an extraction step before the assay is performed. Thromboxane B2 is acted on by liver enzymes to produce an array of soluble urine metabolites, including 11-dehydrothromboxane B2, which is stable and measurable.[53] Immunoassays of urine 11-dehydrothromboxane B2 are employed to characterize in vivo platelet activation.[54] These assays require no special specimen management and can be performed on random urine specimens. The urinary 11-dehydrothromboxane B2 assay also may be used to monitor aspirin therapy and to identify cases of therapy failure or aspirin resistance.[55]

CLOT-BASED PLASMA PROCOAGULANT SCREENS

The Lee-White whole-blood coagulation time test, described in 1913, was the first laboratory procedure designed to assess coagulation.[56] The Lee-White test is no longer used, but it was the first in vitro clot procedure that employed the principle that the time interval from the initiation of clotting to visible clot formation reflects the condition of the coagulation mechanism. A prolonged clotting time indicates a coagulopathy (coagulation deficiency). A 1953 modification, the *activated clotting time* (ACT) test, utilizes a particulate clot activator in the test tube, which speeds the clotting process. The ACT is still widely used as a point-of-care assay to monitor heparin therapy in high-dosage applications such as percutaneous intervention (cardiac catheterization) and coronary artery bypass graft surgery (Chapter 43).

The standard clot-based coagulation screening tests—PT, PTT, fibrinogen assay, and thrombin clotting time (TCT)—use the clotting time principle of the Lee-White test. Many specialized tests, such as coagulation factor assays, tests of fibrinolysis, inhibitor assays, reptilase time, Russell viper venom time, and dilute Russell viper venom time, are also based on the relationship between time to clot formation and coagulation function.

Prothrombin Time

Prothrombin Time Principle

PT reagents, often called *thromboplastin* or *tissue thromboplastin*, are prepared from recombinant or affinity-purified *tissue factor* suspended in phospholipids mixed with a buffered 0.025 M solution of calcium chloride.[57] A few less responsive thromboplastins are organic extracts of emulsified rabbit brain or lung suspended in calcium chloride. When mixed with citrated PPP, the PT reagent triggers fibrin polymerization by activating plasma factor VII (Figure 42-8). Calcium and phospholipids participate in the formation of the tissue factor–factor VIIa complex, the factor VIIIa–factor IXa complex, and the factor Va–factor Xa complex. The clot is detectable visually or by optical or electromechanical sensors. Although the coagulation pathway implies that the PT would be prolonged in deficiencies of fibrinogen, prothrombin, and factors V, VII, VIII, IX, and X, the procedure is most sensitive to factor VII deficiencies, moderately sensitive to factor V and X deficiencies, sensitive to severe fibrinogen and prothrombin deficiencies, and insensitive to deficiencies of factors VIII and IX.[58,59] The PT is prolonged in multiple factor deficiencies that include deficiencies of factors VII and X and is used most often to monitor the effects of therapy with the oral anticoagulant Coumadin (Chapter 43).

Prothrombin Time Procedure

The tissue factor-phospholipid-calcium chloride reagent is warmed to 37° C. An aliquot of test PPP, 50 or 100 μL, is transferred to the reaction vessel, which also is maintained at 37° C. The PPP aliquot is incubated at 37° C for at least 3 and for no more than 10 minutes. Aliquots that are incubated longer than 10 minutes become prolonged as coagulation factors begin to deteriorate or are affected by evaporation and pH change. A premeasured volume of reagent, 100 or 200 μL, is directly and quickly added to the PPP aliquot, and a timer is started. As the

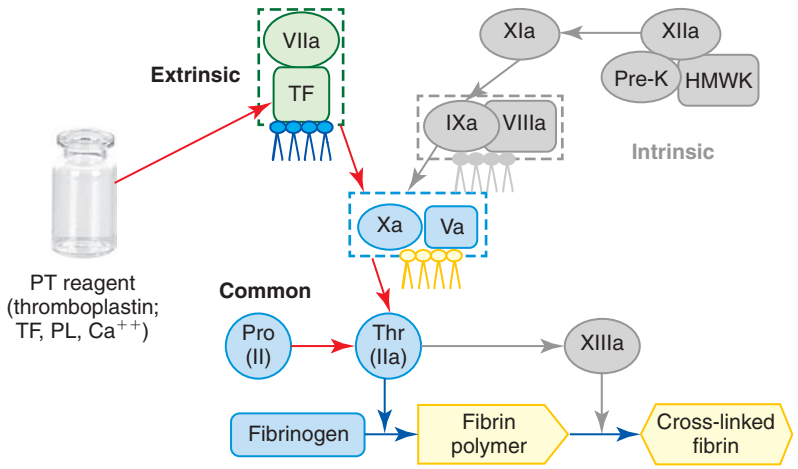

Figure 42-8 Prothrombin time (PT) reagent (thromboplastin) consists of tissue factor (TF), phospholipid (PL), and ionized calcium (Ca^{++}). The reagent activates the extrinsic and common pathways of the coagulation mechanism beginning with factor VII (see colored area in figure). The PT is prolonged by deficiencies of factors VII, X, and V; prothrombin; and fibrinogen when the fibrinogen level is less than 100 mg/dL. The PT is prolonged in Coumadin therapy because Coumadin suppresses production of factor VII, factor X, and prothrombin. Factor VII has a 6-hour half-life and has the earliest effect on the PT. The PT does not detect factor XIII deficiency. *HMWK,* High-molecular-weight kininogen (Fitzgerald factor); *Pre-K,* prekallikrein (Fletcher 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).

clot forms, the timer stops, and the elapsed time is recorded. If the procedure is performed in duplicate, the duplicate values must be within 10% of their mean or the test is repeated for a third time. Most laboratory practitioners perform PTs using automated instruments that strictly control temperature, pipetting, and interval timing (Chapter 44). With automated instruments, duplicate testing is unnecessary.

Prothrombin Time Quality Control

The medical laboratory practitioner tests normal and prolonged control PPP specimens at the beginning of each 8-hour shift or with each change of reagent. Although lyophilized control PPPs are commercially available, the laboratory manager may choose to collect and pool PPP specimens from designated subjects to make "laboratory-developed" controls. In this case, the specimens must be collected and managed using the same tubes, anticoagulant, and protocol that are used for patient plasma specimen collection. The samples are pooled, tested, and aliquotted. Regardless of whether commercial or locally prepared controls are used, the control is tested alongside patient specimens using the same protocol as for patient PPP testing.

The normal control result should be within the reference interval, and the prolonged control result should be within the therapeutic range for Coumadin. If the control results fall within the stated limits provided 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 all the actions taken. Control results are recorded and analyzed at regular intervals to determine the long-term validity of results.

Reporting of Prothrombin Time Results and the International Normalized Ratio

The medical laboratory practitioner reports PT results to the nearest tenth of a second, along with the PT reference interval. If the PT assay is performed in duplicate, the results are averaged, and the average is reported.

For Coumadin monitoring, to compensate for the inherent variations among thromboplastin reagents, most laboratories report the *international normalized ratio* (INR) for patients with a stable anticoagulation response using the following formula:[60]

$$\text{INR} = (\text{PT}_{patient}/\text{PT}_{geometric\ mean\ of\ normal})^{\text{ISI}}$$

where $PT_{patient}$ is the PT of the patient in seconds, $PT_{geometric\ mean\ of\ normal}$ is the PT of the geometric mean of the reference interval, and *ISI* is the *international sensitivity index*. Reagent producers generate the ISI for their thromboplastin by performing an orthogonal regression analysis comparing its PT results on a set of plasmas, with the results obtained using the international reference thromboplastin preparation (World Health Organization human brain thromboplastin). Most responsive thromboplastin reagents have ISIs near 1, the assigned ISI of the WHO reagent. Automated coagulation instruments "request" the reagent ISI from the operator or incorporate it from the reagent label bar code and compute the INR for each specimen. INRs are meant to be computed only for samples from patients who have achieved a stable anticoagulation response with Coumadin. During the first week of Coumadin therapy, the physician should interpret PT results in seconds, comparing them with the reference interval. Chapter 43 provides a full discussion of Coumadin therapy monitoring.

Localized ISI calibration is replacing reagent manufacturer-generated ISIs as it produces a laboratory-specific ISI value that is likely to be more accurate than a distributor-provided ISI.[61] The laboratory practitioner performs PTs on a set of four to five calibrator plasmas—for instance, ISI Calibrate (Instrumentation Laboratory, Bedford, MA). The calibrators arrive with predetermined PT values. If calibrators are not available, the practitioner may use a series of 100 patient specimens. The practitioner prepares a linear graph with the preestablished calibrator PTs or the PT values of the 100 patient specimens using the lab's current PT reagent on the Y scale and local PTs using the new reagent on the X scale and computes the slope. The reference ISI provided by the manufacturer for the new PT reagent is multiplied by the slope value to produce the local ISI of the new PT reagent.

The same approach may be applied to lot-to-lot calibrations of PT reagents; however, in most lot-to-lot validations the operator need only assay a three-level validation plasma set—for instance, ISI Validate (Instrumentation Laboratory, Bedford, MA). If the lot values determined using the new reagent are within predetermined limits, the lot may be placed in everyday operation without a change; if not, it is necessary to recalibrate the ISI value of the new lot of the PT reagent.

Prothrombin Time Reference Interval

The PT reference interval, computed from PT values of healthy individuals, varies from site to site, depending on the patient population, type of thromboplastin used, type of instrument used, and pH and purity of the reagent diluent. Each center must establish its own range for each new lot of reagents, or at least once a year. This may be done by testing a sample of at least 30 specimens from healthy donors of both sexes spanning the adult age range over several days and computing the 95% confidence interval of the results. A typical PT reference interval is 12.6 to 14.6 seconds.

The Prothrombin Time as a Diagnostic Assay

The PT is performed diagnostically when any coagulopathy is suspected. Acquired multiple deficiencies such as disseminated intravascular coagulation (DIC), liver disease, and vitamin K deficiency all affect factor VII activity and are detected through prolonged PT results. The PT is particularly sensitive to liver disease, which causes factor VII levels to become rapidly diminished (Chapter 38).

Vitamin K deficiency is seen in severe malnutrition, during use of broad-spectrum antibiotics that destroy gut flora, with parenteral nutrition, and in malabsorption syndromes. Vitamin K levels

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