are low in newborns, in which bacterial colonization of the gut has not begun. Hemorrhage is likely in vitamin K deficiency, and the PT is the best indicator. To distinguish between vitamin K deficiency and liver disease, the laboratory practitioner determines factor V and factor VII levels. Both factor V and factor VII are reduced in liver disease; only factor VII is reduced in vitamin K deficiency. Chapter 38 provides details regarding liver disease and vitamin K deficiency.

The PT is prolonged in congenital single-factor deficiencies of factor X, VII, or V; profound prothrombin deficiency; and fibrinogen deficiency when the fibrinogen level is 100 mg/dL or less. When the PT is prolonged but the PTT and thrombin clotting time (TCT) test results are normal, factor VII activity may be deficient. Any suspected single-factor deficiency is confirmed with a factor assay. The PT is not affected by factor VIII or IX deficiency, because the concentration of tissue factor in the reagent is high, and those factors are bypassed in thrombin generation.

Minimal Effectiveness of Prothrombin Time as a Screening Tool

Preoperative PT screening of asymptomatic surgical patients to predict intraoperative hemorrhage is not supported by prevalence studies, unless the patient is a member of a high-risk population.[62,63] No clinical data support the use of the PT as a general screening test for individuals at low risk of bleeding, and the PT is not useful for establishing baseline values in Coumadin therapy.[64] The therapeutic target range for Coumadin therapy is based on the INR, not the baseline PT result or PT control value.

Limitations of the Prothrombin Time

Specimen variations profoundly affect PT results (Table 42-7). The ratio of whole blood to anticoagulant is crucial, so collection tubes must be filled to within tube manufacturers' specifications and not underfilled or overfilled. Anticoagulant volume must be adjusted when the hematocrit is greater than 55% to avoid false prolongation of the results. Specimens must be inverted five times immediately after collection to ensure good anticoagulation, but the mixing must be gentle. Practitioners must reject clotted and visibly hemolyzed specimens because they give unreliable results. Plasma lipemia or icterus may affect the results obtained with optical instrumentation.

TABLE 42-7 Factors That Interfere with the Validity of Clot-based Test Results

Problem	Solution
Blood collection volume less than specified minimum	PT falsely prolonged; recollect specimen.
Hematocrit ≥55%	Adjust anticoagulant volume using formula and recollect specimen using new anticoagulant volume.
Clot in specimen	All results are affected unpredictably; recollect specimen.
Visible hemolysis	PT falsely shortened; recollect specimen.
Icterus or lipemia	Measure PT using a mechanical coagulometer.
Heparin therapy	Use reagent known to be insensitive to heparin or one that includes a heparin neutralizer such as polybrene.
Lupus anticoagulant	PT result is invalid; use chromogenic factor X assay instead of PT.
Incorrect calibration, incorrect dilution of reagents	Correct analytical error and repeat test.

Heparin may prolong the PT. If the patient is receiving therapeutic heparin, it should be noted on the order and commented on when the results are reported. The laboratory manager selects thromboplastin reagents that are maximally sensitive to oral anticoagulant therapy and insensitive to heparin. Many reagent manufacturers incorporate polybrene (5-dimethyl-1,5-diazaundecamethylene polymethobromide, hexadimethrine bromide, Sigma-Aldrich, St. Louis, MO) in their thromboplastin reagent to neutralize heparin. The medical laboratory practitioner may detect unexpected heparin by using the TCT test, which is described subsequently.

Lupus anticoagulants (LAs) prolong some thromboplastins. LAs are members of the antiphospholipid antibody family and may partially neutralize PT reagent phospholipids. Coumadin often is prescribed to prevent thrombosis in patients with LAs, but the PT may be an unreliable monitor of therapy in such cases. Patients who have an LA and are taking Coumadin should be monitored using an alternative system, such as the chromogenic factor X assay.[65,66]

Reagents must be reconstituted with the correct diluents and volumes following manufacturer instructions. Reagents must be stored and shipped according to manufacturer instructions and never used after the expiration date.

Partial Thromboplastin Time

Partial Thromboplastin Time Principle

The PTT (also called the *activated partial thromboplastin time,* or APTT) is performed to monitor the effects of unfractionated heparin therapy and to detect LA and specific anticoagulation factor antibodies such as anti-factor VIII antibody. The PTT is also prolonged in all congenital and acquired procoagulant deficiencies, except for deficiencies of factor VII or XIII.[67]

The PTT reagent contains phospholipid (previously called *partial thromboplastin*) and a negatively charged particulate activator such as silica, kaolin, ellagic acid, or celite in suspension. The phospholipid mixture, which was historically extracted from rabbit brain, is now produced synthetically. The activator provides a surface that mediates a conformational change in plasma factor XII that results in its activation (Figure 42-9). Factor XIIa forms a complex with two other plasma components: high-molecular-weight kininogen (*Fitzgerald factor*) and prekallikrein (*Fletcher factor*). These three plasma glycoproteins, termed the *contact activation factors,* initiate in vitro clot formation through the *intrinsic* pathway but are not part of in vivo coagulation. Factor XIIa, a serine protease, activates factor XI (XIa), which activates factor IX (IXa) (Chapter 37).

Factor IXa binds calcium, phospholipid, and factor VIIIa to form a complex. In the PTT reaction system, ionic calcium and

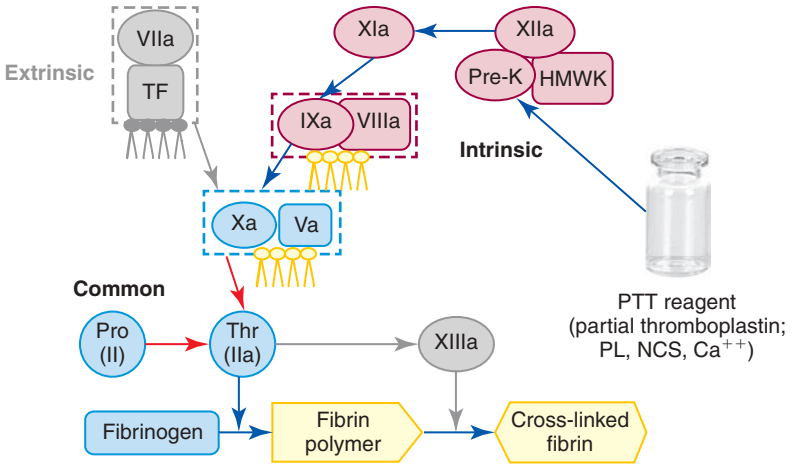

Figure 42-9 Partial thromboplastin time (PTT) reagent (partial thromboplastin) consists of phospholipid (PL), a negatively charged particulate activator (NCS), and ionized calcium. It activates the intrinsic and common pathways of the coagulation mechanism through the contact factors XII, prekallikrein (Pre-K; also called *Fletcher factor*), and high-molecular-weight kininogen (HMWK; also called *Fitzgerald factor*), none of which is significant in the in vivo coagulation mechanism (see colored area in figure). The PTT is prolonged by deficiencies in Pre-K; HMWK; factors XII, XI, IX, VIII, X, and prothrombin; and fibrinogen when the fibrinogen level is less than 100 mg/dL. The deficiencies for which the PTT reagent is specifically calibrated are factors VIII, IX, and XI. The PTT is prolonged in heparin therapy because heparin activates plasma antithrombin, which neutralizes all the plasma serine proteases, particularly thrombin (IIa) and activated factor X (Xa). The PTT is prolonged in the presence of lupus anticoagulant because the anticoagulant neutralizes essential reagent phospholipids. The PTT does not detect factor XIII deficiency. *TF,* Tissue factor; *Pro,* prothrombin (II, zymogen); *Thr,* thrombin (activated factor II, or IIa; serine protease); *Va, VIIIa,* activated factors V and VIII (serine protease cofactors); *VIIa, IXa, Xa, XIa,* activated factors VII, IX, X, XI (serine proteases); *XIIa,* activated factor XII (serine protease, but not part of in vivo coagulation); *XIIIa,* activated factor XIII (transglutaminase).

phospholipid are supplied in the reagent. The factor IXa–calcium–factor VIIIa–phospholipid complex catalyzes factor X (Xa). Factor Xa forms another complex with calcium, phospholipid, and factor Va, catalyzing the conversion of prothrombin to thrombin. Thrombin catalyzes the polymerization of fibrinogen and the formation of the fibrin clot, which is the endpoint of the PTT.

The factors whose deficiencies are associated with hemorrhage and are reflected in prolonged PTT results, taken in the order of reaction, are XI, IX, VIII, X, and V; prothrombin; and fibrinogen, when fibrinogen is 100 mg/dL or less. Most PTT reagents are designed so that the PTT is prolonged when the test PPP has less than approximately 0.3 units/mL (30% of normal) of VIII, IX, or XI.[68] The PTT also is prolonged in the presence of LA, an immunoglobulin with affinity for phospholipid-bound proteins, and is prolonged by anti-factor VIII antibody, antibodies to factor IX and other coagulation factors, and therapeutic heparin. Factor VII and factor XIII deficiencies have no effect on the PTT. Deficiencies of factor XII, prekallikrein, or high-molecular-weight kininogen prolong the PTT but do not cause bleeding.

Partial Thromboplastin Time Procedure

To initiate contact activation, 50 or 100 µL of warmed (37° C) reagent consisting of phospholipid and particulate activator is mixed with an equal volume of warmed PPP. The mixture is allowed to incubate for the exact manufacturer-specified time, usually 3 minutes. Next, 50 or 100 µL of warmed 0.025 M calcium chloride is forcibly added to the mixture, and a timer is started. When a fibrin clot forms, the timer stops, and the interval is recorded. Timing may be done with a stopwatch or by an automatic electromechanical or photo-optical device. If the PTT is performed manually, the test should be done in duplicate, and the two results must match within 10%.

Partial Thromboplastin Time Quality Control

The medical laboratory practitioner tests normal and prolonged control plasma specimens at the beginning of each 8-hour shift or with each new batch of reagent. The laboratory director may require more frequent use of controls. Controls are tested using the protocol for patient plasma testing.

The normal control result should be within the reference interval, and the abnormal control result should be within the therapeutic range for unfractionated heparin (Chapter 43). If the control results fall within the stated limits in the laboratory protocol, the test results are considered valid. If the results fall outside the control limits, the reagents, control, and equipment are checked; the problem is corrected; and the control and patient specimens are retested. The operator records each control run and all the actions taken. Control results are recorded and analyzed at regular intervals to determine the long-term validity of results.

Reagents must be reconstituted with the correct diluents and volumes following manufacturer instructions. Reagents must be stored and shipped according to manufacturer instructions and never used after the expiration date.

Specimen errors that affect the PT similarly affect the PTT (Table 42-7).

Partial Thromboplastin Time Reference Interval

The PTT reference interval varies from site to site, depending on the patient population, type of reagent, type of instrument, and

pH and purity of the diluent. One medical center laboratory has established 26 to 38 seconds as its reference interval. This range is typical, but each center must establish its own interval for each new lot of reagent, or at least once a year. This may be done by testing a sample of 30 or more specimens from healthy donors of both sexes spanning the adult age range over several days and computing the 95% confidence interval of the results.

Monitoring of Heparin Therapy with Partial Thromboplastin Time

Since the early 1970s, the PTT has been the standard method for monitoring unfractionated heparin therapy, which is used to treat patients with venous thrombosis, pulmonary embolism, myocardial infarction, and several other medical conditions.[69] The laboratory practitioner establishes a PTT therapeutic range and publishes it to all inpatient units. A typical therapeutic range is 60 to 100 seconds; however, the range varies widely and must be established locally.[70] The range must be reestablished with each change of PTT reagent, including each lot change, and upon instrument recalibration. Details on monitoring of heparin therapy and establishment of the PTT therapeutic range are provided in Chapter 43.

The Partial Thromboplastin Time as a Diagnostic Assay

The physician orders a PTT assay when a hemorrhagic disorder is suspected or when recurrent thrombosis or the presence of an autoimmune disorder points to the possibility of an LA.[71] The PTT result is prolonged when there is a deficiency of one or more of the following coagulation factors: prothrombin; factor V, VIII, IX, X, XI, or XII; or fibrinogen when the fibrinogen level is 100 mg/dL or less. The PTT also is prolonged in the presence of a specific inhibitor, such as anti-factor VIII or anti-factor IX; a non-specific inhibitor, such as LA; and interfering substances, such as fibrin degradation products (FDPs) or paraproteins, which are present in myeloma.

DIC prolongs PTT results because of consumption of procoagulants, but the PTT results alone are not definitive for the diagnosis of DIC. Vitamin K deficiency results in diminished levels of procoagulant factors II (prothrombin), VII, IX, and X, and the PTT is eventually prolonged. Because factor VII deficiency does not affect the PTT, however, and because it is the first coagulation factor to become deficient, the PTT is not as sensitive to vitamin K deficiency or Coumadin therapy as the PT. The PTT is not prolonged in deficiencies of factor VII or XIII. No clinical data support the use of the PTT as a general screening test for individuals at low risk of bleeding.[72]

Partial Thromboplastin Time Mixing Studies

Lupus Anticoagulants

LAs are IgG immunoglobulins directed against a number of phospholipid-protein complexes.[72] LAs prolong the phospholipid-dependent PTT reaction. Most laboratories employ a moderate-phospholipid or high-phospholipid PTT reagent in their primary PTT assay to monitor heparin therapy and detect coagulopathies. Laboratories use a second low-phospholipid PTT reagent such as PTT-LA (Diagnostica Stago, Parsippany, NJ), which is more sensitive to LA, as their LA screen (Chapter 39). Because they have a variety of target antigens, LAs are called *nonspecific inhibitors.* Chronic presence of LAs confers a 30% risk of arterial or venous thrombosis; every acute care laboratory must provide a means for their detection. Together, chronic and transient LAs are found in 1% to 2% of randomly selected individuals.

Specific Factor Inhibitors

Specific factor inhibitors are IgG immunoglobulins directed against coagulation factors. Specific inhibitors arise in severe congenital factor deficiencies during factor concentrate treatment. Anti-factor VIII, the most common of the specific inhibitors, is detected in 10% to 20% of patients with severe hemophilia, and anti-factor IX is detected in 1% to 3% of factor IX-deficient patients. Autoantibodies to factor VIII occasionally may arise in individuals without hemophilia, usually in young women, where they are associated with a postpartum bleeding syndrome or in patients over 60 with autoimmune disorders. The presence of these types of antibodies is called *acquired hemophilia* (Chapter 38). Alloantibodies and autoantibodies to factor VIII are associated with severe anatomic hemorrhage.

Detection and Identification of Lupus Anticoagulants and Specific Inhibitors

LA testing is part of every thrombophilia profile (Chapter 39). An unexpectedly prolonged screening PTT may also trigger an LA investigation. PTT mixing studies are necessary for the initial detection of LAs.[73] Mixing studies also distinguish LAs from specific inhibitors and factor deficiencies and should be available in all coagulation laboratories.[74]

When the initial PTT is prolonged beyond the upper limit of the reference interval, the laboratory practitioner first determines if heparin is present by performing the TCT. A TCT result that exceeds the upper limit of the TCT reference interval is evidence for the presence of heparin. In fact, heparin often prolongs the TCT to 30 to 40 seconds. Heparin may be neutralized using polybrene or heparinase (Hepzyme; Siemens Healthcare Diagnostics, Tarrytown, NY), and the treated sample may be used for PTT mixing studies.

The heparin-free or heparin-neutralized patient plasma is then mixed 1:1 with *reagent platelet-poor normal plasma* (PNP; Figure 39-1). Several manufacturers make PNP—for example, frozen Cryo*check* Normal Reference Plasma (Precision BioLogic, Inc, Dartmouth, Nova Scotia). A new PTT is performed immediately on the 1:1 mixture. If the mixture PTT corrects to within 10% of the PNP PTT (or to within the reference interval) and the patient is experiencing bleeding, a coagulation factor deficiency (coagulopathy) is presumed.[75]

Some LAs are time dependent and temperature dependent. Most anti-factor VIII inhibitors are temperature-dependent IgG4-class antibodies. If the immediate PTT corrects, a new mixture is prepared and incubated 1 to 2 hours at 37° C. If the incubated mixture's PTT fails to correct to within 10% of the incubated PNP PTT, an inhibitor may be