Hemostasis (Coagulation Factors)

When a body tissue is injured and begins to bleed, it initiates a sequence of clotting factor activities -the coagulation cascade- leading to the formation of a blood clot. This cascade is comprised of three pathways: extrinsic, intrinsic, and common.

Two laboratory tests are used commonly to evaluate coagulation disorders: ***Prothrombin Time*** *(PT) which measures the integrity of the extrinsic system as well as factors common to both systems and* ***Partial Thromboplastin Time*** *(PTT), which measures the integrity of the intrinsic system and the common components.*

Blood Clot Formation

Clotting is a function of plasma. It depends upon the orderly interaction of a group of **plasma proteins** (which are sequentially activated following vascular injury) with some **phospholipid** (from either damaged tissue or platelets) and some **Ca^{++}**. The final stages include the formation of **thrombin**, which then converts:

Soluble plasma protein fibrinogen ---------->insoluble fibrin.

Another factor converts the fibrin into a cross-linked polymer which stabilizes the platelet plug and traps RBCs in the mesh-work to form the actual blood clot. Depending on the type of vascular damage or abnormality, clotting can be initiated and proceed according to two different cascading pathways: the **intrinsic** (initiated by contact with and abnormal/foreign surface) or the **extrinsic** (initiated by exposure to tissue factors). Note that:

- The two pathways converge, so that the final steps are common to the two schemes

- although clotting can be initiated via either the more rapid (15-20 secs) extrinsic scheme or the slower (2-6 mins) intrinsic scheme,
- the division into two pathways is only an artifact of ***In vitro*** testing: the two pathways interconnect at several levels. ***In vivo***, both pathways must be activated for effective hemostasis.
- both coagulation pathways, by a series of feedback mechanisms, control their own activity (e.g. traces of thrombin enhance the activity of earlier factors in the scheme).

Note also that, in addition to the coagulation-promoting factors, there are also substances in blood which inhibit coagulation (e.g., an anti-thrombin factor which inactivates thrombin). Whether or not blood coagulates depends on the balance that exists between the two groups of factors (pro-coagulants and anti-coagulants).

In the extrinsic pathway of blood coagulation, a tissue factor is produced after injury. and released from damaged cells

The tissue factor interacts with factor VIIa to activate factor X to Xa.
Factor Xa then acts on prothrombin according to the common pathway of coagulation.

In the intrinsic pathway:
- factor XII is converted to XIIa on contact with negatively-charged surfaces (subendothelial collagen).
- factor XIIa converts factor XI to XIa
- factor XIa converts factor IX to IXa
- factor IXa converts factor X to Xa; to do this it requires:
  - calcium ions
  - factor VIIIa
- factor Xa then activates the common pathway of coagulation

Click on the titles below to view
the corresponding stages of coagulation pathways:

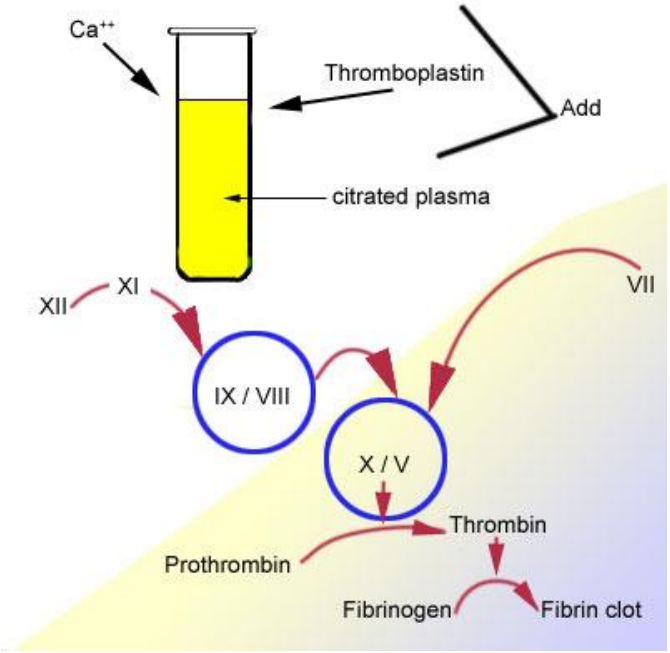
- Fibrin formation
- Intrinsic pathway
- Extrinsic pathway
- Overview of the blood coagulation pathways

Prothrombin time test

The PT test is used to monitor patients taking certain medications as well as to help diagnose clotting disorders.

A sample of the patient's blood is obtained by venipuncture. The blood is decalcified (by collecting it into a tube with oxalate or citrate ions) to prevent the clotting process from starting before the test. The blood cells are separated from the liquid part of

blood (plasma) by centrifugation. The PT test is performed by adding the patient's plasma to some source of Tissue Factor (e.g.: a protein, thromboplastin, from homogenized brain tissue) that converts prothrombin to thrombin. The mixture is then kept in a warm water bath at 37°C for one to two minutes. Calcium chloride (excess quantities of ionized calcium) is added to the mixture in order to counteract the sodium citrate and allow clotting to start. The test is timed from the addition of the calcium chloride until the plasma clots. This time is called the Prothrombin Time.



The prothrombin test specifically evaluates the presence of factors VII, V, and X, prothrombin, and fibrinogen. A prothrombin time within the 11 -15 second range (depends on the source of thromboplastin used) indicates that the patient has normal amounts of the above clotting factors.

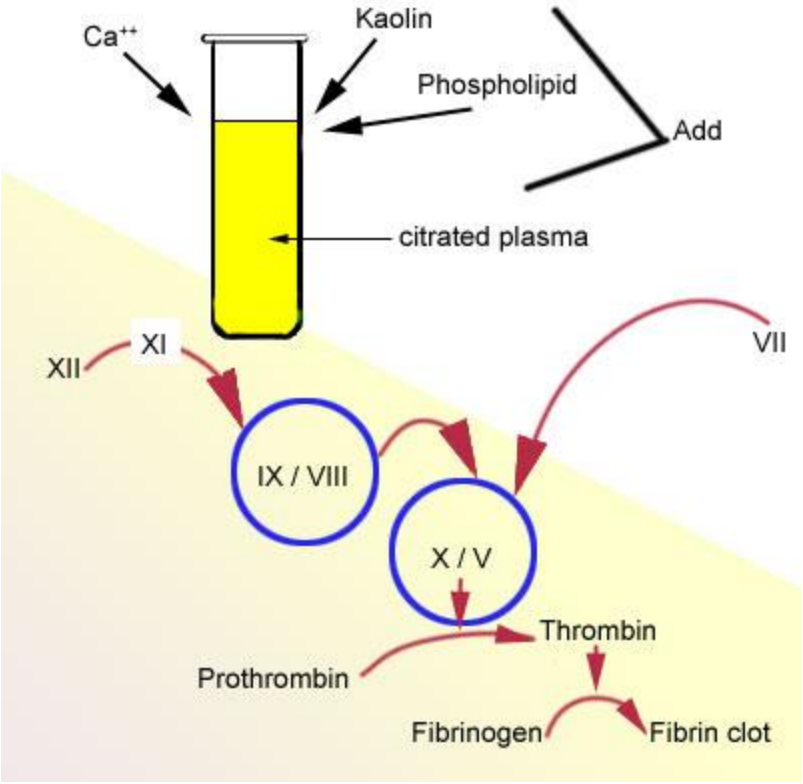
A prolonged prothrombin time indicates a deficiency in any of factors VII, X, V, prothrombin, or fibrinogen. It may mean that the patient has a vitamin K deficiency (vitamin K is a co-factor in the synthesis of functional factors II (prothrombin), VII,

IX and X) or a liver disease (the liver is the site of synthesis of the plasma protein factors). The prothrombin time of patients receiving a vitamin K-competing coumarin drug such as warfarin (anticoagulation therapy used in deep venous thrombophlebitis) will also be prolonged, usually in the range of one and one half to two times the normal PT time.

Activated Partial Thromboplastin Time test

The activated partial thromboplastin time (aPTT) is a test performed to investigate bleeding disorders and to monitor patients taking an anticlotting drug such as heparin which inhibits factors X and thrombin, while activating anti-thrombin.

The aPTT test uses blood which is decalcified to prevent clotting before the test begins. The plasma is separated by centrifugation. (Ionized) Calcium and activating substances are added to the plasma to start the intrinsic pathway of the coagulation cascade. The substances are: kaolin (hydrated aluminum silicate) and cephalin. Kaolin serves to activate the contact-dependent Factor XII, and cephalin substitutes for platelet phospholipids. The partial thromboplastin time is the time it takes for a clot to form, measured in seconds. Normally, the sample will clot in 35 seconds.



PTT measures the integrity of the intrinsic system (Factors XII, XI, VIII, IX) and common clotting pathways.

Increased levels in a person with a bleeding disorder indicate a clotting factor may be missing or defective. At this point, further investigation is needed and warrants the use of sensitive assays for specific coagulation factors. Liver disease decreases production of factors, increasing the PTT.

Clotting time

In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clotting factor, this process is also being assisted by Ca++ and by

factors released by platelets and damaged tissues . The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged. The expected range for clotting time is 4-10 mins.

Partial Thromboplastin Time

Definition

The partial thromboplastin time (PTT) test is a blood test that is done to investigate bleeding disorders and t o monitorpatients taking an anticlotting drug (heparin).

Purpose

Diagnosis

Blood clotting (coagulation) depends on the action of substances in the blood called clotting factors. Measur ing the partialthromboplastin time helps to assess which specific clotting factors may be missing or defectiv e.

Monitoring

Certain surgical procedures and diseases cause **blood clots** to form within blood vessels. Heparin is used to treat theseclots. The PTT test can be used to monitor the effect of heparin on a patient's coagulation system.

Precautions

Certain medications besides heparin can affect the results of the PPT test. These include **antihistamines**, v itamin C(ascorbic acid), **aspirin**, and chlorpromazine (Thorazine).

Description

When a body tissue is injured and begins to bleed, it starts a sequence of clotting factor activities called the coagulationcascade, which leads to the formation of a blood clot. The cascade has three pathways: extrinsi c, intrinsic, and common.Many of the thirteen known clotting factors in human blood are shared by both pat hways; several are found in only one.The PTT test evaluates the factors found in the intrinsic and common pathways. It is usually done in combination withother tests, such as the prothrombin test, which evaluate the factors of the extrinsic pathway. The combination of testsnarrows the list of possible missing or defective fa ctors.

Heparin prevents clotting by blocking certain factors in the intrinsic pathway. The PTT test allows a doctor to check thatthere is enough heparin in the blood to prevent clotting, but not so much as to cause bleeding. T he test is done before thefirst dose of heparin or whenever the dosage level is changed; and again when th e heparin has reached a constant levelin the blood. The PTT test is repeated at scheduled intervals.

The PTT test uses blood to which a chemical has been added to prevent clotting before the test begins. Ab out 5 mL ofblood are drawn from a vein in the patient's inner elbow region. Collection of the sample takes o

nly a few minutes. Theblood is spun in a centrifuge, which separates the pale yellow liquid part of blood (pla sma) from the cells. Calcium andactivating substances are added to the plasma to start the intrinsic pathwa y of the coagulation cascade. The partialthromboplastin time is the time it takes for a clot to form, measured in seconds.

The test can be done without activators, but they are usually added to shorten the clotting time, making the t est moreuseful for monitoring heparin levels. When activators are used, the test is called activated partial th romboplastin time orAPTT.

Test results can be obtained in less than one hour. The test is usually covered by insurance.

Preparation

The doctor should check to see if the patient is taking any of the medications that may influence the test res ults. If thepatient is on heparin therapy, the blood sample is drawn one hour before the next dose of heparin .

Aftercare

Aftercare includes routine care of the puncture site. In addition, patients on heparin therapy must be watche d for signs ofspontaneous bleeding. The patient should not be left alone until the doctor or nurse is sure that bleeding has stopped.Patients should also be advised to watch for bleeding gums, bruising easily, and othe r signs of clotting problems; to avoid
activities that might cause minor cuts or **bruises**; and to avoid using aspirin.

Risks

The patient may develop a bruise or swelling around the puncture site, which can be treated with moist war mcompresses. People with coagulation problems may bleed for a longer period than normal.

Normal results

Normal results vary based on the method and activators used. Normal APTT results are usually between 25 -40 seconds;PTT results are between 60-70 seconds. APTT results for a patient on heparin should be 1.5-2.5 times normal values. AnAPTT longer than 100 seconds indicates spontaneous bleeding.

Abnormal results

Increased levels in a person with a bleeding disorder indicate a clotting factor may be missing or defective. Further testsare done to identify the factor involved. **Liver**
disease decreases production of factors, increasing the PTT.

Low levels in a patient on heparin indicate too little heparin is in the blood to prevent clots. High levels indic ate too muchheparin is present, placing the person at risk of excessive bleeding.

Resources