HANDBOOK OF DIAGNOSTIC HEMOSTASIS AND THROMBOSIS TESTS

Offered by
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Department of Laboratory Medicine
Reference Laboratory Services

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UNIVERSITY OF WASHINGTON
DEPARTMENT OF LABORATORY MEDICINE
COAGULATION LABORATORY

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Consultation Service: Consultation on bleeding patients, thrombosis, fibrinolysis, and test interpretation is available from Dr. Chandler or Dr. La Spada during regular hours Monday through Friday at (206) 598-6131. For assistance after regular hours, weekends, or holidays, call the University of Washington Medical Center paging operator at (206) 598-6190 and request the 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 Reference Laboratory Services at (206) 598-6066 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. Chandler or Dr. La Spada at (206) 598-6131 during regular laboratory hours.

INTRODUCTION

The Coagulation Laboratory offers a wide variety of testing to aid in the diagnosis, management, and monitoring of patients with bleeding disorders, or to aid in evaluating patients with thrombotic or fibrinolytic abnormalities. The laboratory is involved in research and development of diagnostic tests and their clinical application and 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. 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 offered through CAP and the North American Specialized Coagulation Laboratory Association (NASCOLA).

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.

SPECIMEN REQUIREMENTS:
The quality of test samples is very important in providing reliable test results. Please refer to pages 4 and 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 weekdays. Please contact Reference Laboratory Services at (206) 598-6066 or (800) 713-5198 for more information and assistance in determining the best means of transportation.

SUPPLIES:
Reference Laboratory 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. We are not able to provide third party billing for out of state clients. All laboratory work that is ordered from out of state locations will be billed directly to the ordering location.

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

Reference Laboratory Services
Department of Laboratory Medicine
University of Washington
P.O. Box 15290
Seattle, Washington 98115
(206) 598-6066 or (800) 713-5198

CLINICAL COAGULATION LABORATORY OVERVIEW

Since its establishment in 1969, the Clinical Coagulation 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. Chandler or Dr. La Spada at (206) 598-6131 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. Reference Laboratory Services will notify the medical community of these changes. In addition, current information on available clinical tests can be obtained by calling Reference Laboratory Services at (206) 598-6066 or (800) 713-5198. For information and consultation on research assays not listed in this manual, please call Dr. Chandler or Dr. La Spada at (206) 598-6131.

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. Reference Laboratory 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, NCCLS recommends flushing the line with normal saline and then withdrawing 5 mL of blood to be discarded prior to drawing blood for a coagulation sample. This is the recommendation for lines containing or not containing heparin. (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, if 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 to 2 mL whole blood into a glass red top tube or other tube containing **NO ADDITIVE** for discard.

2. Draw blood into buffered citrate collection tube (blue top) filled to the proper level. It is recommended that 0.109M (3.2 %) buffered citrate tubes be used.

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

4. Centrifuge whole blood at 2500 G for ten minutes.

5. Transfer plasma with plastic transfer pipette to a plastic centrifuge tube. Centrifuge plasma at 2500 G for 10 minutes. The second centrifuge step is important to remove all platelets in the sample.

6. Remove supernatant to 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 totally frozen during transport. Partially thawed plasma will have an adverse affect on some coagulation test results.

8. Contact Community Service (206) 598-6066 for complete instructions for transporting sample.

ACID CITRATE PLASMA

1. To avoid tissue fluid contamination, draw 1 to 2 mL whole blood into a glass red top tube or other tube containing **NO ADDITIVE** for discard.

2. Draw blood into a special black top Stabilyte tube containing acidic buffered citrate (available from Reference Laboratory Services at (206) 598-6066, (800) 713-5198 or from Trinity Biotech, Product # 102080). Fill the tube to the proper level.

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

4. Centrifuge whole blood at 2500 G for ten minutes.

5. Transfer plasma with a plastic transfer pipette into a plastic centrifuge tube. Centrifuge plasma at 2500 G 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 Reference Laboratory Services at (206) 598-6066 or (800) 713-5198 for complete instructions for transporting sample.

SERUM for Anti-Phospholipid and C-Reactive Protein, High Sensitivity

1. Draw blood into plain red top or SST tube.

2. Separate serum from cells ASAP.

4. Dispense serum into plastic tubes.

5. Label appropriately.

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

7. Contact Reference Laboratory Services at (206) 598-6066 or (800) 713-5198 for complete instructions for transporting sample.

EDTA WHOLE BLOOD for Prothrombin DNA Screen and Factor 5 DNA

1. Draw blood into lavender topped EDTA tube.

2. Do not centrifuge sample

3. Send sample at **room temperature for overnight delivery.** Contact Reference Laboratory Services at (206) 598-6066 or (800) 713-5198 for complete instructions on transporting sample.

HEMOSTASIS OVERVIEW

Hemostasis is a complex interaction between vessels, platelets and coagulation proteins that, when working properly, stops bleeding while maintaining blood flow in the vessel. 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), 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 thought that coagulation proceeded via two distinct pathways, intrinsic and extrinsic, with the difference between the two being the method of activation. Today, we understand that only a single pathway exists for coagulation activation that occurs in two phases. The first phase of coagulation activation, termed the initiation phase, is dependent on exposure or transport of tissue factor to the site of the wound. Thrombin generation is slow during the initiation phase. As thrombin levels increase, thrombin activates additional platelets and factors V, VIII and XI which dramatically increases the rate of thrombin generation leading to fibrin and clot formation, this is termed the propagation phase. In vivo, almost all coagulation reactions are initiated by exposure of tissue factor and platelet activation. Tissue factor autocatalyzes factor VII to VIIa. Factor VIIa in turn activates factor IX, IXa activates X, Xa 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 “intrinsic” coagulation pathway through factor XIIa. Contact phase factors include prekallikrein, high molecular weight kininogen, and factor XII, which when activated convert factor XI to XIa. Factor XIa converts IX to IXa, which then activates factor X which in turn converts prothrombin into thrombin.

In vivo there are a variety of control mechanisms to limit thrombus formation through the naturally occurring inhibitors protein C, protein S and antithrombin. 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 degrades factors Va and VIIIa. Thrombin in plasma is 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 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 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-1). 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, platelet, vascular or fibrinolytic systems. 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, coagulation and fibrinolysis.

EVALUATION OF A BLEEDING DISORDER

The Coagulation Screen includes PT, APTT, Thrombin time, and Clauss Fibrinogen assays. The DIC panel includes these same tests plus a quantitative D-Dimer and platelet count. If heparin is discovered in either of these testing packages it will be removed and the APTT result will be reported for the sample containing heparin as well as for the sample after heparin removal. The APTT on the sample after heparin removal is a separate test that will be automatically added on in the event heparin is detected in the sample.

Mixing studies may be ordered to determine if bleeding disorders are factor deficiency- or inhibitor- based.

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

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. Petechiae 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 page 9. 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. Von Willebrand Disease type I may present with a mild to marked prolongation of the APTT but other testing more specific for defining the disease is available as the von Willebrand Disease Panel. All of the Hemophilias and the Factor XII deficiency will present with a mild to marked prolongation of the APTT with all other screening 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 in a 1:1 mix and where the 1:1 mix APTT increases as the mix sits in a 37°C water bath is suggestive of a factor VIII inhibitor in a bleeding patient. 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 PLATELET DISORDERS

Platelet count and smear evaluation
Platelet Function Screen
- May be abnormal if platelet count less than 150,000 / uL or Hct less than 30%
- Sample submitted to lab within 1 - 2 hrs of collection or patient comes to lab site for blood collection. Contact Reference Laboratory Services (see below)

Platelet aggregation studies
- May be abnormal if platelet count less than 150,000 / uL or Hct less than 30%
- Patient must not be taking aspirin or NSAID containing medication.
- Test requires patient to come to UWMC blood draw for blood collection.

Please call Reference Laboratory Services at (206) 598-6066 or (800) 713-5198 for assistance and contact the Coagulation Lab at (206) 598-6242 to schedule an aggregation study.

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). 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 part of the GPIb receptor complex 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 Thrombasthemia (missing or defective fibrinogen receptor, GPIIbIIIa) and afibrinogenemia. Defective secretion disorders include storage pool abnormalities, Wiskott-Aldrich syndrome, Hermansky-Pudlak syndrome and Chediak-Higashi. Of this group, storage pool abnormalities are 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 offending drug is discontinued. Heparin-induced thrombocytopenia is a potentially life threatening form of acquired 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) may be 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 inherited bleeding disorders. 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 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 megakarocytes. In vivo, vWF has two important functions. First, it mediates the adhesion of platelets to the injured vessel wall and 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 von Willebrand disease. Factor VIII activity measures coagulant activity of the factor VIII molecule, which binds to and is stabilized by von Willebrand 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.

EVALUATION OF VON WILLEBRAND DISEASE

Von Willebrand Disease 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 von Willebrand factor 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 von Willebrand factor 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 set of reference ranges for von Willebrand factor antigen.

Blood Group	Mean vWF:Ag	Range vWF:Ag (%)
O	75	36-157
A	106	48-234
B	117	57-241
AB	123	64-238

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% of all patients with vWD and 70% of Type 2 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 bands. 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 decreased. Von Willebrand factor activity is typically less than 50% of normal.

Type 2B vWD is characterized by a qualitative defect of von Willebrand factor antigen causing an increased affinity for GP1b complex. Characteristic of this group is the absence of only the high molecular weight multimers and enhanced aggregation to low dose 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 decreased. Von Willebrand factor activity is typically less than 50% of normal. These patients often present with mild to moderate thrombocytopenia.

Ristocetin-induced platelet aggregation on the patient's platelets will differentiate Type 2B vWD from Type 2A vWD and from platelet psuedo-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 normal subjects. In platelet pseudo von Willebrand disease, the vWF levels are normal but the ristocetin response is increased with the patient's 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 and normal levels of intermediate and high MW multimers. At the present time this disorder cannot be reliably diagnosed with current assays. For more information regarding this disorder, please contact Dr. Chandler or Dr. La Spada at (206) 598-6131.

TYPE 2N: This 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. Chandler or Dr. La Spada at (206) 598-6131.

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% with unmeasurable von Willebrand factor antigen and 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's 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. Chandler or Dr. La Spada at (206) 598-6131. For after hours consultation please contact the Laboratory Medicine Resident on call at (206) 598-6190.

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. The endothelium overlying the arteriosclerotic lesion synthesizes reduced amounts of prostacylin 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 without other known risk factors (smoking, hypertension, lipid abnormalities) may indicate an abnormality of the fibrinolytic system or 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 without other risk factors. 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 Workup

PAI-1 activity
tPA antigen
C-reactive Protein

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

Lupus Anticoagulant Panel (panel includes)

Lupus inhibitor assay
Antiphospholipid 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, and further laboratory workup for fibrinolytic abnormalities is generally not considered useful in these patients.

Elevated homocysteine levels in young adults are associated with an increased risk of arterial and possibly 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. Autoimmune diseases may be present and there usually is no family history of arterial thrombosis.

Research studies have indicated that increased levels of fibrinogen may be associated with an increased risk of arterial thrombosis. Presently though, screening of coronary artery disease patients for high fibrinogen 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 system. A predisposition to forming venous thrombi is know 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 antiphospholipid syndrome.

To evaluate a patient for venous thrombophilia it is optimal 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, protein S activity and protein S free antigen 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 Workup

- Activated protein C resistance (APCR)
- Protein C activity
- Protein S free antigen
- Antithrombin activity
- Lupus inhibitor assay

If all other assays are negative order

- Prothrombin (Factor II) DNA screen
- Factor VIII Thrombosis Workup
- Chromogenic Factor VIII, CRP

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 inhibitor S, protein C, protein S or antithrombin. 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. The next most common defect is a persistent elevation of factor VIII activity. The incidence of elevated factor VIII activity is similar to that of APCR/Factor V Leiden. Approximately 2% of the normal population and 10% of thrombophilia patients have the prothrombin polymorphism. Together, hereditary protein S, protein C and anti-thrombin 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 deficiency may be combined with each other but the most common combination is with the R506Q mutation of factor V, elevated factor VIII or the prothrombin mutation. While a single factor

deficiency may be associated with a 2-5 fold increased risk for thrombosis, a 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 antiphospholipid 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 deficiency or malignancy.

REFERENCE CHART OF DIAGNOSTIC TESTS/PANELS AVAILABLE

For a more detailed description of tests, please see page 22. Please refer to page 4 for instructions on the collection, processing and storage of samples.

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
Activated Partial Thromboplastin Time (APTT)	1 mL frozen plasma	22-35 sec	Daily	
Anti-beta 2 glycoprotein I	1 mL frozen serum	< 10 units	T, F	Part of antiphospholipid panel.
Anticardiolipin IgA	1 mL frozen serum	< 12 APL	T, F	Part of antiphospholipid panel.
Anticardiolipin IgG	1 mL frozen serum	< 12 GPL	T, F	Part of antiphospholipid panel.
Anticardiolipin IgM	1 mL frozen serum	< 15 MPL	T, F	Part of antiphospholipid panel.
Antiphospholipid Panel	1 mL frozen serum	see individual tests		Includes anticardiolipin IgG, IgM & IgA plus anti-beta 2 glycoprotein I.
APTT (1:1 mix)	1 mL frozen plasma	call (206) 598-6066	Daily	
Activated Protein C Resistance (APCR)	1 mL frozen plasma	negative	M, Th	Modified Factor V method. Test plasma not affected by oral anticoagulant therapy, heparin or lupus anticoagulant. Part of venous thrombosis panel.
Anticardiolipin IgG / IgM	0.5 mL serum			Test performed in Immunology Division, call (206) 598-6066.
Antithrombin Activity	1 mL frozen plasma	75 - 125 % 1-29 days 41 - 125% 1 month 48 - 125%	M, Th	Chromogenic method. Part of venous thrombosis panel. Patient should be off heparin for at least 3 days prior to testing.
Arterial Thrombosis Panel	3 mL frozen plasma 0.5 mL frozen serum	see individual tests	Weekly	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.
C-Reactive Protein (high sensitivity)	0.5 mL frozen serum			Test performed in Immunology Division, call (206) 598-6066.

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
Chromogenic Factor	1 mL frozen plasma	50 - 150%		Part of a Factor VIII Thrombosis panel. Also, used to measure Factor VIII in the presence of a Lupus anticoagulant.
D-Dimer (quantitative)	1 mL frozen plasma	<0.6 mcg / mL FEU	Daily	LIA method
D-Dimer - research	1 mL frozen plasma	<600ng / mL FEU	research batched	ELISA method
Dilute Russell Viper Venom time	1 mL frozen plasma	Negative	M-F	Clotting assay for lupus anticoagulant testing.
Direct Thrombin Inhibitor Assay	1 mL frozen plasma	Argatroban 60 - 100 sec Lepirudin 90 - 160 sec	Daily	Dilute thrombin time method.
Factor II Activity	1 mL frozen plasma 0-29 days 1 month 3 months	50 - 115 % 33 - 115% 34 - 115% 45 - 115%	M-F	
Factor V Activity	1 mL frozen plasma	50 - 150 %	M-F	
Factor V DNA Screen (Factor V Leiden)	EDTA whole blood			Test performed in Genetics, call (206) 598-6066.
Factor VII Activity	1 mL frozen plasma 0-29 days 1 month 3 months	50 - 150 % 35 - 150% 42 - 150% 39 - 150%	M-F	
Factor VIII Activity	1 mL frozen plasma	50 - 150 %	M-F	
Factor IX Activity	1 mL frozen plasma 0-29 days 1 month 3 months 6 months	60 - 150% 15 - 150 21 - 150% 21 - 150% 36 - 150%	M-F	
Factor X Activity	1 mL frozen plasma 0-29 days 1 month 3 months 6 months	50 - 150% 19 - 150% 31 - 150% 35 - 150% 38 - 150%	M-F	

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
Factor XI Activity	1 mL frozen plasma	50 - 150 %	M-F	
	0-29 days	23 - 150%		
	1 month	27 - 150%		
	3 months	41 - 150%		
	6 months	38 - 150%		
Factor XII Activity	1 mL frozen plasma	30 - 150 %	M-F	
	0-29 days	11 - 150%		
	1 month	17 - 150%		
	3 months	25 - 150%		
	6 months	39 - 150%		
Factor VIII Thrombosis Panel	1 mL frozen plasma 0.5 mL frozen serum	See individual tests	M, Th	Includes a chromogenic factor VIII and C-reactive protein.
Factor Inhibitor Assay	2-3 mL frozen plasma	0 units / mL	M-F	Bethseda assay. Please indicate the specific factor on the requisition form.
Porcine Factor VIII Inhibitor Assay	2 mL frozen plasma	0 units / mL	M-F	Bethseda assay. Assay determines cross reactivity to Porcine VIII in a patient with a factor VIII inhibitor.
Fibrinogen	1 mL frozen plasma	150-400 mg / dL	Daily	
Fibrinolysis Screen	3 mL frozen plasma	See individual tests	Weekly	Includes total tPA antigen, PAI-1 activity tests and C-reactive protein. Draw between 0700 and 1000. Note collection time on requisition.
Heparin activity (unfractionated)	0.5 mL frozen plasma	0.3-0.7 antiXa units / mL	Daily	Sample must be processed within one hour of collection. Please note type of heparin on requisition.
Heparin Activity (low molecular weight)	0.5 mL frozen plasma	Contact lab for therapeutic range	Daily	Sample must be processed within one hour of collection.
Homocysteine	0.5 mL frozen EDTA plasma			Test performed in Chemistry, call (206) 598-6066.
Lupus Anticoagulant Panel	1 mL frozen plasma 0.5 mL serum	See individual tests	M, Th	Includes anticardiolipin IgG / IgM, anti-beta-2 glycoprotein I and Lupus inhibitor assay.

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
Lupus Inhibitor Assay (Lupus anticoagulant)	1 mL frozen plasma	Negative	M, Th	Critical that the plasma is centrifuged twice to remove all platelets. Hexagonal Phospholipid method. Positive patient reported as borderline positive, moderate positive or strong positive.
Plasminogen Activator Inhibitor type 1 (PAI-1)	3 mL frozen plasma	2400-1000: 2.0-15 IU / mL 1000-1600: 1.7-11 IU / mL 1600-2400: 1.7-8.7 IU / mL	Weekly	**Note collection time on tube**
Platelet Aggregation	Citrate whole blood	call 598-6242	By appointment only	Patient must come to the Laboratory to be drawn. Call (206) 598-6242 to schedule.
Platelet Function Screen	Citrate whole blood	PCAO <115 sec PCEO <181 sec	Daily (8AM-11PM)	Screen test for platelet dysfunction. Sample in lab within 1-2 hrs of draw.
Protein C Activity	1 mL frozen plasma	65 - 150 % 0-29 days 20 - 150% 1 month 21 - 150% 3 months 28 - 150% 6 months 37 - 150% 1-5 years 40 - 150% 6-10 years 45 - 150%	M, Th	Chromogenic based assay. Screen test for Protein C deficiency. Part of venous thrombosis panel. Patient should not have ongoing thrombosis. Patient should be off anticoagulant therapy 7 - 10 days.
Protein C Antigen	1 mL frozen plasma	65 - 150 % 0-29 days 20 - 150% 1 month 21 - 150% 3 months 28 - 150% 6 months 37 - 150% 1-5 years 40 - 150% 6-10 years 45 - 150%	Weekly	ELISA assay. See Protein C activity.
Protein S Activity	1 mL frozen plasma	Male: 77 - 164% Female: 60 - 164% 0-29 days 15 - 150% 3 months 35 - 150% 6 months 47 - 150% 1-5 years 49 - 150% 6-10 years 58 - 150%	Th	Clot based. See Protein S free antigen.

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
Protein S Antigen Free	1 mL frozen plasma	Male: 69 - 164% Female: 51 - 164% 0-29 days 15 - 150% 3 months 35 - 150% 6 months 47 - 150% 1-5 years 49 - 150% 6-10 years 58 - 150%	M, Th	LIA. Screen test for protein S deficiency, part of venous thrombosis panel. Patient should not have ongoing thrombosis and should be off oral anticoagulant therapy for 7-10 days.
Protein S Antigen Total	0.5 mL frozen plasma	Male: 78 - 150% Female: 71 - 150% 0-29 days 22 - 150% 1 month 33 - 150% 3 months 54 - 150% 6 months 55 - 150% 1-5 years 54 - 150% 6-10 years 41 - 150%	Weekly	LIA. See Protein S free antigen.
Prothrombin DNA Screen	EDTA whole blood			Test performed in Genetics, call (206) 598-6066.
Prothrombin Time (PT)	0.5 mL frozen plasma	call (206) 598-6066	Daily	
Prothrombin time 1:1 Mix	1 mL frozen plasma	call (206) 598-6066	Daily	
Prothrombin Fragment 1.2	1 mL frozen plasma	0.4-1.1 nmoL/L	Weekly	ELISA method - research only
tPA Activity	1 mL acid citrate frozen plasma	0000 - 1000: 0.3-1.7 IU/mL 1000-1600: 0.6-2.1 IU/mL 1600 - 2400: 0.8-2.6 IU/mL	Weekly	Sample must be drawn in acid citrate. Label tube **acid citrate.** Blood should be collected between 0700-1000. Note time on requisition.
tPA Antigen	1 mL frozen plasma	3 - 12 ng/mL	Weekly	
Thrombin Time	1 mL frozen plasma	16 - 25 seconds	Daily	
Thrombin Time 1:1 mix	1 mL frozen plasma		Daily	
Thromboelastograph	5 mL whole citrate blood	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	Daily	Sample must be received within 4 hours of the time it is collected.
Venous Thrombosis Panel	1 mL frozen plasma	see individual tests	M, Th	Panel includes protein C activity, protein S free antigen, anti-thrombin, APCR and lupus inhibitor. Patient should not have an ongoing thrombosis and should be off oral anticoagulant therapy 7 - 10 days prior to testing.

TEST/PANEL	SAMPLE	REFERENCE RANGE	FREQUENCY OF TESTING	COMMENT
von Willebrand Disease Panel	1 mL frozen plasma	see individual tests	set up T results Th	Panel includes factor VIII activity, vWF antigen and multimer analysis.
von Willebrand Factor Activity	1 mL frozen plasma	50-150 %	T	
von Willebrand Factor Antigen	1 mL frozen plasma	Blood Type O: 36-157% A: 48-234% B: 57-241% AB: 64-238%	M, Th	
von Willebrand Factor Multimer Analysis	1 mL frozen plasma	all multimers present	set up T results Th	

DESCRIPTION OF CLINICAL DIAGNOSTIC TESTS

Diagnostic Panels

ANTIPHOSPHOLIPID 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 high sensitivity c-reactive protein, lupus inhibitor and total homocysteine. 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. Chandler or Dr. La Spada at (206) 598-6131.

COAGULATION SCREEN

Tests included in this screen include PT, PTT, Fibrinogen and Thrombin Time (TT). These tests are used to rule out coagulopathy. The Coagulation screen is reflexive for heparin removal. If heparin is detected in the sample it will be removed and the PTTHR will be reported along with the PTT. The PTTHR reflects the PTT in the sample after heparin is all removed (baseline) and the PTT will reflect the sample as it arrived in the lab, containing heparin.

COMPREHENSIVE VENOUS THROMBOSIS GROUP

Tests included in this panel are APCR, protein C activity, free protein S antigen, antithrombin, lupus inhibitor and Prothrombin DNA screen for Prothrombin mutation. The tests should not be performed during an ongoing thrombosis. The only tests that can be run during this period are the APCR assay and the Prothrombin DNA screen. The patient should be off oral anticoagulant therapy for a minimum of 7 days. See page 13 for a detailed description of venous thrombosis.

If you have any questions regarding the use or interpretation of this panel to screen for potential causes of venous thrombosis, please contact Dr. Chandler or Dr. La Spada at (206) 598-6131.

DISSEMINATED INTRAVASCULAR COAGULATION PANEL

Tests included in this panel are the PT, PTT, fibrinogen, Thrombin Time, Platelet Count and D-dimer assays. This panel of tests may be used to R/O or monitor a DIC process. This panel is also reflexive for heparin removal. If heparin is detected, it will be removed and the results of the PTTHR or PTT after heparin removal as well as the PTT of the sample before heparin removal will be determined.

FACTOR VIII THROMBOSIS PANEL

Persistent elevated factor VIII in the absence of an acute phase response has been associated with an increased risk of thrombosis. Tests included in this panel are the Chromogenic Factor VIII and C-reactive protein. Factor VIII activity can be transiently increased due to an acute phase response. An elevated factor VIII with a normal CRP indicates (no acute phase response) indicates a probable persistent elevation of factor VIII.

LUPUS ANTICOAGULANT PANEL

Antiphospholipid 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.

ONE TO ONE MIX OR MIXING STUDIES

This may involve either a combination of PT and PTT mixing studies or either PT or PTT mixing studies. These are used to differentiate coagulopathy due to factor deficiency from that which is due to the development of an inhibitor. The mixing studies come with an interpretive report.

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 < 1 U / mL and PAI-1 antigen is <2 ng / mL. In some cases the PAI-1 activity may be reduced while the PAI-1 antigen level is normal.

PLATELET FUNCTION SCREEN

Method: PFA 100
This test is a screen test for Platelet Dysfunction. Recommend patient have normal platelet count and Hct >30%.

PLATELET AGGREGATION PANEL

Method: Platelet aggregometry using whole blood lumiaggregation.

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, collagen and ristocetin. The total amount of aggregation and the rate of aggregation are evaluated. Aggregation is detect as an increased in the impedance of the sample measured by an electrode immersed in the whole blood sample. Storage pool release from platelets is assessed by detecting ATP release using lucipherase. As the platelets aggregate in response to an aggregating agonist the resistance across the electrode increases and is registered as an Ohms reading on the Ohms meter. The granular release function of the platelets is measured by the luminescence of a lumiphore added to the reaction mix. The lumiphore will luminesce when platelets release ATP in response to an aggregation initiating agonist. This luminescence is detected and quantified by a photo-multiplier. The

patient Luminescence in the patient sample is compared to that obtained when a standard ATP is introduced into patient blood without platelet activation. This allows the calculation of ATP concentration released during platelet activation.

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 and collagen are absent in patients with homozygous thrombasthenia and diminished in heterozygous thrombasthenia. Variable abnormalities in the platelet release function are seen in storage pool disease, aspirin-like defect and in patients who have taken aspirin or related drugs. Decreased response to ristocetin is suggestive of von Willebrand disease or Bernard Soulier disease.

If desired epinephrine aggregation may be measured using a photo-optical methodology. Both rate and total amount of aggregation are evaluated. Abnormal response to epinephrine occurs in myeloproliferative disorders but also occurs in 25% of the normal population. Therefore, while the epinephrine aggregation is available it is not a part of the usual Platelet Aggregation Panel.

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 (a) 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 (A_{60}) is a measure of fibrinolysis. If the A_{60} 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->A double check).

VENOUS THROMBOSIS PANEL

Tests included in the panel are APCR, protein C activity, protein S free antigen, antithrombin and lupus inhibitor. The tests should not be performed during an ongoing thrombosis. The only tests that can be run during this period are the APCR and lupus inhibitor assays. 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

This is a 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. Direct thrombin inhibitors, such as Argatroban and Lepirudin, will falsely prolong the clotting time.

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) 1:1 MIX

Method: Clot-based assay.

The APTT may be prolonged due to factor deficiencies 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. An interpretive report is given with the result. Direct thrombin inhibitors, such as Argatroban and Lepirudin, will prolong the clotting time.

ACTIVATED PROTEIN C RESISTANCE (APCR)

Method: Clot-based factor V degradation assay.

Activated protein C (APC) resistance is inherited in an autosomal dominant manner. The abnormality is a point mutation in the gene encoding for coagulation factor V and is commonly called the 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 antithrombin, the risk of thrombosis is further increased.

This assays evaluates the degradation of factor Va by APC during coagulation activation. Clotting times are typically prolonged by addition of exogenous APC. If the prolongation is reduced it indicates reduced factor V degradation typically due to the presence of factor V Leiden. Based on the prolongation of the clotting time by APC, it can be determined whether the patient is normal, likely factor V Leiden heterozygote or likely factor V Leiden homozygote. The assay has shown close correlation with the factor V DNA screen. This assay is not affected by oral anticoagulant therapy, heparin therapy or the presence of a lupus inhibitor. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong the clotting times and invalidate this assay.

ANTITHROMBIN ACTIVITY

Method: Chromogenic substrate, back titration method.

Samples for antithrombin III activity should be obtained at least a week after resolution of the acute thrombotic event and a minimum of 3-4 days post heparin therapy. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely increase and invalidate this assay. Congenital antithrombin deficiency is inherited as autosomal dominant. Patients usually have less than 65% antithrombin activity. The disorder occurs in 2-3 % of patients presenting with venous thrombophilia. Acquired antithrombin 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 activity should return to baseline levels within 72 hours. Elevated antithrombin III activity has no known clinical significance.

CHROMOGENIC FACTOR VIII ASSAY

Method: Chromogenic substrate

Based on the study by Koster et al. a baseline factor VIII activity of >150% is a risk factor for venous thrombosis. Providing that the sample is drawn when there is no inflammation or acute clinical illness, no aerobic exercise in the past several to 24 hours and no use of estrogen therapy, a baseline value of >150% is a significant risk factor for individual subjects. This finding is very common: approximately 20-25% of patient with thrombophilia have an elevated factor VIII. Factor VIII levels are most appropriately considered in subjects after venous thrombotic events if they have a strong family history.

D-DIMER ASSAY - QUANTITATIVE (cross-linked fibrin degradation products)

Method: LIA assay for clinical samples and ELISA for research (batched) samples.

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. It has been used as a screening test when large proximal deep venous thrombosis is suspected.

DIRECT THROMBIN INHIBITOR ASSAY

Method: Clot-based dilute thrombin time.

Heparin-induced thrombocytopenia (HIT) is a serious autoimmune complication of heparin therapy. Heparin anticoagulation is not an option, as continued heparin therapy would fuel the underlying autoimmune reaction. Direct thrombin inhibitors, such as argatroban or lepirudin, are effective alternatives for anticoagulation in patients with acute HIT. These direct thrombin inhibitors are generally followed with the activated partial thromboplastin time (aPTT). In the setting of HIT with a lupus inhibitor, the aPTT may not be an accurate indication of direct thrombin inhibitor effect due to prolongation of the aPTT by the lupus inhibitor. The thrombin time reagent contains no phospholipid and thus has no interference from a lupus inhibitor. This assay uses a 1:4 dilution of the patient's plasma into normal plasma to minimize interfering substances and standardize the therapeutic range.

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 20-40% 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. Isolated factor deficiencies for factors VIII, IX, XI, or XII will present with a borderline to marked prolongation of the APTT and all other screen procedures are normal. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting times and falsely decrease the factor concentrations for these assays.

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 an 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 effect 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 Bethseda assay can be used to 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 Bethseda assay. Many non-hemophilic inhibitors are of type II.

The spontaneous development of a 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 post 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: Clot based Clauss or Kinetic method.

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 an 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 has a 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 an antigenic fibrinogen or total clottable fibrinogen determination. Typically the kinetic fibrinogen will be lower than the antigenic or total clottable method fibrinogen. To specifically classify the type of dysfunctional fibrinogen it is necessary to perform specific research assays to identify the abnormality in the protein.

Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting times and falsely decrease the fibrinogen concentration.

HEPARIN ACTIVITY (UNFRACTIONATED AND LOW MOLECULAR WEIGHT)

Method: Chromogenic, anti-Xa activity assay.

This assay is calibrated using a hybrid unfractionated and low molecular weight heparin curve. Generally patients on unfractionated 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.

The aPTT cannot be used to monitor low molecular weight heparin therapy. Low molecular weight heparin inhibits factor Xa more potently than thrombin. The Heparin Activity assay is the assay of choice to monitor patients on Low Molecular Weight Heparin.

LUPUS INHIBITOR (ANTICOAGULANT)

Method: Hexagonal (II) phase phospholipid clotting assay

It is very important that all the platelets are removed from the sample prior to freezing, as residual platelets will adversely affect the test results. Freezing will rupture the platelet membrane 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 modified aPTT reagent that is very sensitive to lupus inhibitors. If the modified aPTT is prolonged, phospholipid is substituted for buffer and the aPTT repeated. If the phospholipid corrects the aPTT then a lupus inhibitor is present. Patients are reported as negative, borderline positive, moderate positive or strong positive. A patient with a borderline positive result may have a transient lupus inhibitor associated with infection or reaction to some drugs. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting time and invalidate this assay.

Lupus inhibitors (antiphospholipid antibodies) are immunoglobulins that interfere with phospholipid dependent coagulation assays. Typically, the aPTT is the first assay affected. If a strong lupus inhibitor is present, the aPTT may be moderately to markedly prolonged and will not completely correct on 1:1 mix. The prothrombin time may also be 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 inhibitors; these patients are at risk for bleeding. Lupus inhibitors are associated with arterial and venous thrombosis.

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 tPA.

Fibrinolytic activity in blood follows a circadian rhythm. Peak levels of PAI-1 occur in the morning with the lowest levels occurring in the evening. For this reason the optimal 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. Absent PAI-1 activity and antigen indicate PAI-1 deficiency which is associated with a mild to moderate delayed bleeding syndrome.

PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI-1) ANTIGEN

Method: Enzyme immunoassay

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 occur in the morning with the lowest levels occurring in the evening. For this reason the optimal 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: Chromogenic based 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 that 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 the 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 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.

PROTEIN C ANTIGEN

Method: Enzyme immunoassay

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 on-going 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 dominant. 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.

Trimester	Range Activity
First	43-111%
Second	26-80%
Third	11-55%

As a clot based assay, the protein S activity level may be influenced by other clotting proteins and conditions. Elevated factor VIII activity (>250%) may falsely lower apparent protein S activity. The presence of a lupus inhibitor or a heparin concentration greater than 0.6 units / mL may lead to falsely elevated protein S activity. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting times and invalidate this assay. **The free protein S antigen assay is recommended over the protein S clottable assay to screen for protein S deficiency.**

PROTEIN S ANTIGEN (FREE)

Method: Latex immunoassay (LIA)

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.

Trimester	Range of Free Protein S Antigen	Range of Total Protein S Antigen
First	55-132%	64-127%
Second	48-134%	48-120%
Third	38-84%	47-91%

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 that 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.

PROTEIN S ANTIGEN (TOTAL)

Method: Latex immunoassay (LIA)

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. Patients 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 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 / or 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. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting times in this assay.

PROTHROMBIN TIME 1:1 MIX

The prothrombin time (PT) 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 (Research test only)

Method: ELISA

Prothrombin fragment 1.2 is a polypeptide released from prothrombin when it is activated to thrombin by the prothrombinase complex (activated factor X and V, calcium and phospholipid). Elevated F 1.2 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 those patients on oral anticoagulant therapy. Elevated levels are observed in inherited thrombophilia (protein C deficiency, protein S deficiency, and antithrombin deficiency). Increased levels occur in thrombosis, pulmonary embolism, DIC, trauma, septicemia and complications 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 <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. The thrombin time will be >100 sec with heparin levels >0.05 unit / mL. The thrombin time reagent uses bovine thrombin. A patient with a prolonged thrombin time that is not due to heparin and 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. Direct thrombin inhibitors, such as argatroban and lepirudin, will falsely prolong clotting times in this assay.

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 fibrinogen deficiency. No correction or only a partial correction indicates the presence of an inhibitor.

tPA ACTIVITY (Tissue Plasminogen Activator Activity)

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

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.

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. Samples for tPA activity must be drawn into a special acid citrate Stabilyte tube.

tPA ANTIGEN (Tissue Plasminogen Activator Antigen)

Method: Enzyme immunoassay

This assay measures total tPA in plasma, including active tPA and tPA / PAI-1 complex. Since PAI-1 activity is usually in excess compared to tPA activity, and the reagent between the two continues after the sample is obtained (in citrate plasma), most of the tPA in this assay is in the form of tPA / PAI-1 complex. Elevated tPA antigen typically is associated with elevated PAI-1 activity and indicates a reduction in fibrinolytic activity. tPA antigen and activity may be elevated after endogenous release or exogenous infusion of tPA. Reduced tPA antigen is usually associated with PAI-1 deficiency. tPA antigen levels increase with age, exercise, and stress. Venous stasis will result in increased tPA levels.

VON WILLEBRAND FACTOR ACTIVITY

Method: Enzyme immunoassay

Antibody directed towards to GPIb complex binding site on von Willebrand factor. Levels parallel total von Willebrand factor antigen levels in normal patients and type 1 von Willebrand disease. Levels are lower that total von Willebrand factor levels in patients with type 2 von Willebrand disease.

VON WILLEBRAND FACTOR ANTIGEN

Method: Latex immunoassay

Decreased von Willebrand factor antigen is associated with von Willebrand disease. See page 9 for a detailed description of von Willebrand disease and its subtypes.

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 some situations lack the intermediate weight multimers. Type 3 patients will not have any visible multimers due to the extremely low level or unmeasurable 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.

RESEARCH ASSAYS

Listed below are assays offered through our Research and Development division for investigational purposes only. The availability of these assays is 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. Chandler or Dr. La Spada at (206) 598-6131 for consultation. If you are interested in an assay not listed below, please call 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.

Beta thromboglobulin antigen
C4b-binding protein antigen
CD 40 Ligand (soluble)
Chromogenic factor II
E-selectin antigen
Endothelial Protein C Receptor
Factor VII antigen
Factor X antigen
Fibrinopeptide A
High Molecular Weight Kininogen factor activity
Myeloperoxidase
Neuron Specific Enolase
P-Selectin antigen
Platelet Factor 4 antigen
PMN Elastase
Prekallikrein factor activity
Prothrombin fragment 1.2
S100
Soluble endothelial protein C receptor
Tissue Factor antigen, soluble
Thrombin antithrombin complex antigen
Thrombomodulin antigen
Tissue Factor antigen
Tissue Factor Pathway Inhibitor antigen