Coagulation Studies—Waukesha Memorial Hospital (WMH)

Blood Collection for Coagulation Studies
To ensure the best possible specimen, follow collection requirements as closely as possible.

1. **Patient should be fasting**, if possible; for certain tests, patient cannot be receiving anticoagulant medication (heparin or warfarin/Coumadin®).

2. **Draw blood from patient into light blue-top (sodium citrate) vacuum tube(s)** (those used for prothrombin time/activated partial thromboplastin time containing 3.2% or 3.8% sodium citrate; 3.2% preferred). If patient’s hematocrit is ≥50%, volume of anticoagulant in tube should be adjusted. Use the following formula to determine correct anticoagulant volume:
   \[
   \text{anticoagulant volume} = \frac{(100 - \text{hematocrit})}{(595 - \text{hematocrit})} \times \text{volume of specimen}
   \]
   Tubes must fill completely. A clean venipuncture is essential to avoid activation of coagulation by tissue thromboplastin. Specimens containing fibrin clots or moderate to gross hemolysis will be rejected.

3. **The specimen must be double-centrifuged to prepare a platelet-free plasma specimen.** Immediately centrifuge at ≥1,500 x G for 10 minutes, at 4° C, if possible. Carefully remove plasma from cells avoiding platelet/buffy coat. Dispense into a plastic tube and centrifuge plasma in plastic tube at ≥1,500 x G for 10 minutes, at 4° C, if possible. Remove top portion of plasma leaving approximately 250 µL in bottom to *discard*. The double-centrifuged plasma should be aliquoted (0.5-1 mL each) into clearly labeled plastic tubes. *(Glass vial is not acceptable.)* The number of tests ordered will determine aliquots needed; generally 1 aliquot per test.

4. **Patient specimens should be frozen at ≤-40° C, if possible, and sent together in same container with at least 5 lbs of dry ice. They must arrive in a frozen state.**

5. Affix **FROZEN SPECIMEN** label to transport bag. Alert courier of frozen specimens.

6. **Please include requested information** (see individual test descriptions) as testing and interpretations are dependent on clinical history in many of the more complex abnormalities.

7. Careful specimen handling will most often ensure acceptable specimens and valid results.

Antiphospholipid Syndrome
Although antiphospholipid antibodies (APA) have been associated with a variety of clinical phenomena, the term “antiphospholipid syndrome” is generally used to link a variety of thromboembolic events to antibodies directed against specific proteins involved in blood coagulation. Thrombotic events are thought to occur in up to 30% of patients harboring APA. These most often include venous thrombosis and/or pulmonary embolism (PE), as well as cerebral artery thrombosis. Obstetrical complications include recurrent spontaneous abortion, fetal demise, or fetal gross retardation. Other clinical manifestations of APA syndrome include hemolytic anemia, livedo reticularis, skin necrosis, and neuropsychiatric events. Asymptomatic patients may also present with unexplained prolongation of PTT (Partial Thromboplastin Time).

Two types of antiphospholipid syndromes have been described:

The presence of APA may be associated with systemic lupus erythematosus or other autoimmune disorders, neoplastic disease, and other pathologic conditions. APA represents a large heterogeneous family. Testing generally includes evaluation for lupus anticoagulant and anticardiolipin antibodies. Since either of these aforementioned antibodies present similarly, it is recommended that initial work-up include testing for both. If an antibody is detected and characterized, patient can be followed for that specific abnormality. The diagnosis of APA requires a positive test on 2 separate occasions, at least 8 to 12 weeks apart, in setting of thrombosis, thrombocytopenia, or recurrent fetal loss.

Indications for APA testing (general guidelines):

- recurrent spontaneous miscarriage (especially late second to third trimester), fetal demise, or unexplained fetal growth retardation
- part of the work-up for venous thrombosis at any age
- part of the work-up for arterial thrombosis in young patients (usually <50 years of age) or patients with no evidence of atherosclerosis
- unexplained prolongation of APTT

Assessment of Thrombosis
A delicate balance exists between clot formation and clot dissolution and any condition which shifts the balance of direction of clot formation may lead to thrombosis. Early onset of venous thrombosis (<50 years of age) usually is associated with inherited deficiencies of specific coagulation factors. Specific abnormalities are further defined below. Patients who develop venous thromboses later in life usually have associated acquired deficiencies. Moreover, other medical conditions, especially myeloproliferative disorders and malignancy can lead to thrombosis and should be considered in differential diagnosis.
Activated Protein C (APC) Resistance
APC resistance is the most common known hereditary predisposition to thrombosis. Protein C is cleaved to APC by thrombin. APC is an anticoagulant which functions by inactivating factor Va and VIIIa. APC resistance is most frequently caused by a genetic mutation in the factor V gene, factor V Leiden. Patients who are heterozygous for factor V Leiden have a 3- to 7-fold increased risk for venous thrombosis. Homozygotes have an 80-fold increased risk. Risk is also further increased in the presence of a second risk factor such as oral contraceptive use, pregnancy, protein S deficiency, hyperhomocysteinemia, or advanced age.

The functional assay for APC utilizes APTT (Activated Partial Thromboplastin Time)-based method to screen for APC resistance. In normal plasma, the PTT is prolonged with addition of APC. A ratio of APTT with and without added APC is used to determine resistance.

In presence of a positive screening test, a DNA assay for factor V Leiden is required for confirmation of diagnosis.

Antithrombin (AT) Deficiency
AT deficiency is seen in 2% to 4% of patients with venous thrombosis. Genetically deficient AT patients usually have their first thrombotic event before the age of 50 years.

AT deficiencies are quantitative (Type I) or qualitative (Type II). As with PC and PS, a functional assay that measures AT activity is done as it can detect both types. If abnormal, an antigenic assay can be done to distinguish type of deficiency.

Acquired AT deficiency is seen in liver disease, disseminated intravascular coagulation (DIC), heparin therapy, nephrotic syndrome, colitis, post-operative state, and oral contraceptives. If results show a decreased level of AT, liver function tests, urine protein, and a DIC panel are recommended to rule out acquired etiologies.

Homocysteine - Methylenetetrahydrofolate Reductase (MTHFR)
Hyperhomocysteinemia was detected in approximately 20% of patients under the age of 40 who were presenting with their first episode of venous thrombosis. A mutation in MTHFR enzyme is the most common genetic factor leading to increased homocysteine levels. Increased homocysteine is now recognized as an independent risk factor for arterial and venous thrombosis.

Homocysteine levels can be measured in our laboratory and are best done on fasting specimens. Presence of MTHFR mutation can be detected by polymerase chain reaction (PCR)-based DNA analysis. A mutation predicts presence of hyperhomocysteinemia, but levels must still be determined.

Acquired causes of hyperhomocysteinemia include renal failure, carcinoma, hypothyroidism, and treatment with methotrexate, theophylline, or phenytoin. Deficiencies in vitamin B₆ or folate can also increase homocysteine levels.

Protein C (PC)
PC is a vitamin K-dependent protein, which is activated by thrombin becoming a serine protease, which inactivates factor Va and factor VIIIa. Approximately 2% to 4% of patients with venous thrombosis may have either a congenital or acquired PC deficiency. The first thrombotic event usually presents between the ages of 10 years to 50 years.

PC deficiencies may be quantitative (Type I) or qualitative (Type II). In our laboratory, the functional PC activity is performed which can detect either type of deficiency. If PC activity is decreased, a PC antigen may be performed to quantitate the level of PC molecule and distinguish type of deficiency.

Coumadin® treatment, liver disease, acute thrombosis, vitamin K deficiency, and DIC will reduce PC activity. PC cannot be reliably determined during oral anticoagulation, and it is best done at least 10 days after discontinuing treatment. If results show a decreased level of PC, liver function tests and a DIC panel are recommended to rule out acquired etiologies.

Protein S (PS)
PS is a vitamin K-dependent protein synthesized in liver, vascular endothelium, and megakaryocytes. PS functions as a cofactor for activated PC to facilitate degradation of factors Va and VIIIa. In plasma, 40% of PS circulates as a free molecule, which represents functionally active form. The remaining 60% is complexed with C4b binding protein, the plasma protein related to classic complement pathway.

Levels of <50% of normal may be associated with venous thrombosis. Like PC, PS-deficient individuals tend to present with the first thrombotic event before the age of 50 years.
PS deficiencies are quantitative (Type I) or qualitative (Type II). A functional assay that measures PS activity is used as the initial screening test as it can detect both types. If result is abnormal, a free and total S antigen should be pursued. C4b binding protein is commonly elevated during acute-phase reactions, consequently reducing the level of free PS. Additionally, markedly elevated factor VIII levels (also an acute-phase reactant) can artifactually reduce the level of functional PS. In either of these settings, repeat testing is suggested when acute-phase reaction has subsided.

Other causes of acquired disease PS include oral contraceptives, estrogen replacement therapy, pregnancy, nephrotic syndrome, HIV infection, and inflammatory bowel disease.

Like PC, PS levels are decreased by Coumadin® therapy, liver disease, vitamin K deficiency, DIC, and acute thrombosis. PS cannot be reliably determined during oral anticoagulation, and it is best done at least 10 days after discontinuing treatment. If results show a decreased level of PS, liver function tests, urine protein, and a DIC panel are recommended to rule out acquired etiologies.

**Prothrombin 20210A Allele**
Recently, a mutation in the prothrombin gene has been described and thought to represent an approximate 3-fold increased risk for venous thrombosis in heterozygous state. This genetic risk factor is associated with increased plasma prothrombin levels and is seen in 1% to 2% of the healthy population. Testing for this abnormality is usually performed by PCR-based DNA analysis.

**D-Dimer**
The level of D-Dimer rises during coagulation activation as a consequence of fibrin formation and subsequent degradation of fibrin clot by fibrinolytic system. The D-Dimer is valuable in detecting presence of, or monitoring presence of fibrin-based thrombotic events, such as DIC, deep vein thrombosis (DVT), and pulmonary embolism (PE).

**DIC**
D-Dimer is useful for early diagnosis and ongoing monitoring of DIC. D-Dimer should be used in conjunction with clinical evaluation as well as a battery of other appropriate coagulation studies for DIC.

**PE**
A non-detectable D-Dimer level is very close to a 100% negative predictor for PE. However, a positive or detectable level of D-Dimer is not a positive predictor for PE.

**Summary**
Increased levels are found in the following:

1. PE
2. DVT
3. DIC
4. Hemorrhage
5. Surgery
6. Metastatic carcinoma
7. Cirrhosis
8. Intrauterine device
9. Gram-negative sepsis

**von Willebrand Disease (vWD)**
vWD is caused by an inherited deficiency of von Willebrand factor (vWF) and has an estimated prevalence of approximately 2% of the population in the United States. Approximately 80% of vWD disease patients have a quantitative Type I deficiency characterized by easy bruising and prolonged bleeding from mucosal surfaces.

vWF is synthesized by endothelial cells and megakaryocytes and participates in platelet adhesion and aggregation. vWF also stabilized factor VIII:C (anti-hemophilic factor). Deficiency of factor VIII:C causes classic hemophilia A while deficiency of vWF causes vWD. vWF exists in multimers ranging from 500,000 to 2 million molecular weight. High molecular weight multimers are the most effective components for interaction with platelets.

vWD disease work-up should include an APTT, bleeding time, and factor VIII:C activity as well as a qualitative (functional) vWF. The functional or activity determination is performed using ristocetin cofactor assay, which is based on platelet aggregation. A decreased vWF level should be followed up with a quantitative determination the vWF antigen and vWF multimer study.