



Thrombophilia: Laboratory Support of Risk Assessment and Diagnosis

Clinical Background

Thrombophilia is characterized by hypercoagulability and an increased propensity for thrombosis. Almost 2 million Americans succumb annually to a thromboembolic event,¹ with venous thrombosis the third most common cardiovascular disease after ischemic heart disease and stroke. Venous thrombosis affects 1 to 2 in 1000 individuals every year and is associated with life-threatening conditions such as pulmonary embolism (PE).¹ Though less clearly delineated, hypercoagulability is also believed to play a role in the pathogenesis of arterial thrombosis.²

Conditions associated with an increased risk of venous thrombosis can be either inherited or acquired (Tables 1 and 2). One or more predisposing factors are identifiable in 80% of individuals with a first episode of thrombosis, and an inherited cause of thrombophilia can be identified in approximately 30% to 35% of individuals with a first thrombotic episode.³ Manifestations include deep vein thrombosis (DVT) of the lower limbs, PE, superficial thrombophlebitis, mesenteric or cerebral vein thrombosis, fetal loss (spontaneous abortion or stillbirth), preeclampsia, and neonatal purpura fulminans.

Individuals at high risk for venous thrombosis include those with a personal or family history of thrombosis, inherited coagulation disorders, homocystinuria, paroxysmal nocturnal

hemoglobinuria, essential thrombocythemia, polycythemia vera, recurrent spontaneous abortion and stillbirth, and malignancy. Additional risk factors include surgery, trauma, physical inactivity (bed confinement or paralysis), warfarin induced skin necrosis, diabetes, hyperlipidemia, vasculitis, thrombocytopenia, sepsis, congestive heart failure, and use of purified prothrombin complex concentrates. Other disorders that may be associated with increased thrombotic risk are thrombomodulin mutations, plasminogen deficiency, and elevations of histidine-rich glycoprotein, plasminogen activator inhibitor-1, interleukin 8, lipoprotein (a), D-dimer, and thrombin-activatable fibrinolysis inhibitor.

The risk of thrombosis increases with the number of defects or risk factors present; ie, individuals with multiple conditions associated with thrombosis are at greater risk than those with only one condition.¹ Risk factors for thrombophilia are generally not associated with risk of arterial thrombosis, with the exception of hyperlipidemia, antiphospholipid syndrome (Appendix 1), elevated homocysteine, and dysfibrinogenemia.²

The identification of thrombotic risk factors and diagnosis of thrombophilia contributes to patient management in multiple ways (Table 3). Such diagnosis is based on personal and family history of thrombosis (especially during adolescence and young adult years), clinical manifestations, and laboratory testing.

Table 1. Inherited Conditions Associated with Venous Thrombosis

Condition	Frequency (%) in Healthy Individuals	Frequency (%) in Patients with Venous Thrombosis*	Relative Risk (%) of Thrombosis
Activated protein C resistance/factor V mutation ^{††4,5}	5	21	3-7
Antithrombin deficiency ⁶	0.02-0.17	1	15-40
Factor VIII excess ^{§7}	11	25	6
Hyperhomocysteinemia ^{§§8,9}	5-10	10-25	3-4
Protein C deficiency ⁶	0.3	3	5-12
Protein S deficiency ⁶	0.7	2	4-10
Prothrombin (factor II) 20210G→A mutation ^{††10,11}	2	6	2-3

Other rare inherited conditions that may be associated with inherited venous thrombosis include sticky platelet syndrome, heparin cofactor II deficiency, factor XII deficiency, and dys- and hyperfibrinogenemia. Deficiency of antithrombin, protein C, and protein S may also be acquired.

*Data is for heterozygotes.

†Affects primarily Caucasian population.

‡Homozygosity of the Factor V HR2 allele increases the risk of venous thrombosis 3- to 4-fold in the presence of the Factor V Leiden mutation (no increase if Factor V Leiden mutation is absent).

§Can also be acquired.

||MTHFR 677→T or 1298A→C mutation, or dietary deficiency of folate, and/or vitamins B6 and B12.

Table 2. Acquired Conditions Associated with Venous Thrombosis

Antiphospholipid syndrome (<i>most common cause</i>)
Autoimmune disorders (eg, systemic lupus erythematosus)
Combined oral contraceptives
Elevated factor IX, XI
Endocrine disorders (eg, diabetes mellitus, Cushing's syndrome)
Heparin-induced thrombocytopenia (HIT)*
Hormone replacement therapy
Liver disease
Malignancy [†]
Myeloproliferative disorders (eg, polycythemia vera, chronic myelogenous leukemia)
Nephrotic syndrome
Obesity
Paroxysmal nocturnal hemoglobinuria
Pregnancy and puerperium
Thrombotic thrombocytopenic purpura

*Should be considered in any individual who has received heparin within the 30 days preceding a thrombotic episode and has a decrease in platelet count to <100,000/ μ L or more than 50% of baseline.

[†]A thrombotic event can precede the diagnosis of malignancy by months to years.

Clear guidelines how to best manage individuals with a family or personal history of documented risk factors and who have not experienced a thrombotic episode have not been established. The decision for prophylactic therapy should be based on an individual's clinical history.¹² Screening general populations for inherited disorders associated with venous thrombosis is not recommended; however, the clinical utility of global screening assays in high risk populations is being evaluated.

Patients with acute thrombosis are treated with intravenous heparin or oral anticoagulants such as warfarin (Coumadin[®]). Prophylactic treatment is provided to diagnosed patients when in high risk situations, eg, surgery, prolonged immobilization, pregnancy and puerperium. Lifelong prophylactic therapy may be considered for those with recurrent thrombotic episodes, high risk disorders, or with multiple risk factors and may include plasma transfusions (eg, antithrombin concentrates), oral anticoagulants, low dose aspirin, and heparin.¹² Heparin is of limited benefit post thrombosis in patients with antithrombin deficiency, however, and heparin selection for pregnant women should be individualized due to risk of bone fracture.¹³ Low molecular weight heparin (LMWH) may be a better option for those at risk of osteoporosis since LMWH does not cause bone thinning. Individuals with hyperhomocysteinemia may be treated with vitamin supplementation (folic acid, cobalamin, pyridoxine).

Individuals Suitable for Testing

- Symptomatic individuals
- Individuals with a personal or family history of thrombosis or thrombophilia-associated mutations
- High risk individuals predisposed by surgery, trauma, immobility, pregnancy, oral contraceptives, etc.

Note: high risk pregnant women include those with a personal or family history of thrombosis, previous neural tube defect affected fetus, recurrent spontaneous abortions, severe early onset preeclampsia, cesarean section, obesity, advanced maternal age, higher parity, and prolonged immobilization.¹³

Test Availability

Tests available to assist in diagnosis and management of thrombophilia disorders are listed in Appendix 2. Additionally, Quest Diagnostics offers panels that include multiple tests, thereby simplifying the test ordering process. Refer to the Quest Diagnostics Directory of Services for information on these panels, which are typically named according to the medical condition.

Test Selection

A venous thrombosis laboratory work-up for high-risk or symptomatic individuals begins with a personal and family history. Test selection may vary for each individual based on his/her history as well as a particular defect's prevalence in specific populations. For example, venous thrombosis in a pediatric patient suggests the likelihood of an inherited disorder; in an individual with SLE, antiphospholipid syndrome should be considered; and in an older individual, malignancy. Testing for multiple etiologies is recommended since venous thrombosis is a polyfactorial disorder, and presence of multiple etiologies increases the risk for thrombosis.^{14,15} Generally accepted testing guidelines suggest the use of first and second line testing in the thrombophilia diagnosis (Figure).^{14,15}

First line testing for an individual with venous thrombosis typically includes a CBC with smear and APTT; activated protein C resistance (APCR); functional (activity) assays for antithrombin, proteins C and S, and factor VIII; prothrombin 20210G→A mutation detection; homocysteine; and anticardiolipin and antiphospholipid antibodies (see Appendix 1. Antiphospholipid Syndrome). APCR and prothrombin 20210G→A mutation detection need not be performed initially in non-Caucasian individuals since these disorders are primarily observed in Caucasians. Likewise, if a first thrombotic event occurs after the age of 50, testing for protein C, S, and antithrombin deficiency may be postponed as hypercoagulability due to these disorders usually manifests as thrombosis earlier than the fifth decade. Testing for heparin induced thrombocytopenia should be considered for any individual who has received heparin within the 30 days preceding a thrombotic episode and has a decrease in platelet count to <100,000/ μ L or more than 50% of baseline.

Table 3. Value of Thrombophilia Diagnosis

Identify pathologic basis of thrombotic event
Aid in selection of appropriate therapy
Determine duration and intensity of treatment
Determine need for prophylaxis
Estimate future thrombotic risk
Determine degree of risk associated with oral contraceptives and estrogen replacement therapy
Determine need for evaluation of family members

Table 4. Confounding Effects of Anticoagulant Therapy and Acute Thrombosis on Testing Used in the Diagnosis of Thrombophilia

Test	Confounding Effect		
	Warfarin	Heparin	Acute Thrombosis
Antithrombin	None (rarely increases)*	Decreases	May decrease [†]
Antiphospholipid antibodies	None	Potential false positive	None
APCR/Factor V Leiden [‡]	Nonsensical results	None [§]	None
APTT	May increase	Increases	Increases
Homocysteine	None	None	None
Lupus anticoagulant	Potential false positive	Nonsensical results	None
Protein C	Decreases	None [§]	May decrease [†]
Protein S	Decreases	None [§]	May decrease [†]
Prothrombin gene mutation	None	None	None
Reptilase time	Increases	None	Increases
Thrombin time	Increases	Increases	Increases

APCR, activated protein C resistance; APTT, activated partial thromboplastin time.

*Can be increased to normal range in individuals with heterozygous deficiency.

[†]Results are inaccurate during acute thrombosis; however, a normal level during an acute thrombotic event essentially excludes deficiency of these proteins.

[‡]Mutation analysis not affected.

[§]Heparin up to 1 U/mL does not affect results.

Additional testing directed toward diagnosis of other causes of acquired thrombophilia such as systemic lupus erythematosus, liver disease, nephrotic syndrome, polycythemia vera, chronic myelogenous leukemia, diabetes mellitus, Cushing's syndrome, etc. may be indicated (see Table 2).

Positive functional assays can be confirmed by genetic testing in some cases or by demonstration of the abnormality in another family member. For example, a borderline or positive APCR test can be confirmed with factor V (Leiden) mutation analysis. Such analysis differentiates homozygous and heterozygous states, providing additional prognostic information. Factor V HR2 allele mutation analysis provides even more prognostic information in factor V (Leiden) carriers. Homocysteine elevations may be due to an acquired nutritional deficiency (vitamin B₁₂, B₆, or folate), methylenetetrahydrofolate reductase (MTHFR) mutation, or cystathionine β-synthase mutation. Acquired causes for antithrombin, protein C, and protein S deficiencies can be ruled out by a liver function testing, disseminated intravascular coagulation screen (D-dimer, fibrin degradation product, PT, APTT, fibrinogen, platelet count), and a proteinuria test (urine albumin).¹⁶ Decreased antithrombin and protein C and S activities (function) can be further characterized as a deficiency (type I or III) or dysproteinemia (type II) by using antigenic assays; however, such characterization will not affect treatment decisions.

If all of the aforementioned testing is negative, the patient may have a rare disorder that can be identified by testing for fibrinogen, plasminogen activity (function), plasminogen activator inhibitor-1 (PAI-1), lipoprotein (a) [Lp(a)], tissue plasminogen activator, heparin cofactor II, etc (Figure). Testing for rare disorders is only recommended for individuals with a strong personal and family history of thrombosis and negative first line tests or in whom clinical suspicion is high. Since all thrombophilia etiologies are not yet known, it is possible for all of these tests to be negative.

Test Interpretation

Acute thrombosis, anticoagulant therapy, drug therapy, and certain medical conditions can affect the results and interpretation of tests used to diagnose causes of thrombophilia (Tables 4 and 5). Additional interpretive information, specific to each test, is provided below.

Activated Partial Thromboplastin Time (APTT)

The APTT will be prolonged if there is deficiency or inhibition of factors of the intrinsic pathway including high molecular weight kininogen (HMWK), prekallikrein, factors V, VIII, IX, X, XI and XII, prothrombin, and fibrinogen. Prolongation is also seen in individuals with lupus anticoagulant.

Activated Protein C Resistance (APCR)

A decreased ratio of dRVVT clotting times obtained with and without activation of endogenous protein C is suggestive of an activated protein C (APC) inhibitor, a factor V (Leiden) mutation, and increased risk of deep vein thrombosis. Assay sensitivity and specificity approach 100%, even in the presence of anticoagulants and heparin (≤ 1 IU/mL plasma).^{17,18} Falsely decreased ratios (false-positive test) are observed in patients with lupus anticoagulants and specimens with platelet counts $>20,000/\mu\text{L}$. In $<5\%$ of cases, an APC inhibitor is found without a corresponding factor V (Leiden) mutation, perhaps indicative of an unknown mutation. Such cases are also associated with increased venous thrombosis risk.¹⁹

Antithrombin

Decreased levels of antithrombin are associated with an increased risk of both arterial and venous thrombosis and are seen in individuals with hereditary antithrombin deficiency, nephrotic syndrome, colitis, liver disease, active thrombosis, disseminated intravascular coagulation (DIC), those receiving L-asparaginase therapy or oral contraceptives, and individuals who are pregnancy or have undergone surgery. Levels are also decreased in individuals receiving heparin. Levels in neonates are approximately half of the adult level, which is reached by 6 months of age. Low levels in both the activity

Table 5. Conditions That Affect Testing for Inherited Causes of Thrombophilia

Condition	Effect
Lupus anticoagulant	Decreases APCR, protein S, and factor VIII
Deficiency of Vitamin B12, B6, or folate Methotrexate, phenytoin, theophylline Hypothyroidism, malignancy, menopause	Increases homocysteine
Oral contraceptives Estrogen replacement	Decreases antithrombin and protein C and S
Pregnancy and puerperium	Decreases APCR and protein C and S Increases homocysteine
Acute phase reaction, inflammation, infection	Increased factor VIII Decreases protein S
Kidney disease/nephrotic syndrome	Decreases antithrombin and protein S
DIC, liver disease, sepsis, L-asparaginase therapy	Decreases antithrombin and protein C and S
Surgery, recent thrombosis	Decreases antithrombin and protein C and S Increases homocysteine
Vitamin K deficiency	Decreases protein C and protein S

APCR; activate protein C resistance, DIC; disseminated intravascular coagulation.

and antigen assays indicate type I deficiency, whereas low activity levels in the presence of normal antigen levels indicate type II deficiency (dysproteinemia). Increased levels may be due to oral anticoagulants or heparin cofactor II.

C4 Binding Protein

Approximately 65% of protein S circulates in plasma bound to C4 binding protein. Increased levels of C4 binding protein may cause decreased levels of free protein S, and subsequent increased risk of thrombosis, and are associated with inflammation, pregnancy, diabetes mellitus, SLE, AIDS, allograft rejection, estrogen and progesterone administration, and smoking.

Cardiolipin Antibodies

Anticardiolipin antibodies of the IgA, IgG, and IgM isotype are associated with the antiphospholipid syndrome and, when >40 GPL units, increase the risk for venous thrombosis 5- to 8-fold. IgG antibodies appear to be more predictive of disease activity, while IgM antibody occurs more often in drug-induced disorders and infectious disease (eg, syphilis). Higher antibody titers are generally correlated with greater thrombotic risk (see Appendix 1. Antiphospholipid Syndrome).

Cytochrome P450 2C9 Genotype

The cytochrome P450 enzyme CYP2C9 participates in the metabolism of a number of important drugs, including warfarin. Individuals carrying variants in the CYP2C9 gene have reduced metabolism of warfarin and those with 2 copies of variant alleles are at high risk of life-threatening side effects.

D-Dimer

Elevated levels are associated with myocardial infarction, deep vein thrombosis, pulmonary embolism, DIC and other coagulation disorders, surgery, trauma, sickle cell disease,

liver disease, severe infection, sepsis, inflammation, malignancy, obstetric complications, and hyperfibrinolysis.

dRVVT Screen with Reflex to Phospholipid Neutralization

This test evaluates the activity of factors II, V, and X (common clotting pathway). A confirmed dRVVT test result (ie, increased ratio) is indicative of a lupus anticoagulant and/or phospholipid antibody since excess phospholipid shortens (overrides) the prolonged dRVVT. A falsely prolonged dRVVT test may occur when heparin is >1.0 IU/mL. A false-negative dRVVT test may be due to platelet contamination of the plasma. Samples with moderate or severe icterus or lipemia are contraindicated.

Factor V HR2 Allele DNA Mutation Analysis

The HR2 allele is associated with APCR and increased risk of venous thrombosis in individuals also heterozygous for the factor V (Leiden) mutation. Such co-inheritance increases the risk of venous thromboembolism 3- to 4-fold when compared with factor V (Leiden) alone. An individual heterozygous positive for the HR2 allele and negative for factor V (Leiden) is not at increased risk of thrombosis compared to factor V (Leiden) alone. However, homozygosity for factor V HR2 is associated with increased risk of thrombosis even in the absence of a factor V (Leiden) mutation.

Factor V (Leiden) Mutation Analysis

The 1691G→A factor V Leiden mutation results in the laboratory finding of APCR. Factor V (Leiden) confers approximately a 7-fold increase in venous thromboembolic events in heterozygous individuals and an 80-fold increase in homozygous subjects.²⁰ When a heterozygous mutation is coupled with oral contraceptive use, the risk increases synergistically to 30-fold.²¹ The mutation is also associated with arterial thrombosis (especially in smokers),

complications of pregnancy (including fetal loss),²² and increased levels of factor VIII. Although this test is highly specific, identification of a mutation may occur in the absence of APCR in rare cases. Sensitivity of this test for APCR is 94%;²³ thus, a negative result does not rule out APCR or an increased risk of venous thrombosis.

Factor VIII

Factor VIII is an acute phase reactant and increased levels are found during periods of stress, postoperatively, and in inflammatory conditions. Elevated levels are also found at birth and during pregnancy. Increased levels are associated with increased risk for venous thrombosis,²⁴ whereas decreased levels are associated with hemophilia A. A factor VIII activity:fibrinogen ratio >0.75 is considered diagnostic of factor VIII excess.

Fibrin Monomer

The presence of soluble fibrin monomer complexes in plasma is diagnostic of DIC.

Fibrinogen

Increased levels are associated with acute phase reactions, pregnancy, and increased risk of thrombosis. Low fibrinogen activity levels are associated with afibrinogenemia, hypofibrinogenemia, or dysfibrinogenemia (which may be associated with thrombophilia in rare instances), as well as with DIC, systemic fibrinolysis, pancreatitis, severe hepatic dysfunction, and L-asparaginase or valproate treatment. Individuals with afibrinogenemia or hypofibrinogenemia will have decreased activity and antigen levels. Individuals with dysfibrinogenemia will typically have decreased activity levels and normal or decreased antigen levels.

Fibrinogen Degradation Products (FDP)

FDP result from the breakdown of fibrinogen, as well as fibrin, by plasmin. Normally, the fibrinolytic process is localized to fibrin, however, during conditions such as DIC, fibrinolysis spreads and becomes systemic. Elevated levels of FDP are seen in many clinical states (eg, DVT and PE); thus measurement of FDP is useful for their diagnosis. Persistent elevations indicate that abnormal fibrinolysis and fibrinogenolysis are occurring.

β_2 -Glycoprotein I Antibodies

β_2 -Glycoprotein I antibodies of the IgA, IgG, and IgM isotype are associated with the antiphospholipid syndrome, and their presence is more specific but less sensitive than cardiolipin antibodies for the diagnosis of antiphospholipid syndrome. Individuals who are positive for cardiolipin antibodies and negative for β_2 -glycoprotein I antibodies are more likely to have an infection (varicella, rubella, adenovirus, HIV) or drug exposure (amoxicillin, chlorpromazine, hydralazine) than antiphospholipid syndrome (see Appendix 1).

Heparin, Low Molecular Weight (Xa Inhibition)

LMWH are prepared by the chemical or enzymatic degradation of unfractionated heparin, and are used in the prevention and treatment of thromboembolic conditions. Measurement of LMWH in plasma is used to monitor therapeutic levels. The therapeutic range for LMWH is 0.7 to 1.3 anti-Xa units/mL measured 4 to 6 hours after a

subcutaneous dose, and the prophylactic range is 0.3 to 0.7 anti-Xa units/mL measured 4 to 6 hours after a subcutaneous dose. (This assay was validated using enoxaparin; thus may not be appropriate for optimal evaluation of patients receiving other LMWH.)

Heparin, Unfractionated (Xa Inhibition)

Unfractionated heparin is used for the prevention and treatment of thromboembolic conditions and measurement is used to monitor therapeutic levels. When administered as an intravenous infusion, the therapeutic range is 0.35 to 0.7 IU/mL.

Homocysteine

Levels are increased in the following: cardiovascular disease, vitamin B₁₂ and folate deficiencies, chronic renal disease, homocystinuria, hypothyroidism, selected malignancies, individuals whose diet is rich in methionine (high meat intake), cigarette smokers, and in individuals treated with corticosteroids, methotrexate, cyclosporin, vitamin B₆ antagonists (isoniazid, azauridine, penicillamine, procarbazine), anticonvulsants (phenytoin, carbamazepine), and S-adenosyl-methionine. When coupled with the factor V (Leiden) mutation, venous thrombosis risk increases synergistically.²⁵ Falsely increased levels may occur if serum or plasma is not separated from the red cells within 1 hour of collection.

Homocysteine is decreased in pregnancy (except in some women carrying a fetus with a neural tube defect), individuals less than 15 years of age, and individuals taking oral contraceptives or hormone replacement therapy.

Human Platelet Antigen 1 (HPA-1) Genotype

The HPA-1b platelet antigen polymorphism is associated with increase platelet thrombogenicity, alloimmune thrombocytopenia, and post-transfusion purpura.

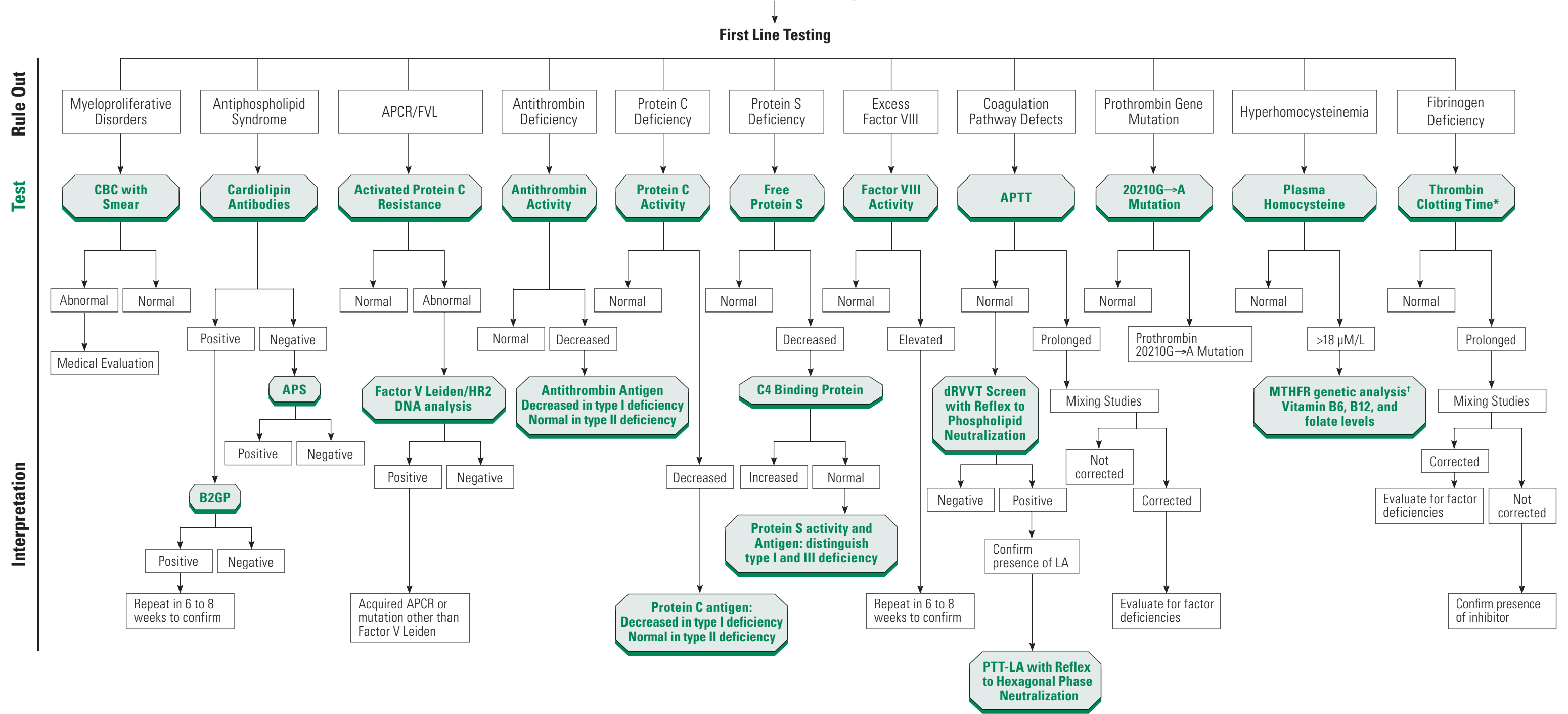
Lipoprotein (a) [Lp(a)]

Increased levels of Lp(a) are observed in patients with coronary artery disease, stroke, cerebrovascular and peripheral vascular disease, and venous thrombosis. Substantial increases are secondarily (not genetically related) observed in nephrotic syndrome and end-stage renal disease. Decreased Lp(a) levels may be seen in several rare disorders (lecithin:cholesterol acyltransferase [LCAT] deficiency, lipoprotein lipase [LPL] deficiency, liver disease). Normal levels in the African American population may be 2 to 3 times the values in Caucasian and Asian populations. Native Americans and Mexican Americans have lower normal levels (no lower than one half) relative to the Caucasian and Asian populations.

Methylenetetrahydrofolate Reductase (MTHFR) DNA Mutation Analysis

A homozygous MTHFR mutation (677C→T) may be associated with hyperhomocysteinemia, an increased risk for arterial and venous thrombosis, and an increased risk for obstetrical complications (eg, preeclampsia, abruptio placenta, fetal growth retardation, and stillbirth).²² Heterozygosity for this mutation, in the absence of vitamin deficiency, usually is associated with normal plasma

Individual with Documented Thrombotic Episode or at High Risk



Individual With Documented Thrombotic Episode(s), Strong Family History of Thrombosis, and Negative First Line Tests

Second Line Testing‡

Coagulation factors V, VII, IX, X, XI

Fibrinogen

Heparin cofactor II

Lipoprotein (a)

Plasminogen

Plasminogen activator inhibitor-1

Plasminogen activator (urokinase-like)

Reptilase time

Thrombomodulin

Tissue plasminogen activator

Figure. Testing algorithm for the diagnosis of thrombophilia in individuals with a history of thrombosis or those at high risk. An individual with a documented thrombotic episode should undergo a complete medical evaluation to rule out conditions associated with thrombophilia not diagnosed by first line testing, eg, nephrotic syndrome, diabetes mellitus, etc. High risk individuals are those with a strong family history of thrombosis and/or those with acquired risk factors, eg, obesity, prolonged immobilization, etc. All non-genetic testing should be repeated in 6 to 8 weeks to reduce the likelihood of false-positives. Some assays are affected by anticoagulants or the acute thrombotic process. APCR/FVL indicates activated protein C resistance/factor V Leiden; APS, antiphosphatidylserine antibody; B2GP, beta2-glycoprotein I; LA, lupus anticoagulant.

*Some authors consider thrombin clotting time to be a second line test.

†Mutation analysis is considered optional by some authors as treatment is not changed by the presence or absence of the MTHFR mutation.

‡Second line testing is for the identification of rare causes of thrombophilia and recommended for individuals with a documented thrombotic episode(s), a strong family history of thrombosis, and negative first line tests.

homocysteine levels. When a 677C→T mutation is combined with a 1298A→C mutation (ie, double heterozygosity), the risk is the same or greater than that of a 677C→T homozygote.

Mixing/Correction Study

Test results are consistent with an intrinsic factor deficiency when a prolonged APTT is normalized after mixing patient plasma with normal plasma and the normalized result does not reverse after incubation of the mixed sample. A specific factor inhibitor, lupus inhibitor, fibrinolysis, or fibrinogenolysis is suggested when 1) the PT is normal, and the APTT is prolonged initially, normalizes after mixing, and reverses to prolonged after incubation of the mixed sample; 2) the PT is normal, and the APTT remains prolonged after mixing studies; 3) a prolonged PT is corrected in the mixing study, and a prolonged APTT remains prolonged; 4) a prolonged PT remains prolonged, and a prolonged APTT normalizes after mixing, and reverses to prolonged after incubation. A prolonged PT that is corrected in the mixing study, along with a normal APTT, suggests a factor VII deficiency. Test results are consistent with a single or multiple deficiency of factors II, V, or X (common pathway) when a prolonged PT is normalized after mixing studies and when a prolonged APTT normalizes after mixing studies and remains normalized after incubation.

Phosphatidylserine Antibodies

Antibodies to phosphatidylserine are associated with antiphospholipid syndrome, an increased risk of venous thrombosis, thrombocytopenia, and recurrent fetal loss.

Plasminogen Activator Inhibitor-1 (PAI-1)

Increased levels of PAI-1 antigen and PAI-1 activity are associated with decreased fibrinolytic activity and increased risk for venous thrombosis and coronary artery disease. Interpretation of increased levels is confounded by circadian variation (morning values being about 2-fold higher than afternoon values), increases associated with the acute phase response (eg, following myocardial infarction, major surgery, severe trauma, or sepsis), as well as increases associated with normal pregnancy and disorders such as endotoxemia, liver disease, obesity, hyperinsulinemia, hypertriglyceridemia, and malignancy. Severely decreased or undetectable levels may be associated with bleeding problems. The antigenic, but not the activity test can help distinguish between a constitutional deficiency of PAI-1 and a dysproteinemia.

Plasminogen Activator Inhibitor-1 (PAI-1) 4G/5G Polymorphism

The 4G allele is associated with increased PAI-1 antigen and activity levels. Similar to PAI-1 antigen and activity levels, data regarding utility of the 4G/5G polymorphism in predicting venous thrombosis is conflicting.²⁷ It may be more useful when co-inherited with another thrombophilia marker. When co-inherited, the 4G allele may further increase the risk of thrombophilia. For example, Visanji et al found the risk for venous thrombosis increased approximately 2-fold in patients with at least 1 copy of the 4G allele (4G/4G or 4G/5G genotypes) relative to that in patients with the 5G/5G genotype.²⁷ All patients were heterozygous for factor V

(Leiden) mutation and had experienced at least 1 venous thromboembolic event. Furthermore, Zoller et al reported an approximate 4-fold increase in the risk of pulmonary embolism among subjects with hereditary protein S deficiency who were homozygous for the 4G allele.²⁸

Plasminogen

Decreased levels are associated with liver disease, DIC, thrombolytic agents, primary fibrinolysis, tissue plasminogen activator and, rarely, with venous thrombosis and pulmonary embolism (homozygous state only). Increased levels are associated with trauma, infection, acute myocardial infarction, surgery, and chronic inflammation. A functional assay is usually the preferred assay because the presence of a normal amount of antigen does not exclude a qualitative defect of the protein. The antigen level is most often obtained to assess a quantitative abnormality of the protein.

Protein C

Decreases are associated with venous thrombosis, recurrent superficial thrombophlebitis, neonatal purpura fulminans, arterial thrombosis (rarely), oral anticoagulant-induced skin necrosis, DIC, infection, acute illness such as the flu or a gastrointestinal disorder, malignancy, liver disease, vitamin K deficiency, surgery, and L-asparaginase therapy. Falsely low values may be obtained in individuals on oral anticoagulant therapy and those who are APC resistant. Heparin levels up to 1 IU/mL do not interfere with test results. Protein C levels are significantly decreased in neonates; adult levels are reached only after 10 years of age. Low levels in both activity and antigen assays are suggestive of type I deficiency, whereas low activity levels in the presence of normal antigen levels indicate type II deficiency (dysproteinemia). Increases are associated with oral contraceptives, and pregnancy. Although not affected by increased factor VIII or acute phase reactions, overall specificity for inherited deficiency is low, and positive results (ie, low levels) should be confirmed by demonstration of a deficiency in another family member.

Protein S

Decreases are associated with increased risk for venous, and possibly arterial, thrombosis, oral anticoagulant-induced skin necrosis, neonatal purpura fulminans, DIC, acute phase reactions, oral anticoagulants, APC resistance, vitamin K deficiency, liver disease, surgery, L-asparaginase therapy, oral contraceptives, estrogen replacement therapy, pregnancy, nephrotic syndrome, infections (HIV, varicella), Crohn's disease, and ulcerative colitis. Levels are significantly decreased in neonates; however, adult levels are reached by 6 months of age. Low levels in both activity and antigen (free and total) assays are suggestive of type I deficiency, whereas low activity in the presence of normal total antigen levels indicate type II deficiency (dysproteinemia). Type III deficiency is characterized by low levels in the activity and free antigen assays, but normal levels in the total antigen assay. Increases may be observed in lipemic samples. Although protein S levels are not affected by heparin (up to 1 IU/mL) or factor VIII (up to 250%), overall specificity of protein S measurement for the diagnosis of inherited deficiency is low, and positive results (ie, low levels) should be confirmed by demonstration of a deficiency in another family member.

Prothrombin (Factor II) 20210G→A Mutation

This mutation is associated with increased prothrombin levels, increased risk for venous thrombosis,^{11,24} increased risk for obstetric complications (eg, preeclampsia, abruptio placenta, fetal growth retardation, and stillbirth),²¹ and, possibly, premature coronary heart disease. Venous thrombosis risk increases synergistically in the presence of oral contraceptive use.²⁹ The combination may also lead to cerebral sinus and spinal vein thromboses.

Prothrombin Fragment 1.2

Prothrombin fragment 1.2 is the amino terminus fragment of prothrombin released when prothrombin is converted to thrombin. Elevated levels are associated with an increased risk of thrombosis and found in patients with trauma, eclampsia, pre-eclampsia, DIC, DVT, and PE. Levels are also increased in individuals with antithrombin deficiency.

PTT-LA with Reflex to Hexagonal Phase Neutralization

Lupus anticoagulants are non-specific antibodies that extend the clotting time of phospholipid-dependent clotting assays, and lupus anticoagulant antibodies bind to hexagonal phase phospholipids. A reduction of the APTT by more than 8 seconds after the addition of hexagonal phase phospholipid is considered confirmation of the presence of a lupus anticoagulant and an increased risk of thrombosis. The sensitivity and specificity of this test are 96% and >95%, respectively. While false-negatives are rare, if clinical suspicion of LA is high, dRVVT with confirm is suggested.

Red Cell CD55 and CD59 Expression

A percentage of CD55 and/or CD59 deficient red blood cells >15% is diagnostic of paroxysmal nocturnal hemoglobinuria (PNH). Individuals with PNH are at markedly increased risk of thrombosis, particularly of the intra-abdominal and cerebral veins.

Reptilase Clotting Time

Unlike thrombin, reptilase is not affected by the presence of heparin or hirudin; thus, a prolonged thrombin time in an individual with a normal reptilase time is consistent with contamination or the presence of heparin. Reptilase clotting time is also prolonged in individuals with a-, hypo-, and dysfibrinogenemias.

Serotonin Release Assay (SRA)

A value >20% is considered positive and strongly suggests heparin induced thrombocytopenia. False-positive test results are automatically excluded by running a parallel study with anti-Fc(γ)RIIa.

Soluble P-Selectin (SPS)

P-selectin is a cell adhesion molecule found on the surface of activated platelets after release from platelet alpha granules. A soluble form of P-selectin, which has a lower molecular mass, is secreted into plasma. Elevated levels of SPS are found in individuals with thrombotic disease states, eg, DVT, atherosclerosis.

Thrombin-Antithrombin (TAT) Complex

Elevated TAT complex is a risk factor for thrombosis and

found in individuals with DIC, malignancies, and those receiving heparin and fibrinolysis therapy.

Thrombin Clotting Time

Prolonged clotting times may indicate abnormal fibrinogen levels (elevated or decreased), dysfibrinogenemia, the presence of heparin, hirudin, paraproteins, uremia, or increased levels of fibrin degradation products. Normalization of clotting time after mixing indicates hypo- or dysfibrinogenemia, whereas continued prolongation indicates the presence of heparin, hirudin, paraproteins, uremia, or increased levels of fibrin degradation products.

Thrombin Generation

Thrombin generation is a global test for hypercoagulability and, as such, is useful for identifying individuals at risk for thrombosis. It may uncover unexplained clinical thrombophilia when no known causes have been identified. Factor VIII levels >250% have been associated with increased thrombin generation potential. This assay is also useful in monitoring anticoagulant therapy.

Tissue Factor (TF)

TF, released after vascular injury, activates Factor VII, and the TF:Factor VIIa complex activates the coagulation protease cascade. Elevated levels of TF are seen in atherosclerosis, cancer, and sepsis and may play a role in venous thrombosis.

Tissue Plasminogen Activator (TPA)

TPA converts plasminogen to plasmin, which in turn degrades fibrin to soluble degradation products. TPA is inhibited by plasminogen activator inhibitor-1. Elevated TPA levels are associated with an increased risk of atherosclerosis, myocardial infarction, stroke, and recurrent venous thrombosis.

von Willebrand Factor Protease (ADAMTS-13) Activity with Reflex to Protease Inhibitor

von Willebrand factor is released into circulation as high molecular weight multimeric forms that are broken down into smaller, less active multimers by von Willebrand factor cleaving protease (ADAMTS-13). The persistence of high weight multimers is associated with platelet aggregates and thrombi. Deficiency of von Willebrand factor cleaving protease is associated with thrombotic thrombocytopenic purpura.

Sample Collection Considerations

Anticoagulants may interfere with some test results (Table 4). When clinically indicated, replace an oral anticoagulant with heparin for 7 to 10 days, then stop the heparin and draw the sample 12 to 24 hours later.⁶

Certain medicines and medical conditions may also affect some test results (Table 5).

Platelets significantly decrease the sensitivity of antiphospholipid antibody testing; thus, the specimen must be centrifuged for 15 minutes at 1,500 g and/or filtered through a 0.22 micron screen to remove platelets prior to freezing.³⁰ The final platelet count must be <10,000 platelets/ μ L of plasma (<5,000 platelets/ μ L preferred; note that 1 μ L=1 mm³).

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Appendix 1. Antiphospholipid Syndrome

Antiphospholipid syndrome, the most common cause of acquired thrombophilia, is characterized by the presence of antiphospholipid antibodies such as lupus anticoagulants (LA), anticardiolipin antibodies (ACA), and phosphatidylserine antibody. The syndrome is associated with both arterial and venous thrombosis, systemic lupus erythematosus (SLE), connective tissue and autoimmune disorders, malignancy, HIV infection, drug ingestion, and obstetric, hematologic, neurologic, dermatologic, and cardiac complications (Appendix 1 Table).

Although no single test is 100% sensitive or specific for LA, the diagnosis usually begins with demonstration of prolonged phospholipid-dependent clotting times (eg, APTT).^{1,2} When the APTT is prolonged, a mixing study is highly recommended to rule *in* presence of an inhibitor, signified by lack of clotting time correction. Following an uncorrected mixing study result, use of the dRVVT and the PTT-LA tests is highly recommended. When the dRVVT or the PPT-LA is prolonged, a confirmatory test is automatically performed (at an additional charge and CPT code). A prolonged dRVVT is confirmed with a phospholipid neutralization test (dRVVT confirm), while a prolonged

Appendix 1 Table. Complications Associated with Antiphospholipid Syndrome

Obstetric – Maternal	Hematologic	Dermatologic
Deep vein thrombosis	Thrombocytopenia	Skin necrosis
Chorea	Hemolytic anemia	Ulceration
Postpartum syndrome	Evans syndrome	Livedo reticularis
Eclampsia/preeclampsia	Low complement 3 & 4	Digital gangrene
Obstetric – Fetal	Neurologic	Cardiac
Intrauterine fetal death	Chorea	Coronary artery disease
Recurrent spontaneous abortion	Stroke	Libman-Sacks endocarditis
Fetal growth retardation	Multi-infarct dementia	Arterial graft occlusion
Prematurity	TIA/CVA	
Neonatal thrombosis	Transverse myelitis	
	Migraine	

PTT-LA test is confirmed with the hexagonal phase neutralization test. Traditional mixing studies to confirm prolonged dRVVT and PTT-LA tests are not needed since normal plasma is included in the confirmation test. Normal plasma will correct prolonged clotting times if there is an underlying factor deficiency, whereas in the presence of an inhibitor, the test remains prolonged.

Although highly sensitive, ACA testing is not specific for thrombotic risk. Transient LA or ACA may be due to infectious diseases (varicella, rubella, adenovirus, HIV) or drug exposure (amoxicillin, chlorpromazine, hydralazine). Since transient LA or ACA are not associated with clinical complications, 2 positive tests, more than 3 months apart, are recommended for diagnosis of clinically significant antiphospholipid antibodies.³

The diagnosis of antiphospholipid syndrome may be improved by using the β_2 -glycoprotein I antibody assay. β_2 -Glycoprotein I antibodies of the IgA, IgG, and IgM isotype are associated with the antiphospholipid syndrome, and their presence is more specific but less sensitive than cardiolipin antibodies for the diagnosis of antiphospholipid syndrome. Individuals who are positive for cardiolipin antibodies and negative for β_2 -glycoprotein I antibodies are more likely to have an infection (varicella, rubella, adenovirus, HIV) or drug exposure (amoxicillin, chlorpromazine, hydralazine) than antiphospholipid syndrome.

A specific phosphatidylserine antibody test can also be used to identify an antiphospholipid antibody.

Appendix 1 References

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Appendix 2. Tests Used in The Diagnosis of Thrombophilia

The Appendix 2 Table contains a comprehensive list of tests used in the diagnosis of thrombophilia.

Appendix 2 Table. Tests Used in The Diagnosis of Thrombophilia

Test Code	Test Name	Method	Description
763X	Activated Partial Thromboplastin Time (APTT)	Photo-optical clot detection	A silica-synthetic phospholipid reagent is mixed with patient plasma, and calcium chloride is then added to initiate clot formation. The time elapsed for formation of a fibrin clot is measured.
22X	Activated Protein C Resistance	dRVVT clot-based	Endogenous protein C is activated prior to performing a dRVVT test. Results are reported as the ratio of dRVVT clotting times obtained with and without activation of endogenous protein C.
216X	Antithrombin, Activity	Chromogenic	Antithrombin in the patient's sample binds to thrombin; excess thrombin cleaves a synthetic thrombin substrate, releasing a fluorescent substance, the amount of which is inversely proportional to the amount of antithrombin activity in the plasma.
5158X	Antithrombin, Antigen	Nephelometry	Antithrombin antigen is measured in a fixed rate time nephelometry assay.
15914X	C4 Binding Protein ¹	Radial immunodiffusion	The patient's sample is mixed with agarose gel containing sheep anti-human C4 binding protein antibodies. Antigen-antibody complexes are detected as a visible precipitating ring.
36189X	Cardiolipin Antibody Screen with Reflex to IgA, IgG, and IgM	EIA	The level of antibodies directed against various plasma proteins that bind to phospholipid surfaces (eg, damaged endothelial membranes, monocytes, tumor cells, etc) is measured. The method is highly sensitive but not specific for β_2 -glycoprotein I. Individual tests for IgA, IgG, and IgM antibodies are available (separate order codes).
11294X	Cytochrome P450 2C9 Genotype ¹	PCR	Single nucleotide primer extension and detection of fluorescent extension products are utilized to detect the 2 most common variants in the CYP2C9 gene, CYP2C9*2 and CYP2C9*3.
8659X	D-Dimer	Immunoturbidimetric	The increase in light absorption caused by the agglutination of D-dimer antibody-coated latex particles with endogenous D-dimer is measured.
15780X	dRVVT Screen with Reflex to Phospholipid Neutralization	Photo-optical clot detection	The time elapsed for clot formation following Russell viper venom activation of factor X is determined. When the dRVVT is prolonged, another dRVVT test is performed (at an additional charge [additional CPT code]) after adding excess phospholipid in the presence of a heparin neutralizer. The ratio of the 2 dRVVT tests is calculated, and the result is reported as "confirmed" or "not confirmed".
17902X	Factor V HR2 Allele DNA Mutation Analysis ^{2,3}	PCR, oligonucleotide ligation, chemiluminescent detection	The HR2 allele (4070G→A) in the factor V gene is detected by amplification of the gene region with PCR, followed by oligonucleotide ligation and hybridization to color-coded microspheres. Reaction products are detected by chemiluminescent detection of the microspheres.
17900X	Factor V (Leiden) Mutation Analysis ³	PCR, luminescent detection	The 1691G→A factor V Leiden mutation is detected using allele specific probes. Results are reported as negative, heterozygous positive, or homozygous positive.
16049X	Factor VIII Activity,	Chromogenic Chromogenic	Factor VIII in the patient sample is activated by mixing with thrombin and activated factor IX. Activated factor VIII then accelerates the factor IXa-mediated conversion of factor X to Xa. Factor Xa activity, which is proportional to factor VIII activity, is measured by the change in a chromogenic substrate. This assay provides increased sensitivity (relative to the factor VIII clotting assay) at levels >150% and is insensitive to the effects of direct thrombin inhibitors and heparin. It is, therefore, the preferred assay when a lupus anticoagulant is present.

continued

Test Code	Test Name	Method	Description
347X	Factor VIII Activity, Clotting	Photometric clot detection	Patient plasma is mixed with factor VIII-deficient normal plasma. The APTT clotting time of the mixed plasma is compared to that of reference plasma. Results are reported as percent of normal factor VIII activity.
11074X	Fibrin Monomer	Hemagglutination	Patient plasma is mixed with human erythrocytes coated with purified fibrin monomer. The presence of soluble fibrin monomer complexes in plasma results in agglutination.
461X	Fibrinogen	Photo-optical clot detection	Fibrinogen in the patient sample is converted to fibrin in the presence of excess thrombin. The clotting time obtained is inversely proportional to the fibrinogen level (activity).
458X	Fibrinogen Degradation Products	Agglutination	In the presence of corresponding antigens, latex particles coated with monoclonal anti-FDP antibodies agglutinate to form macroscopic clumps.
37801X	Fibrinogen, Quantitative, Nephelometry	Nephelometry	Patient plasma is reacted with highly specific antiserum to form insoluble antigen-antibody complexes, which are detected in solution as turbidity.
30340X	β_2 -Glycoprotein I Antibodies (IgA, IgG, IgM)	EIA	This enzyme immunoassay semi-quantitatively measures antibodies directed against β_2 -glycoprotein I (β_2 -GPI, apolipoprotein H). Individual tests for IgA, IgG, and IgM are available (separate order codes).
30292X	Heparin, Low Molecular Weight (Xa Inhibition) ¹	Chromogenic	The level of low molecular weight heparin in plasma is measured by the inhibition of activated factor X (Xa) activity in the presence of excess antithrombin.
404X	Heparin, Unfractionated (Xa Inhibition)	Chromogenic	The level of unfractionated heparin in plasma is measured by inhibition of activated factor X (Xa) activity in the presence of excess antithrombin.
31789X	Homocysteine	Competitive immunoassay	The level of total homocysteine (ie, protein-bound, oxidized, and free, reduced homocysteine) is measured in a competitive immunochemiluminometric assay.
10707X	Human Platelet Antigen 1 Genotype ²	DNA based capture/binding	After amplification of the segment of the GPIIIa gene that includes the HPA-1 polymorphism, allele specific probes are used in a chromogenic assay to determine the genotype.
34604X	Lipoprotein (a)	Immunoprecipitin	Agglutination occurs between lipoprotein (a) in the patient sample and anti-lipoprotein (a) antibodies adsorbed to latex particles and is measured photometrically.
17911X	Methylenetetrahydrofolate Reductase (MTHFR) DNA Mutation Analysis ³	PCR, oligonucleotide ligation assay	This test detects the 677C→T and 1298A→C point mutations. Results for both mutations are reported as negative, heterozygous positive, or homozygous positive.
8922X	Mixing/Correction Study	Clot Detection	APT and/or APTT is measured after mixing patient plasma and normal plasma to determine the cause (eg, factor deficiency, factor inhibitor, and/or nonspecific inhibitor) of a prolonged PT and/or APTT test result.
36595X	Phosphatidylserine Antibodies	Chromogenic	The presence of IgA, IgG, and IgM antibodies to phosphatidylserine is detected in an enzyme immunoassay.
36555X	Plasminogen Activator Inhibitor-1 (Antigen)	EIA	The concentration of plasminogen activator inhibitor-1 (antigen) is measured in an enzyme immunoassay.
10491X	Plasminogen Activator Inhibitor-1 Activity	Chromogenic	The amount of functional PAI-1 in plasma is detected based on PAI-1 inhibition of tissue plasminogen activator (tPA), which catalyzes the conversion of plasminogen to plasmin.
11368X	Plasminogen Activator Inhibitor-1 4G/5G Polymorphism ³	PCR	This test employs PCR amplification of a 150-bp fragment of the PAI-1 gene, followed by restriction enzyme digestion and gel electrophoresis separation of the products.

continued

Test Code	Test Name	Method	Description
4458X	Plasminogen Activity	Chromogenic	Plasminogen in the patient sample forms a complex with streptokinase. Hydrolysis of this complex produces a color, the intensity of which is directly proportional to the percent of normal plasminogen activity.
5164X	Plasminogen, Antigenic	Nephelometry	Specific antiserum to plasminogen forms antigen-antibody complexes that are detected in solution as turbidity.
1777X	Protein C Activity	Chromogenic	Snake venom is used to activate protein C in the patient sample. The activated protein C then stimulates the breakdown of a chromogen inducing production of a color that is measured photometrically.
4948X	Protein C Antigen	EIA	Protein C antigen is measured via an enzyme immunoassay.
1779X	Protein S, Activity	Clot detection	Protein S in the patient sample enhances the anticoagulant action of activated protein C, resulting in a prolonged clotting time. The increase in clotting time is directly proportional to the percent of normal protein S activity.
5165X	Protein S, Total Antigen	Immunoassay	The level of total protein S (bound and free) is measured in a microlatex particle-mediated immunoassay.
10170X	Protein S, Free	Immunoturbidimetric	The increase in light absorption caused by the agglutination of free protein S antibody-coated latex particles with endogenous free protein S is measured.
17909X	Prothrombin (factor II) 20210G→A Mutation ^{1,3}	PCR	In this single nucleotide polymorphism genotyping system, PCR amplification is followed by hybridization to wild-type and mutation-specific probes in separate reaction wells. A perfect match between probe and patient DNA sequence is required for light generation and identification of wild-type and mutant DNA. Results are reported as negative, heterozygous, or homozygous positive for the 20210G→A mutation.
37674X	Prothrombin Fragment 1.2	EIA	Anti-prothrombin fragment 1.2 is used to measure the level of prothrombin fragment 1.2
17408X	PTT-LA with Reflex to Hexagonal Phase Neutralization	Clot-based PTT	Dilute cephalin is used, thus increasing this assay's sensitivity to weak phospholipid antibodies. Prolonged PTT-LA test results are confirmed (at an additional charge [additional CPT code]) with a hexagonal phase neutralization test.
36737X	Red Cell CD55 and CD59 Expression ²	Flow cytometry	Single color flow cytometry utilizes anti-CD55 and anti-CD59 monoclonal antibodies to detect the relative amounts of CD55- and CD59-deficient red blood cells.
37700X	Reptilase Clotting Time ¹	Clotting	Reptilase, an enzyme derived from the venom of <i>Bothrops atrox</i> , cleaves fibrinopeptide A from fibrinogen resulting in the production of fibrin and subsequent clot formation. Clotting time is determined by measuring the increase in viscosity after reptilase is added to test plasma.
14627X	Serotonin Release Assay (SRA) ¹	Radiobinding ¹⁴ C serotonin	Fc(γ)RIIIa receptor-phenotyped platelets from highly reactive donors are incubated with ¹⁴ C-serotonin. Porcine heparin (0.1, 0.5, and 100 U) is then added along with patient serum; the presence of antibodies to the heparin-Fc(γ)RIIIa receptor complex in the sera of patients with HIT causes the release of serotonin. Results are reported as percent serotonin released.
10296X	Soluble P-Selectin, ELISA ²	ELISA	Soluble P-selectin is quantitatively determined in a solid phase ELISA assay.
10162X	Thrombin-Antithrombin (TAT) Complex	ELISA	Thrombin is inhibited by antithrombin and the resulting inactive proteinase/inhibitor complex is measured quantitatively by ELISA.
883X	Thrombin Clotting Time	Clot detection	Thrombin is mixed with the patient plasma and clotting time is determined photometrically.

continued

Test Code	Test Name	Method	Description
19413X	Thrombin Generation ¹	Fluorogenic, kinetic	The coagulation process is initiated by the addition of tissue factor, anionic phospholipid membrane, and calcium chloride to plasma. The amount of thrombin generated is then measured by the fluorescence of a thrombin-specific chromogenic substrate. The fluorescent intensity is proportional to the concentration of thrombin and monitored continuously.
10656X	Tissue Factor ¹	EIA	This EIA utilizes a murine anti-human tissue factor monoclonal antibody to determine the level of tissue factor.
29816X	Tissue Plasminogen Activator (tPA)	EIA	This EIA utilizes goat anti-tPA IgG to determine the level of tPA.
14532X	von Willebrand Factor Protease (ADAMTS-13) Activity with Reflex to Protease Inhibitor ¹	Electrophoresis	The patient sample is incubated with protease free multimers and the extent of degradation is determined by electrophoresis. The presence of inhibitors is determined in a mixing assay (at an additional charge [additional CPT code]).

EIA, enzyme immunoassay; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

¹This test was developed and its performance characteristics determined by Quest Diagnostics Nichols Institute. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Performance characteristics refer to the analytical performance of the test.

²This test is performed using a kit that has not been approved or cleared by the FDA. The analytical performance characteristics of this test have been determined by Quest Diagnostics Nichols Institute. This test should not be used for diagnosis without confirmation by other medically established means.

³Polymerase chain reaction (PCR) is performed pursuant to a license agreement with Roche Molecular Systems, Inc.

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