unit is defined as the amount of inhibitor that inactivates 50% of the factor in the normal plasma pool.

Factor VIII inhibitors are time and temperature dependent which necessitates the incubation period of two hours. This is not true for the other clotting factors; an incubation period of thirty minutes is usually sufficient to express the full affect of these inhibitors.

**Factor VIII inhibitors** associated with hemophilia A are the most common. There are two types of inhibitors. In a type I inhibitor there is complete inactivation of the factor. The Bethesda assay can be used accurately quantitate the inhibitor level. The majority of the hemophilia A patients with anti-factor VIII antibodies fit into this category. Approximately 15% of all hemophilia A patients and 95% severe hemophiliacs will develop inhibitors. An inhibitor is suspected when the usual therapy does not have the expected results or bleeding has become more significant. Type II inhibitors show a partial, competitive type of inhibition which precludes accurate measurement of the inhibitor level using the Bethesda assay. Many non-hemophilic inhibitors are of type II.

The spontaneous development of factor VIII inhibitor in a non hemophilic patient is rare. Clinically, these patients may present with bleeding similar to hemophilia A such as gastrointestinal bleeding, joint or muscle bleeding, excessive bruising, hematuria, post operative or post trauma bleeding. This bleeding may lead to life threatening central nervous system or retropharyngeal hemorrhage. This disorder may occur in autoimmune disease, lymphoproliferative malignancy, non-hematologic malignancy, drugs, dermatological disorders and post partum and in older men and women with no other underlying disease.

**Factor V inhibitors** have been described with after surgery, post transfusion, with infections, insect bite and after interoperative use of bovine topical thrombin which contains small amounts of bovine factor V. This inhibitor is associated with clinical bleeding.

Approximately 3% of hemophilia B patients develop **factor IX inhibitors**. This occurs most often in severe hemophilia B. Factor IX inhibitors are not time or temperature dependent. Spontaneous factor IX antibodies are very rare and are usually associated with an autoimmune disorder.
FIBRINOGEN

Method: nephelometric method based on total light scattering of clot formed during prothrombin time assay.

Acquired abnormalities of fibrinogen can be quantitative or qualitative and may be associated with a bleeding problem or with thrombosis. Decreased quantities of fibrinogen are noted in liver disease, renal disease, ascites, acute DIC and asparaginase therapy. Acquired dysfunctional fibrinogen is observed in nephrotic syndrome and DIC. Fibrinogen is an acute phase reactant protein. It is markedly increased in inflammation and infection. During pregnancy the fibrinogen level rises rapidly with a two to three fold increase noted by the end of the third trimester. An elevated fibrinogen is an indication that a acute phase response is occurring that may lead to increased levels of factor VIII, von Willebrand factor and PAI-1. Recent studies suggest that chronically increased fibrinogen levels are associated with an increased risk of arterial thrombosis including stroke and myocardial infarction.

Inherited abnormalities may be due to a lack of fibrinogen (afibrinogenemia, autosomal recessive), deficiency of fibrinogen (hypofibrinogenemia, autosomal dominant or recessive), or a dysfunctional fibrinogen molecule (dysfibrinogenemia, autosomal dominant). All of the mentioned conditions are rare. Dysfunctional fibrinogens are the most common and may be asymptomatic or associated with an increased risk of bleeding or thrombosis depending on the specific defect. The prothrombin time has a slight to moderate prolongation; the APTT is normal to slight prolongation; the thrombin time is moderately prolonged. On a 1:1 mix the thrombin time may show partial correction. The diagnosis of a dysfunctional fibrinogen can be confirmed with a kinetic fibrinogen determination. Typically the kinetic fibrinogen will be lower than the nephelometric total fibrinogen. To specifically classify the type of dysfunctional fibrinogen it is necessary to perform specific research assays to identify the abnormality in the protein.

HEPARIN ACTIVITY (UNFRACTIONATED)

Method: chromogenic, anti-Xa activity assay.

Note: It is important to note the type of heparin the patient is receiving when requesting this assay. This assay is calibrated using standard unfractionated heparin. For measurement of low molecular weight heparin see assay below. Generally patients on heparin therapy are monitored with the APTT. Conditions other than heparin may be present that alter the APTT making the test ineffective as a heparin monitoring tool. The most common cause of interference is the presence of a lupus anticoagulant. Lupus inhibitors do not interfere with the heparin activity assay.

HEPARIN ACTIVITY (LOW MOLECULAR WEIGHT)

Method: chromogenic, anti-Xa activity assay.

Note: It is important to note the type of heparin the patient is receiving when requesting this assay. The principle of the assay is the same as unfractionated heparin, but a low molecular weight heparin is used to construct the calibration curve. The APTT cannot be used to monitor low molecular weight heparin therapy. Low molecular weight heparin inhibits factor Xa more potently than thrombin. Currently this assay is calibrated using Enoxaparin, if another type of low molecular weight heparin is used please indicate on the request form and provide a sample of pure low molecular weight heparin for use in preparing a standard curve.
LUPUS INHIBITOR (ANTICOAGULANT)

**Method:** hexagonal (II) phase phospholipid clotting assay.

It is very important that all the platelets be removed from the sample prior to freezing as residual platelets will adversely affect the test results. Freezing will rupture the platelet membrane potentially resulting in neutralization of some of the antiphospholipid antibody, which may cause a false negative result in the assay.

A mixture of patient plasma, normal plasma pool, and a buffer are added to a special APTT reagent that is very sensitive to the lupus inhibitor. If the APTT is prolonged, phospholipid is substituted for buffer and the APTT repeated. If the phospholipid corrects the APTT, a lupus inhibitor is present. Patients are reported as negative, weak positive, positive or strongly positive. A patient that is weakly positive may have a transient lupus inhibitor associated with infection and reaction to some drugs. It is recommended that a weak positive lupus inhibitor be repeated in two months.

Lupus inhibitors (antiphospholipid antibodies) are immunoglobulins that interfere with phospholipid dependent coagulation assays. Typically the APTT is the first assay to be affected. In these patients the APTT is moderately to markedly prolonged and will not completely correct on 1:1 mix. The prothrombin time may be in the upper limits of the normal reference range or slightly prolonged. The prothrombin time prolongation may be due to either the antiphospholipid antibody and/or decreased factor II. Hypoprothrombinemia (decreased factor II) has been reported in patients with lupus inhibitor; these patients are at risk for bleeding.

Lupus inhibitors are associated with arterial and venous thrombosis. If a patient has a thrombotic episode and it is necessary to anticoagulate the patient, it may be necessary to use an alternate method to monitor treatment. If the patient is placed on heparin therapy, it will be necessary to monitor heparin therapy using an APTT reagent that is insensitive to the lupus inhibitor. An alternate method is to quantitate the amount of heparin activity with the heparin assay (see above).

PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI-1) ACTIVITY

**Method:** Immunofunctional assay.

Citrated plasma is added to a microtiter plate containing bound, active TPA. Active PAI-1 in the sample binds TPA forming TPA/PAI-1 complex. The amount of bound PAI-1 is quantitated using a peroxidase conjugated anti-PAI-1 antibody. The amount of active PAI-1 in the sample is proportional to the amount of PAI-1 bound to the plate. One unit of PAI-1 activity is defined as the amount of PAI-1 that inhibits one international unit of single chain tPA.

Fibrinolytic activity in blood follows a circadian rhythm. Peak levels of PAI-1 occurs in the morning with the lowest levels occurring in the evening. For this reason the optimum time to draw blood is between 0800 and 0900. Blood drawn between 0700 and 1000 is acceptable. It is important that the time of blood collection be indicated on the requisition form.

Increased PAI-1 activity indicates impaired fibrinolytic function and may be associated with increased risk of arterial thrombosis. PAI-1 is an acute phase reactant. Increased levels are found in pregnancy, sepsis, deep venous thrombosis and after myocardial infarction.
PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI-1) ANTIGEN

Method: ELISA assay.

Measures total level of PAI-1 in blood including active PAI-1 (see PAI-1 activity above), TPA/PAI-1 complex and latent PAI-1. Typically parallels PAI-1 activity. Useful for diagnosis of elevated PAI-1 in patients with arterial thrombotic disease and in patients with PAI-1 deficiency associated bleeding.

Care must be taken when drawing the sample to avoid release of PAI-1 from platelets.

Fibrinolytic activity in blood follows a circadian rhythm. Peak levels of PAI-1 occurs in the morning with the lowest levels occurring in the evening. For this reason the optimum time to draw blood is between 0800 and 0900. Blood drawn between 0700 and 1000 is acceptable. It is important that the time of blood collection be indicated on the requisition form.

PROTEIN C ACTIVITY

Method: APTT based clotting assay.

The patient should be off oral anticoagulant therapy for at least a week and should be tested at least one month after resolution of the thrombus.

Protein C is a vitamin K dependent protein synthesized in the liver. Protein C is present in plasma as a proenzyme which is converted to its active form by the thrombin/thrombomodulin complex on the endothelial surface. When activated, protein C in the presence of protein S regulates thrombosis by inactivating factors Va and VIIIa.

A qualitative or quantitative protein C deficiency may exist. Type I deficiency has decreased function (activity) and antigen levels. Type II patients have decreased function and normal antigen determination. There is no difference in risk of thrombosis between the two types of protein C deficiency. The protein C activity assay screens for both types of deficiency. Hereditary protein C deficiency is inherited as autosomal dominant. Approximately 3 % of patients with venous thrombophilia have protein C deficiency. Half of patients heterozygous for protein C deficiency will present with venous thrombosis before age 40. Thrombosis of the lower extremity occurs most often, less common is thrombosis of the mesenteric or cerebral veins. Surgery, pregnancy, or immobilization may be an added risk factor in these patients. A protein C deficiency combined with the factor V Leiden mutation increases the risk of venous thrombosis 80 fold. Homozygous or double heterozygous protein C deficiency is extremely rare and is associated with neonatal purpura fulminans.

Decreased protein C levels occur during oral anticoagulant therapy, liver disease, vitamin K deficiency, cirrhosis, DIC, and during an acute thrombotic event. Since this is a clotting based assay, other clotting protein levels or conditions may interfere with the assay. Elevated factor VIII levels (>250%) will underestimate protein C activity. One may over estimate protein C levels in the presence of a lupus inhibitor or if the heparin concentration in the plasma is greater than 0.6 units/mL.
PROTEIN C ANTIGEN

Method: ELISA

The patient should be off oral anticoagulant therapy for at least a week and should be tested at least one month after resolution of the thrombus.

The protein C antigen determination is recommended when the protein C activity falls below 65% provided the patient is not on oral anticoagulant therapy, and does not have vitamin K deficiency, liver disease, DIC, or ongoing thrombosis. The antigen determination is used to classify the type of hereditary protein C deficiency. Type I deficiency has a decreased activity and antigen. Type II deficiency has a low protein C activity and a normal or elevated antigen level.

PROTEIN S ACTIVITY

Method: APTT based clotting assay

Protein S is a vitamin K dependent protein synthesized in the liver. Protein S acts as a cofactor for activated protein C, enhancing the anticoagulant function of activated protein C by increasing its affinity for phospholipid membranes.

Congenital protein S deficiency is inherited as autosomal dominate. Clinically it is very similar to protein C deficiency described above. Patients have an increased risk of venous thrombosis. It is estimated that approximately 3% of patients with venous thrombophilia have protein S deficiency. Acquired protein S deficiency occurs in oral anticoagulant therapy, liver disease, nephrotic syndrome, DIC, ongoing thrombosis, inflammation and pregnancy. There is a marked decrease in pregnancy. Since there is increased risk of thrombosis due to the pregnancy, one needs to be careful in evaluating for hereditary protein S deficiency during pregnancy.

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Mean Activity</th>
<th>Range Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>78</td>
<td>48-108</td>
</tr>
<tr>
<td>Second</td>
<td>51</td>
<td>23-79</td>
</tr>
<tr>
<td>Third</td>
<td>36</td>
<td>18-54</td>
</tr>
</tbody>
</table>

As a clotting based assay the protein S activity assay is influenced by other clotting proteins or conditions. Elevated factor VIII (>250 %), elevated factor VII levels, and positive activated protein C resistance may lead to an underestimate of protein S activity. The presence of a lupus inhibitor or heparin concentration greater than 0.6 units/mL may overestimate protein S activity.

If acquired deficiency of protein S is excluded, a decreased protein S activity should be verified with a free protein S antigen determination.
PROTEIN S ANTIGEN (FREE)

Method: ELISA

As was true for protein S activity, the reference range for free protein S and total protein S are decreased during pregnancy. This needs to be taken into consideration when evaluating a possible hereditary protein S deficiency in a pregnant woman.

<table>
<thead>
<tr>
<th>Trimester</th>
<th>mean free (%)</th>
<th>range free (%)</th>
<th>mean total (%)</th>
<th>range total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>83</td>
<td>62-104</td>
<td>92</td>
<td>67-136</td>
</tr>
<tr>
<td>Second</td>
<td>93</td>
<td>55-131</td>
<td>93</td>
<td>62-120</td>
</tr>
<tr>
<td>Third</td>
<td>75</td>
<td>37-93</td>
<td>93</td>
<td>62-120</td>
</tr>
</tbody>
</table>

Protein S is a vitamin K dependent cofactor of protein C. Protein S exists in two forms in plasma, free form and complexed to C4b binding protein (C4b-BP). The free form represents approximately 40% of the total protein S. This free form acts as the cofactor for activated protein C and is the only form that has anticoagulant activity. The free protein S level should correlate with the protein S activity. In inflammation there is an increase in C4b-BP which results in an increase of bound protein S. This leads to a decrease of free protein S antigen and corresponding decrease in protein S activity.

If all acquired deficiencies are excluded, a sample with decreased free protein S antigen should be evaluated for total protein S antigen to classify the type of protein S deficiency.

PROTEIN S ANTIGEN (TOTAL)

Method: ELISA

Total protein S antigen determination is only used to classify type of protein S deficiency when the protein S activity and free protein S antigen are decreased. Patient with type I deficiency have decreased total protein S and free protein S. Type II deficiency has normal total protein S and decreased free protein S. There is a rare form described where there is normal total and free protein S antigen with decreased protein S activity. The majority of the inherited protein S deficiency are of type I.

PROTHROMBIN (Factor II) DNA SCREEN

Method: DNA based PCR assay.

Prothrombin (factor II) is one of the blood coagulation factors. Recent work has identified a variant (base 20210G->A) in the 3’ untranslated region of the prothrombin gene that is associated with an increased risk for venous thrombosis. Approximately 20% of Dutch patients with a family history of venous thrombosis are heterozygotes for the 20210A allele, as compared to about 1% of healthy controls. In a population-based study, the 20210A allele appears to increase the risk of venous thrombosis about 3-fold for adults of both sexes. This test determines the presence or absence of the 20210G (normal) and 20210A (variant) alleles in the prothrombin gene.
PROTHROMBIN TIME

Method: clot based assay

The prothrombin time (PT) is used to detect abnormalities in the extrinsic and common pathway of coagulation. Specifically, the PT is prolonged if there is a decrease in factor II, V, VII, X, and fibrinogen. The degree of prolongation is dependent upon the severity of the deficiency and the number of clotting proteins decreased. The PT is affected by elevated factor II, V, VII, and X resulting in shortening of the PT. Since each lot of thromboplastin is slightly different, reference ranges need to be determined for the specific lot used. The sensitivity of the thromboplastin to various factor levels as a single factor deficiency or combined as multiple factor deficiency is influenced by the international sensitivity index (ISI) value of the thromboplastin. Our laboratory uses a thromboplastin with an ISI value of less than 1.4.

PROTHROMBIN TIME 1:1 MIX

The prothrombin time may be prolonged due to a factor(s) deficiency or an inhibitor. A 1:1 mix is used to differentiate between the two. Equal parts of patient plasma and normal plasma pool are mixed together and a PT performed on the mixture. Correction of the patient prolonged PT on the mixture indicates a factor deficiency. Non correction or partial correction indicates an inhibitor.

PROTHROMBIN FRAGMENT 1.2

Method: ELISA

Prothrombin fragment 1.2 is a polypeptide released from prothrombin when prothrombin is activated to thrombin by the prothrombinase complex (activated factor X and V, calcium and phospholipid). Elevated F1.2 levels occur when prothrombin is converted in vivo at an accelerated rate and can be used as a marker for in vivo thrombin generation. Prothrombin fragment 1.2 is a quantitative marker to assess the degree to which the coagulation system has been activated.

The assay has been used to assess thrombotic risk and monitor the lowest dose of warfarin needed to prevent a thrombotic event in patients on oral anticoagulant therapy. Elevated levels are observed in inherited thrombophilia (protein C deficiency, protein S deficiency, and At-III deficiency). Increased levels occur in thrombosis, pulmonary embolism, DIC, trauma, septicemia, and complication of pregnancy. Patients on oral anticoagulant therapy have reduced levels of F1.2.

THROMBIN TIME

Method: clot based assay.

The thrombin time will be prolonged when the fibrinogen concentration is less than 100 mg/dL. Levels need to fall below 50 mg/dL before a marked prolongation is noted. The thrombin time is also prolonged in the presence of a dysfunctional fibrinogen, high levels of fibrin degradation products, and anti-thrombin antibodies. The thrombin time is very sensitive to heparin and will be >100 sec at heparin levels greater than 0.05 unit/mL.

A patient with a prolonged thrombin time that does not correct on 1:1 mix who has recently been exposed to topical bovine thrombin should be checked for a bovine thrombin inhibitor. In the presence of this inhibitor the thrombin time will often be normal if human thrombin is used in the test system.
THROMBIN TIME 1:1 MIX

A 1:1 mix of the thrombin time is used to distinguish an inhibitor from a decreased fibrinogen level. Equal parts of patient plasma and normal plasma pool are mixed and the thrombin time repeated. Complete correction indicates a factor deficiency. No correction or only a partial correction indicates the presence of an inhibitor.

TPA ACTIVITY

Method: Immunofunctional, plasminogen-chromogen substrate (amidolytic) assay.

Fibrinolytic activity in blood follows a circadian rhythm with the lowest level of TPA activity in the morning and peak level in the evening. Therefore, it is recommended that blood be collected between 0800 and 0900 to determine the minimal level of tPA activity. Acceptable collection time is 0700-1000. Please record the collection time on the requisition form.

Tissue-type plasminogen activator (tPA) is a serine protease that has a crucial function in the fibrinolytic process. It catalyses the conversion of the zymogen d(Glu-)plasminogen to the active serine protease plasmin which in turn is responsible for the degradation of fibrin in the thrombus. TPA is synthesized and secreted by vascular endothelial cells.

TPA ANTIGEN

Method: ELISA

A decrease in tPA may lead to impaired fibrinolysis resulting in an increased risk of thrombosis. An elevated tPA level means there is endothelial hyperactivity. This may be due to a lesion or blood stasis and is considered an endothelial marker. An elevated tPA level does not necessarily mean there is an increase in fibrinolysis as there may be an increase in PAI-1 at the same time. Increased tPA antigen levels may occur in respiratory distress, myocardial infarction, shock, septicemia, and severe hepatic disorders. Tissue plasminogen activator levels increase with age, exercise, and stress. Venous stasis will increase tPA level. There have been some reports of a congenital bleeding disorder associated with elevated tPA and normal level of PAI-1.

VON WILLEBRAND FACTOR ACTIVITY (ristocetin cofactor assay)

Method: aggregation assay.

The von Willebrand factor activity assay (also known as ristocetin cofactor assay) is a semi-quantitative measurement of von Willebrand factor activity. A dilution of patient plasma is added to formalin fixed normal platelets. The time to aggregation is compared to a standard curve of known von Willebrand factor activity.

The assay can be used as a screen for von Willebrand disease. A value > 70 % makes vWD unlikely unless the patient has an ongoing acute phase response. Levels < 40 % suggests vWD, a von Willebrand Disease Panel is recommended to confirm the diagnosis and determine the subtype. Values between 40 -70 % are questionable and the entire vWD panel is recommended.

The functional assay has been recommended to monitor vWD patients who are infused with commercial concentrates. In these situations a base and post transfusion level should be obtained and compared accordingly for correct patient response to infused material.
VON WILLEBRAND FACTOR ANTIGEN (VWF:AG)

Method: ELISA


VON WILLEBRAND FACTOR MULTIMER ANALYSIS

Method: SDS gel electrophoresis.

Multimeric sizing of the von Willebrand factor protein is used to characterize von Willebrand disease (vWD). Classification of von Willebrand disease is dependent upon a quantitative and/or qualitative defect of the von Willebrand factor protein. In type 1 all the multimers are present but will be decreased in quantity. Type 2 patients lack the high molecular weight multimers and in most situations lack the intermediate weight multimers. Type 3 patients will not have any visible multimers due to the extremely low level or unmeasureable antigen.

A low resolution gel (1% agarose/SDS) permits the high molecular weight, intermediate weight, and low molecular weight multimers to enter the gel and be separated. Once the separation is completed the multimers are transferred to a polyvinyl fluoride membrane using a semi-dry electroblot transfer system. Immunolocalization of the multimeric bands is visualized by application of alkaline phosphatase conjugated antibodies.
Listed below are assays offered through our Research and Development division for investigational use only. The availability of these assays are dependent upon the number of tests, frequency of testing, and the cost of reagents. In addition to performing these assays, the laboratory offers consultation for research protocol design and data interpretation. If you are interested in any of these assays, please contact Dr. Schmer, Dr. Chandler or Dr. La Spada at (206) 548-6131 for consultation. If you are interested in an assay not listed below, please call Dr. Schmer, Dr. Chandler or Dr. La Spada to determine whether it could be run in our laboratory. This list will change as new assays are being evaluated. In the future some of these assays may be offered as clinical diagnostic assays.

- Activated Factor VII
- Anti-Bovine Thrombin Antibody
- Anti-Bovine Factor V Antibody
- Antiplasmin Activity
- Aprotinin Activity
- Beta Thromboglobulin Antigen
- C4b-Binding Protein Antigen
- Collagen Binding Assay (von Willebrand factor function)
- Dilute Russell’s Viper Venom Time
- Factor VII Antigen
- Factor X Antigen
- Factor IX Antigen
- Factor XIII Quantitative
- Fibrin Monomers
- Fibronectin Antigen
- Heparin Cofactor II Antigen
- High Molecular Weight Kininogen
- Platelet Factor 4 Antigen
- Plasmin Antiplasmin Complex Antigen
- Plasminogen
- Prekallikrein
- Reptilase Time
- Tissue Factor Antigen, Soluble
- Thrombomodulin Antigen
- Thrombospondin Antigen
- Tissue Factor Pathway Inhibitor Antigen
- Urokinase Plasminogen Activator Antigen
- Von Willebrand Factor Multimer Analysis
- High Resolution Gel (separate low molecular weight multimers)
- Ultra High Molecular Weight Multimers