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Activated partial thromboplastin time (aPTT)

Typical normal range is 25-38 seconds\(^1\)

**Overview**
- aPTT assesses the intrinsic and common coagulation pathways\(^2\)
- aPTT is the best single screening test for coagulation disorders. When properly performed, aPTT is abnormal in 90% of patients with coagulation disorders\(^3\)
- aPTT is used to\(^3\):
  - Monitor heparin therapy
  - Screen for hemophilia A and B
  - Detect clotting inhibitors

**Method**\(^2\)
- aPTT measures the time it takes for clots to form after recalcification and addition of a phospholipid and an activator of the intrinsic coagulation system to the citrated plasma

**Important notes**
- Testing for aPTT may vary from one lab to another\(^2\)
- The normal range for the lab in which aPTT testing occurs should be used in the interpretation\(^2\)
- aPTT reagents are variably sensitive to the effects of a lupus anticoagulant. If sensitive, a false-positive aPTT elevation may result from this cross-reactivity rather than from a bleeding tendency\(^2,4\)

**References**


**Anti–factor Xa (FXa) activity assay**

Typical range is 0 IU/mL, 0.3-0.7 IU/mL (unfractionated heparin), or 0.5-1.0 IU/mL (LMWH)\(^1,2\)

**Overview**
- The anti-FXa activity assay levels determine the amount of FXa inhibition in a sample\(^3\)
- Inhibition of FXa is an important part of the mechanisms of action of several anticoagulants, including heparin, LMWH, and fondaparinux\(^1,4\)
- These agents can be monitored with an anti-FXa activity assay\(^3\)

**Method**\(^2\)
- Chromogenic assays are the preferred methodology. There are also clot-based assays. In a chromogenic assay, FXa activity is measured using a chromogenic substrate assay. FXa normally cleaves the substrate and releases a colored compound that can be detected by a spectrophotometer. When an inhibitor of FXa is present, less colored compound is released and the anti-FXa activity can be extrapolated
**Important notes**

- Anti-FXa activity assays are not standardized and there is considerable interassay variability

**References**


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**Bethesda assay**

**Typical normal range is 0 Bethesda units (BUs)**

**Overview**

- The Bethesda assay is used to detect the presence of an inhibitor, quantify it, and allow follow-up response to therapy
- The assay is based on the ability of patient plasma to inactivate the selected factor in normal plasma

**Method**

- Patient plasma is serially diluted in normal plasma, and after incubation for 2 hours at 37°C, factor activity is measured using a one-stage factor assay

**Important notes**

- The specificity and reliability of the Bethesda assay was improved with the Nijmegen method, which included 2 important modifications that help prevent false-positives such as low-titer inhibitors. The normal plasma and control mixture are now buffered with imidazole to a pH of 7.4, and the standard control mixture is replaced with plasma depleted of FVIII
- One Bethesda unit corresponds to the amount of antibody that destroys 0.5 units of factor after 2 hours of incubation at 37°C

**Nijmegen modification**

![Diagram of Bethesda assay](image)

**References**

Bone marrow examination
Cellularity to fat ratio is 100% at birth and declines ≈10% each decade.1 Cell types by percentage in adults include: erythrocytes, 18%-30%; granulocytes, 50%-70%; and lymphocytes, 3%-17%.2

Overview1
• A bone marrow examination is a microscopic examination of tissue useful in the evaluation of thrombocytopenia and thrombocytotic disorders3,4
• The test can be used to help diagnose myeloproliferative disorders, myelophthisic disorders, or suggest a reactive process3
• Bone marrow examination is used to:
  o Assess number and morphology of megakaryocytes, the platelet precursor cells
  o Reveal the quantity and morphology of both red and white blood cells and their precursors

Method1
• Samples are usually aspirated from the iliac crest with a needle or removed surgically3,4
• Analysis includes the study of bone marrow aspirate smears, clot sections, and core biopsy material4

Important notes
• Bone marrow examinations can be used to diagnose some cancers (eg, leukemia, lymphomas, multiple myeloma, metastases), nonhematopoietic neoplasms, dysproteinemias and plasma cell disorders, aplastic anemia, and other conditions1
• The test is not helpful in the evaluation of platelet dysfunction with a normal platelet count4

References

Electron microscopy
Provides detailed imaging of cells and subcellular components with magnification up to 105 and resolution of 0.2 nm1

Overview1
• Electron microscopy is used in the classification of platelet disorders through the ultrastructural evaluation of platelets1,3
• Magnification is used for the identification of the platelet ultrastructure: platelet membranes, cytoplasmic organelles (granules), and cytoskeleton1
  o Can detect a decreased number or abnormal morphology of platelet α-granules and δ-granules4
  o Other typical electron microscopy findings include a scarce platelet canalicular system in Bernard-Soulier syndrome and polymorphonuclear leukocyte Döhle-body-specific structures in MYH9-related disorders3

Method1
• Sample processing steps involved include: fixation, dehydration, embedding with resin, ultrathin sectioning, collection using copper or nickel grids, and contrast enhancement with heavy-metal staining
• An electron beam passes through the prepared sample and creates an image on a high-resolution photographic plate
Important notes

• Since the test requires special processing and an electron microscope, it is not widely available in most labs

References

Factor II (FII) assay

Typical normal range is 60%-140% activity or 100 mcg/mL

Overview
• The FII assay measures the activity of FII (prothrombin), part of the common coagulation pathway
• FII is a vitamin K–dependent protein synthesized in the liver and converted to thrombin during coagulation

Method
• The FII assay is a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

Important notes
• In general, lower FII levels are associated with more severe bleeding

References

Factor IX (FIX) assay

Typical normal range is 60%-140% activity or 5 mcg/mL

Overview
• The FIX assay measures the activity of FIX, part of the intrinsic coagulation pathway
• FIX is a vitamin K–dependent protein synthesized in the liver and activated by FIII (tissue thromboplastin) and FVII
• Decreased FIX levels may indicate
  o Congenital hemophilia B (Christmas disease)
  o Acquired deficiency due to vitamin K deficiency, liver disease, warfarin effect, nephritic syndrome, or FIX immunoglobulin inhibitors

Method
• Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma
Important notes
• FIX levels >129% are associated with a risk of venous thrombosis

References

Factor V (FV) assay
Typical normal range is 60%-140% activity or 10 mcg/mL

Overview
• The FV assay measures the activity of FV (proaccelerin or labile factor)
• FV is a protein synthesized in the liver that assists with thrombin formation
• Decreased FV levels may indicate:
  o Congenital FV deficiency
  o Liver disease
  o DIC

Method
• Clotting factor assays are usually performed as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

Important notes
• In general, lower FV levels are associated with more severe bleeding
• Patients with clinical bleeding issues often have FV levels <5% that of normal levels

References

Factor VII (FVII) assay
Typical normal range is 60%-140% activity or 0.5 mcg/mL

Overview
• The FVII assay measures the activity of FVII (proconvertin), part of the extrinsic coagulation pathway
• FVII is a vitamin K-dependent protein synthesized in the liver
• Decreased FVII levels may indicate:
  o Congenital deficiency
  o Liver disease
  o Vitamin K deficiency or warfarin therapy
Method
Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma.

References

**Factor VIII (FVIII) assay**
Typical normal range is 50%-200% activity or 0.1 mcg/mL

Overview
- The FVIII assay measures the activity of FVIII (antihemophiliac factor)
- FVIII is an acute-phase reactant synthesized in the liver. It is required for the first phase of the intrinsic coagulation pathway
- Decreased FVIII levels may indicate:
  - Congenital hemophilia A
  - Acquired hemophilia

Method
- Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

Important notes
- FVIII is an acute-phase reactant and may be elevated in clinical settings involving tissue damage, inflammation, or stress
- Tests measuring FVIII should have linearity <1%
- FVIII levels reflect the severity of the deficiency measured in a lab

References

**Factor X (FX) assay**
Typical normal range is 60%-140% activity or 10 mcg/mL

Overview
- The FX assay measures the activity of FX (Stuart-Prower factor), which plays a role in both the intrinsic and extrinsic coagulation pathways
  - Activated by FIX in the intrinsic coagulation pathway
  - Activated by FVII in the extrinsic coagulation pathway
- FX is a vitamin K–dependent protein synthesized in the liver and activated by FVII and FIX
- Decreased FX levels may indicate:
  - Liver disease
  - Vitamin K deficiency
  - Warfarin therapy
**Method**
- Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

**Important notes**
- FXI levels increase during pregnancy or with the use of estrogen therapy

**References**

**Factor XI (FXI) assay**
- Typical normal range is 60%-140% activity or 5 mcg/mL

**Overview**
- The FXI assay measures the activity of FXI (plasma thromboplastin antecedent), part of the intrinsic coagulation pathway
- FXI is synthesized in the liver and megakaryocytes and activates FIX

**Method**
- Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

**Important notes**
- Patients with decreased FXI levels may bleed postoperatively but do not usually experience spontaneous bleeding

**References**

**Factor XII (FXII) assay**
- Typical normal range is 60%-140% activity or 30 mcg/mL

**Overview**
- The FXII assay measures the activity of FXII (Hageman factor), part of the intrinsic coagulation pathway
- FXII is activated by collagen, basement membranes, or activated platelets

**Method**
- Clotting factor assays are usually done as a one-stage assay. A one-stage assay is a modification of aPTT or PT in which patient plasma is serially diluted in specific factor-deficient plasma

**Important notes**
- Patients with decreased FXII levels are prone to thrombosis but do not usually display hemorrhagic symptoms

**References**
Factor XIII (FXIII) quantitative assay

Overview
• A FXIII quantitative assay should be performed to determine FXIII activity when urea clot lysis assays (FXIII qualitative tests) are positive for lysis1
• FXIII can be measured quantitatively either through immunoassay or with activity assays2,3
• If plasma FXIII activity is decreased, the subtype of FXIII deficiency should be established using quantitative assays for FXIII-A_B2 antigen; if that is also decreased, measure FXIII A-subunit and FXIII B-subunit antigens or alternatively isolated A-subunit and B-subunit antigens3

Method
• FXIII activity assays spectrophotometrically measure the ammonia released during the transglutamine reaction4
  o Typical normal range is 50%-220% for FXIII activity1
• FXIII antigen assays are used to help classify FXIII deficiencies5
  o These assays may use enzyme-linked immunosorbent assays (ELISAs) that help examine concentrations of plasma FXIII A-subunit, B-subunit, and A2B2 antigen

Important notes
• Dissolution of a fibrin clot within 1 hour of incubation in 5M urea or 1% monochloroacetic acid suggests FXIII levels of <1%6
• Urea clot lysis assays cannot detect heterozygous, mild, moderate, or acquired states2
• Within ammonia release assays, thresholds for detection can vary; Berichrom® FXIII sensitivity is limited (5%-10% activity) while Technochrom® FXIII cites a 0.6%-300% operating range2,7,8

References

Fibrinogen

Typical normal range is 200-400 mg/dL1

Overview
• Fibrinogen, or factor I (F1), is a glycoprotein synthesized in the liver. It is modified by thrombin to produce fibrin1,2
• Decreased levels of fibrinogen may indicate1:
  o DIC
  o Liver disease

Method3
• Fibrinogen can be assessed both quantitatively, using immunologic methods, and qualitatively, with clotting assays similar to one-stage assay methods used for other clotting factors

Important notes
• Increased (≥400 mg/dL) or decreased (<80 mg/dL) fibrinogen can affect coagulation testing4,5
• Fibrinogen levels increase during pregnancy or with the use of estrogen therapy1,6
  o Normal ranges2:
    – 13 to 28 weeks gravid—8.5-16.8 mcml/L (289-571 mg/dL)
    – 29 to 42 weeks gravid—9.5-19.1 mcml/L (323-650 mg/dL)
Coagulation Toolkit Coagulation assays and tests

• Fibrinogen is an acute-phase reactant and may be elevated in clinical settings involving tissue damage, inflammation, or stress.

References

Fibrinogen clottable/antigen ratio
Typical normal range is >80% ratio.

Overview
• Fibrinogen clottable/antigen ratio is used to look for the production of abnormal fibrinogen.
• Fibrinogen is a glycoprotein synthesized in the liver. It is modified by thrombin to produce fibrin.
• Inherited fibrinogen disorders can appear as defects in the amount of FI produced (ie, no FI [afibrinogenemia], not enough FI [hypofibrinogenemia]) or defects in the quality of FI (dysfibrinogenemia).

Method
• Fibrinogen can be assessed both quantitatively, using immunologic methods, and qualitatively, with clotting assays similar to one-stage assay methods used for other clotting factors.
• Dysfibrinogenemia occurs when a sample shows the total clottable fibrinogen is <80% of the total immunologic fibrinogen.

References

Fibrinolytic testing
Overview
• Fibrinolytic testing involves the measurement of plasminogen activator inhibitor-1 (PAI-1) activity, PAI-1 antigen, α2-antiplasmin activity, and either total tissue plasminogen activator (tPA) antigen or tPA–PAI-1 complex.
• Often used when patients present with a history of bleeding but negative results on common coagulation assays.

Method
• Tests of plasma samples can differentiate between quantitative PAI deficiency (absence of both PAI-1 activity and PAI-1 antigen with low tPA antigen) and qualitative PAI deficiency (absence of PAI-1 activity, low or normal PAI-1 antigen, and low tPA antigen).
• α2-antiplasmin activity is measured using a back titration assay with purified plasmin in which the ability to inhibit plasmin is assessed.
Important notes
• A falsely low PAI-1 activity can occur when tPA levels are elevated, which may happen with prolonged tourniquet time during blood draw. Evaluation of PAI-1 deficiency should therefore include assessment of tPA antigen or tPA–PAI-1 complex as a control

References

Flow cytometry
Overview
• Flow cytometry is used to study platelet structure and function and to help in the diagnosis, characterization, and monitoring of hematologic malignancies

• Platelet flow cytometry tests:
  o Detect the activation state of circulating platelets
  o Study the reactivity of platelets to specific agonists
  o Study platelet function in a very small sample with a relatively low platelet count
  o Detect the presence, decreased expression, or deficiency of typical platelet surface glycoproteins
  o Identify platelet-associated immunoglobulins

Method
• Specimens can come from peripheral blood, bone marrow aspirates and core biopsies, fine-needle aspirates, fresh tissue biopsies, or any bodily fluids

• Cell surface proteins with fluorescently labeled antibodies are detected, allowing the expression of a panel of proteins to be analyzed for each platelet

• Cells in a buffer suspension pass through a laser beam; the forward and side light scatter show cell size and granularity, respectively

Important notes
• Flow cytometry for platelet function testing should be performed within 1 hour of phlebotomy since platelets progressively activate during in vitro storage

References

International normalized ratio (INR)
Expressed as a ratio of patient PT/reference PT

Overview
• INR standardizes the results of PT tests performed with different reagents

• INR is used to:
  o Assess anticoagulation due to reduction in vitamin K–dependent factors
  o Evaluate liver disease, especially when comparing values between different labs

Method
• INR is calculated by comparing a patient’s results to the mean PT value in that lab, yielding a ratio. This is then corrected using an International Sensitivity Index (ISI) that reflects the sensitivity of the reagents used in the PT assay
Important notes
• INR can vary among labs based on instrumentation

References

**Low-dose, ristocetin-induced, platelet-aggregation–based (RIPA-based) plasma/platelet mixing study**

**Overview**
• The low-dose RIPA-based plasma/platelet mixing study is used to distinguish platelet-type vWD from vWD type 2B
• Both disorders show enhanced responsiveness to low-dose ristocetin (≤0.5 mg/dL)
  - With vWD type 2B, abnormal aggregation to low-dose RIPA persists if subject plasma is mixed with normal platelets but corrects when subject platelets are mixed with normal plasma
  - With platelet-type vWD, abnormal aggregation to low-dose RIPA persists if subject platelets are mixed with normal plasma but corrects when subject plasma is mixed with normal platelets (or another source of vWF)

**Method**
• RIPA is typically performed using a dual channel aggregometer. Platelet count is adjusted in platelet-rich plasma (PRP) to 250,000 per mcL with autologous platelet-poor plasma
  - PRP is incubated at 37°C for 2 minutes and stirred before platelet aggregation is induced with ristocetin
  - Aggregation is measured continuously by light transmittance

**Important notes**
• Used as an alternative to cryoprecipitate challenge since cryoprecipitate challenge may provide a false-positive platelet-type vWD identification in patients with vWD type 2B
• In addition to low-dose RIPA-based plasma/platelet mixing studies, vWD type 2B and platelet-type vWD can also be distinguished using DNA sequencing of exon 28 of the vWF gene. An abnormal exon 28 suggests vWD type 2B

References

**Peripheral blood smear**

**Overview**
• The peripheral blood smear is used to microscopically examine quantity and morphology of red blood cells (RBCs), white blood cells (WBCs), and platelets
• The test should be performed to confirm thrombocytopenia and identify the underlying cause, as well as to look for myeloproliferative disorders
• Peripheral blood smears may also be used to provide a range of information concerning the cause of anemia; coexistent neutropenia, thrombocytopenia, and anemia may indicate bone marrow failure or lack of a nutritional substance to provide adequate bone marrow production
• They may also be used to identify lymphoproliferative disorders

**Method**
• Blood samples should be collected into an ethylenediaminetetraacetic acid (EDTA) anticoagulant and mixed to prevent in vitro clotting
• Platelet morphologic analysis can be determined through an air-dried, peripheral, Wright-stained smear made from the EDTA specimen
• Abnormal blood cell shapes can be recognized by an automated calculator; more accurate smears require a review by a pathologist
Important notes

• Basophilic stippling in RBCs may indicate increased bone marrow production.

References

Plasma D-dimers

Typical normal range is <0.5 mcg/mL

Overview

• Plasma D-dimers are a primary marker of fibrinolysis and an indirect marker of coagulation.
• Plasma D-dimers are the smallest degradation products released when cross-linked fibrin is lysed.
• Plasma D-dimers are used to:
  o Detect DIC
  o Exclude deep venous thrombosis and pulmonary embolism.

Method

• Plasma D-dimers level in whole blood can be measured by red cell agglutination. In plasma, latex agglutination or immunoassay is used.

Important notes

• There is no standard cutoff value; results and values vary between test manufacturers.
• Values are increased in a variety of medical conditions, including pregnancy, kidney, liver, and heart failure, and after major injury or surgery.

References

Platelet aggregation studies

Typical normal range is >65% aggregation in response to ADP, epinephrine, collagen, ristocetin, and arachidonic acid.

Overview

• Platelet aggregation studies are used to distinguish intrinsic platelet disorders involving surface glycoproteins, signal transduction, and platelet granules.
  o Abnormalities may be in adhesion, release, or aggregation.
• Measures the ability of agonists to cause platelet activation in vitro and platelet-platelet binding.

Method

• These studies stimulate platelet aggregation by introducing agonistic agents in vitro.
  o For example, ADP (first wave and second wave), epinephrine, collagen, ristocetin, and arachidonic acid.
  o Ristocetin-induced platelet aggregation (RIPA) can be assessed at both high and low doses of ristocetin, allowing detection of both increased and decreased sensitivity to ristocetin.
• Blood should be obtained from peripheral venipuncture, kept at room temperature, transported to the laboratory, and tested quickly.
• Aggregation is measured using a turbidimeter and expressed graphically.
Important notes

- Platelet aggregation studies are rarely useful in evaluating acquired bleeding disorders.
- Tests should be repeated to confirm reproducibility of results.

References


**Platelet count**

**Typical normal range is 150,000-400,000 per mL.**

Overview

- Performing a platelet count test is one of the first steps in identifying a platelet disorder.
- MPV and platelet size distribution curve are measured simultaneously.
- Assessment of the platelet count may influence the interpretation of platelet function studies.
- Counts <100,000 per mL indicate thrombocytopenia; counts >400,000 per mL indicate thrombocytosis.

Method

- Blood samples should be collected into an ethylenediaminetetraacetic acid anticoagulant and mixed to prevent in vitro clotting.
  - The platelet count is usually stable for up to 24 hours postcollection.
- Measured by automated analyzers using electrical impedance or light scattering.

Important notes

- Normal platelet count ranges are different for premature infants (100,000-300,000 per mL), newborns (150,000-300,000 per mL), and infants (200,000-475,000 per mL).
- Low platelet counts can occur during pregnancy due to gestational, or incidental, thrombocytopenia. Women with gestational thrombocytopenia typically have platelet counts between 70,000 per mL and 150,000 per mL, are otherwise healthy, and have no prior history of ITP.

References


**Platelet size/mean platelet volume (MPV)**

**Typical normal values range from 7-11 fl.**

Overview

- MPV is useful for diagnosing thrombocytopenic disorders through the measurement of the average platelet size.
- Since new platelets from bone marrow tend to be larger, increased MPV can also indicate platelet turnover.
- MPV is used in the:
  - Diagnosis of hematologic disorders
  - Assessment of platelet function
  - Evaluation of thrombocytopenia
  - Evaluation of the need for platelet transfusion in thrombocytopenic patients.
Coagulation Toolkit
Coagulation assays and tests

Method
• MPV should be determined in 1-3 hours after obtaining the sample since platelet size increases over time

Important notes
• MPV correlates with bleeding tendency in thrombocytopenic patients
• If platelet size is out of the reference range, automated cell counters may underestimate or overestimate platelet size since the largest and smallest platelets could be excluded from analysis
• Automated assessment of MPV may be less accurate in the presence of macrothrombocytopenia or microthrombocytopenia

References

Prothrombin time (PT)
Typical normal range is 11-13 seconds

Overview
• PT assesses the extrinsic and common coagulation pathways
• PT is primarily used to:
   o Monitor long-term use of anticoagulant therapy through the INR
   o Evaluate liver function
   o Evaluate coagulation disorders

Method
• PT measures the time it takes for clots to form after recalcification and thromboplastin is added to the citrated plasma

Important notes
• Testing for PT may vary from one lab to another
• The normal range for the lab in which PT testing occurs should be used in the interpretation

References
Prothrombin time/activated partial thromboplastin time (PT/aPTT) 1:1 mixing studies
Typical normal range is 11-13 seconds (PT) and 25-38 seconds (aPTT)¹

Overview²
• Mixing studies are used to evaluate abnormal PT and/or aPTT
• The results distinguish between a factor deficiency and an inhibitor

Method²
• Normal plasma and patient plasma are mixed 50:50
• Values are determined immediately and at various times after incubation at 37°C

Important notes²
• With factor deficiencies, PT and/or aPTT will correct with mixing and remain corrected with incubation
• With acquired neutralizing antibodies, adding normal plasma may or may not immediately correct the prolonged PT and/or aPTT
  o Acquired antibodies can be dependent on time and temperature, so the longer the sample is incubated, the more prolonged the results will be. Initial values may be normal or somewhat prolonged, but should be more prolonged at 2 hours
• Prolonged results due to lupus anticoagulant will not show correction in immediate mixing or after incubation
• Other inhibitors or substances (eg, heparin, fibrin split products, paraproteins) may cause the study to fail to correct

References
Reptilase time (RT)
Typical normal range is 16-24 seconds

Overview
- RT assesses the conversion of fibrinogen to fibrin by reptilase, a thrombinlike enzyme derived from snake venom
- The test is not affected by heparin and generally is more sensitive to dysfibrinogenemias than TT
- RT is used to:
  - Evaluate prolonged aPTT
  - Exclude dysfibrinogenemia

Method
- Reptilase is derived from the venom of Bothrops atrox. RT is the time it takes for clots to form after reptilase is added

References

Tests for lupus anticoagulant

Overview
- Lupus anticoagulant prolongs phospholipid-dependent coagulation reactions
- Lupus anticoagulant is suspected when one of several screening assays is prolonged, most commonly the aPTT

Method
- The following tests can assist in diagnosing lupus anticoagulant:
  - Dilute Russell’s viper venom test measures the clotting time in the presence of Vipera russelli, a substance that activates FX, bypassing the intrinsic and extrinsic coagulation pathways
  - Kaolin clotting time is a variation of the Lee-White whole blood clotting time where clot formation is accelerated using the contact activation initiator kaolin
  - Tissue thromboplastin inhibition test is a dilute version of the PT

Important notes
- aPTT reagents are variably sensitive to the effects of a lupus anticoagulant. If sensitive, a false-positive aPTT elevation may result from this cross-reactivity rather than from a bleeding tendency
- Lupus anticoagulant does not typically present as a hemorrhagic disorder

References
Thrombin time (TT)
Typical normal range is 16-24 seconds

Overview
• TT assesses clot formation in response to thrombin
• Prolonged TT occurs when there is a deficiency or structural abnormality of fibrinogen or inhibition of the thrombin-fibrinogen reaction
• TT is used to detect:
  o Decreased or abnormal fibrinogen
  o Unreported therapeutic heparin
  o Other antithrombins

Method
• TT measures the time it takes for clots to form after thrombin is added to the citrated plasma

References

Urea clot lysis assay
Results are expressed as positive or negative for presence of deficiency

Overview
• The urea clot lysis assay measures the activity of FXIII
• FXIII, with the help of calcium, turns a polymerized fibrin clot into an initial clot

Method
• The most common screening test to assess FXIII levels is the clot lysis test. Plasma is clotted, a clot lysis agent (urea) is added, and the time to clot dissolution is measured. Fibrin clots with FXIII deficiency are soluble in 5M urea

Important notes
• All standard clotting tests appear normal. In whole blood, clot appears qualitatively friable

References

Von Willebrand factor (vWF) activity assay
Normal range depends on blood type

Overview
• The vWF activity assay quantifies the functionality of vWF protein rather than the quantity
• Normal vWF activity levels vary by blood type:
  o Type O: 75% mean of normal
  o Type A: 105% mean of normal
  o Type B: 115% mean of normal
  o Type AB: 125% mean of normal
Method\(^1,2\)
- The ristocetin cofactor (vWF:RCo) test measures vWF activity. The antibiotic ristocetin induces vWF binding to platelets and the degree of platelet aggregation is measured. Collagen binding assays can also be done.

Important notes\(^3\)
- Von Willebrand disease (vWD) is a group of bleeding disorders with >20 subtypes—no one lab test can detect all forms of vWD.

References

Von Willebrand factor antigen (vWF:Ag) assay
Normal range depends on blood type\(^1\)

Overview
- The vWF:Ag assay measures the level of vWF protein in the blood.\(^2\)
- vWF is a blood protein used in platelet adhesion and as a carrier for FVIII.\(^3\)
- Normal vWF:Ag levels vary by blood type:\(^1,2\)
  - Type O: 75% mean of normal
  - Type A: 105% mean of normal
  - Type B: 115% mean of normal
  - Type AB: 125% mean of normal

Method\(^2\)
- vWF:Ag measures the level of vWF protein in the blood by immunoassay.

Important notes
- Von Willebrand disease (vWD) is a group of bleeding disorders with >20 subtypes—no one lab test can detect all forms of vWD.\(^4\)
- vWF:Ag is an acute-phase reactant that may be elevated in clinical settings such as trauma, surgery, and clotting.\(^4,5\)

References

Von Willebrand factor (vWF) multimers
Results are expressed as normal or abnormal\(^1\)

Overview\(^2\)
- vWF multimers are the forms of vWF protein that are found after vWF dimers assemble to form high-molecular-weight multimers.

Method\(^1\)
- The distribution of vWF multimers is analyzed by visualization on a 1% to 2% agarose gel.
**Important notes**
- Von Willebrand disease (vWD) is a group of bleeding disorders with >20 subtypes—no one lab test can detect all forms of vWD

**References**

**Warfarin gas chromatography**

**Overview**
- Warfarin is a dicumarol derivative. It exerts an anticoagulatory effect by inhibiting the gamma carboxylation of vitamin K–dependent coagulation factors. This leads to lower levels of active coagulation factors

**Method**
- Warfarin and superwarfarins in the blood are identified through the use of special assays
- Chromatography separates compounds by using their different interactions in the mobile and stationary system phases. As compounds travel through a support medium, those that interact more strongly with the stationary phase will be retained longer

**Important notes**
- Patients without liver disease who have a tendency toward severe bleeding should be suspected for surreptitious warfarin use. These patients may have deliberately induced bleeding symptoms

**References**