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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

Imagine a world without balance – a body where a tiny cut could lead to uncontrollable bleeding, or where blood could spontaneously clot, blocking the vital flow to our organs. Such a scenario highlights the extraordinary precision of our body's internal control systems. Among the most critical of these is **haemostasis**, the intricate process that prevents and stops bleeding, ensuring our blood remains within its designated vessels. It is a finely tuned symphony, where every instrument must play its part perfectly to maintain the delicate balance between preventing excessive bleeding and avoiding unwanted clot formation (thrombosis).

In the complex world of clinical medicine, understanding and monitoring this delicate balance is paramount. This is where the clinical laboratory steps in, acting as the vigilant conductor of this physiological orchestra. Coagulation tests, such as the Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and Thrombin Time (TT), are indispensable tools for assessing an individual's coagulation status, providing critical insights into their risk of bleeding or thrombosis. These tests are windows into the dynamic processes occurring within a patient's bloodstream.

The reliability of these laboratory results is a matter of convenience and a direct determinant of patient safety. This highlights the fundamental importance of Quality Assurance (QA) in haemostasis testing. QA in the laboratory ensures that every result generated is accurate, precise, and specific. Without robust internal quality control (IQC) and external quality assurance (EQA) schemes, discrepancies can arise, leading to potentially catastrophic patient outcomes. Consider the Prothrombin Time (PT) and its derived International Normalized Ratio (INR), used to monitor patients on anticoagulants like Warfarin. The calculation of INR relies on the International Sensitivity Index (ISI) of the thromboplastin reagent used. If a laboratory fails to recognize a change in ISI with a new batch of reagents and continues to use an outdated ISI, patients could be dangerously underdosed, risking thrombosis, or overdosed, leading to a severe bleeding risk. This vivid example highlights why IQC and EQA must be a fundamental part of any test a laboratory offers, ensuring consistency and comparability of results across different settings and methodologies.

This edition of The Crux aims to illuminate the intricacies of haemostasis and its key laboratory assessments. We will journey through the mechanisms of blood clotting, explore the diagnostic power of PT, aPTT, and TT, delve into the nuances of interpreting their results, and address common challenges and their solutions in daily laboratory practice. Our goal is to provide an educative and engaging narrative, fostering a deeper appreciation for the vital role laboratory professionals play in safeguarding patient health.

DISEASE DIAGNOSIS

Unravelling Coagulation Mysteries – How Lab Tests Point the Way

Blood an elixir of human biology is a vital body fluid that performs essential functions, including transporting oxygen and nutrients, fighting infections, and regulating body temperature. Blood circulates throughout the human body and forms a part of the cardiovascular system.

HOMEOSTASIS

Blood takes part in the various physiologic activities of the body like Gas Transport, Metabolism, Immunologic Défense, Cellular Communication via Hormones. And the normal functioning of all the above is termed as Homeostasis (homeo-same/like, stasis-standing). The basic precondition being that blood is maintained in its fluid state.

HEMOSTASIS

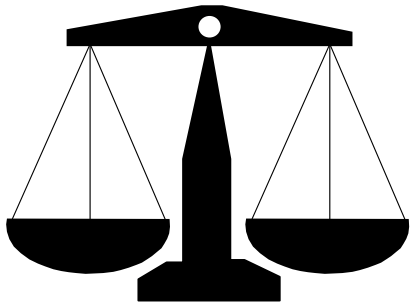
To effectively carry out the above-mentioned functions, the blood must remain in:

- In fluid state
- Within the confines of the vasculature

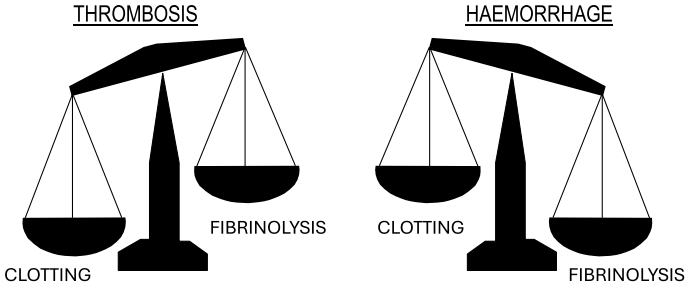
If blood clots in an artery, without an injury, then the tissues being supplied blood by that artery, can die. Such non-specific clots must be removed or dissolved fast, to prevent a mishap. On the other hand, if the blood fails to clot at the site of the injury, there will be excessive loss of blood, which will affect the health of the body as a whole. Hence a proper balance needs to be maintained between clot formation and clot dissolution activities. This balance is referred to as 'haemostasis'. Haemostasis is derived from the Greek word 'haima' or blood and 'stasis' or 'halt' and literally means 'arrest bleeding'. Haemostasis is a remarkable physiological process designed to prevent and stop bleeding, keeping blood safely contained within damaged blood vessels. Haemostasis is achieved through a series of interactions between the inner surfaces of the various blood vessels which has a thin layer of cells called 'endothelial cells', circulating platelets and the coagulation factors. Haemostasis is a complex process involving a series of sequential and highly Coordinated events, traditionally broken down into three major steps: 1. Vasoconstriction, 2. Platelet plug formation, and 3. blood coagulation (fibrin clot formation). These steps occur rapidly following vascular injury, ensuring prompt cessation of blood loss.

The Phases of Haemostasis:

1. **Vasoconstriction:** Immediately upon injury, the smooth muscle cells within the walls of the damaged blood vessel constrict. This initial response, largely controlled by the vascular endothelium, significantly reduces blood flow to the injured area, thereby limiting the amount of blood lost.
2. **Platelet Plug Formation (Primary Haemostasis):** This phase is primarily driven by platelets (also known as thrombocytes). When platelets encounter a damaged vascular surface, particularly exposed collagen, they become activated.
3. **Blood Coagulation (Secondary Haemostasis):** While the platelet plug provides a temporary seal, a more robust and permanent clot is needed. This is achieved through the coagulation cascade, a complex series of biochemical reactions involving various clotting factors that circulate in the blood plasma in an inactive state. The Coagulation System comprises of a balance between the Clotting Mechanism and the Fibrinolytic Mechanism.



Clotting Mechanism: This is responsible for the formation of a clot whenever there is a damage to the tissues or the circulatory system of the body. The objective, being to prevent excessive loss of blood.
Fibrinolytic Mechanism: This is responsible for the clot dissolution to maintain the flow of blood. A healthy balance of the two mechanisms is essential for normal Haemostasis. If excess clotting takes place, then Thrombosis occurs and if Fibrinolysis is in excess then Haemorrhage occurs.



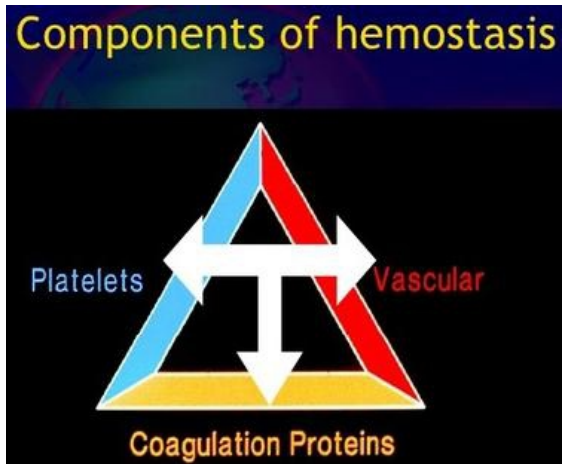
Haemostasis is achieved through a series of interactions between the inner surfaces of the various blood vessels which has a thin layer of cells called 'endothelial cells', circulating platelets and the coagulation factors.

THE 3 ELEMENTS OF HEMOSTASIS

Haemostasis can be conceptualized as a triad composed of three interacting elements.

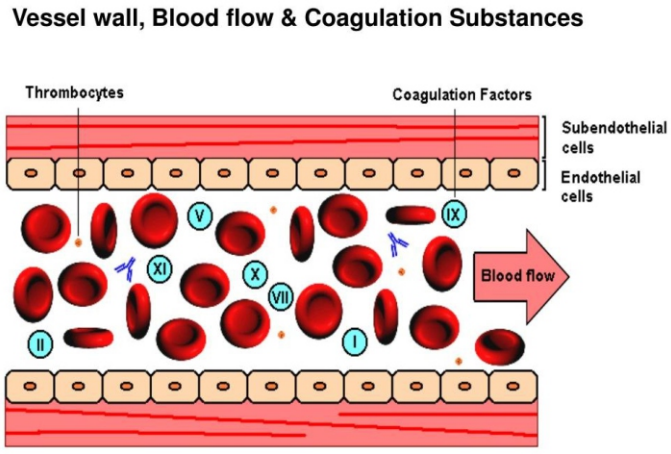
- Vasculature
- Platelets
- Coagulation System

HEMOSTASIS TRIAD



VASCULATURE

Structure of vascular endothelium



The Vascular Endothelium or (Vessel Wall) consists of 3 layers inner, middle and outer layer. It has 3 major functions in relation to haemostasis

- Provide a non-thrombogenic (non-clotting) surface.
- Provide the initial stimuli for thrombus formation whenever there is an injury or trauma.
- Provide platelet inhibitors and activators for clot clearance.

The inner surface of the various blood vessels has a thin layer of endothelial cells, which is important in maintaining a non-thrombogenic (non-clotting) surface for the vasculature.

Prothrombotic properties of endothelial cells

These endothelial cells interact with the other members of the haemostasis triad whenever the vascular integrity is disturbed due to trauma.

- They bind with platelets
- Form platelet plug
- Activate the coagulation mechanism

The product of this is the formation of a blood clot at the site of injury.

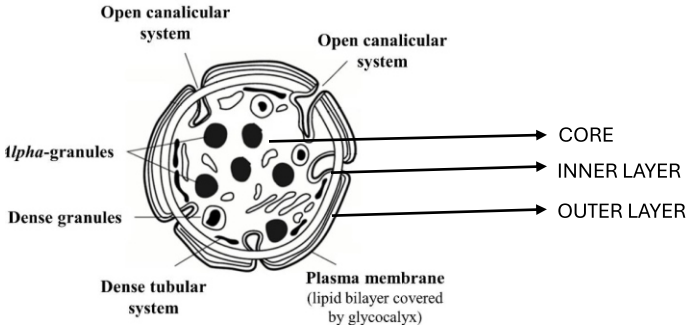
Anti thrombotic properties of the vascular endothelial cells

- The outer membrane of the VE cells is covered with a special outer coating. This outer coating produces a natural anticoagulant called antithrombin III (AT III) and prevents clot formation.
- The outer membrane also contains certain receptor (e.g. Thrombomodulin) which imparts anti thrombotic properties by activating Protein C. This activated Protein C
  - o Inhibits Factor V and Factor Vill
  - o Stimulates the VE to release Tissue Plasminogen activator (tpA)
- The tissue Plasminogen activator converts Plasminogen to plasmin, which in turn breaks down Fibrin, thereby retaining the fluid state of the blood.

PLATELETS

Platelet structure

The structure of Platelets can be divided into 3 layers Outer Layer, Inner Layer, Core. The outer layer is bipolar. This is involved in the function of platelet adhesion and aggregation. Platelets are negatively charged due to the sialic acid on the membrane. Platelet Factor 3 is produced from the membrane just below the outer layer, which participates in clotting mechanism. The inner layer Provides support for cell structure and mechanism for cell contraction. During its circulation in the plasma, platelets absorb the plasma coagulation factors. These factors are present in platelets in similar concentration as present in plasma and are readily available when platelets are activated. Calcium ions are also found which are required for platelet activation. The core of the platelets is concerned with respiratory and excretory functions as well as the production, storage and release of energy. They also regulate the platelet's chemical and physiologic response to stimuli.



Platelet activity

Platelet activating factor is responsible for platelet activation. It is produced by endothelial cells, PMNs, monocytes, and platelets. It is also liberated during vascular injury. When the vascular endothelial cells are injured, platelets are activated. When platelets are activated they change shape, and the outer membrane surface is modified which helps in adhering to each other more readily. Platelet adhesion is mediated by interaction of Von Willebrand factor. So, deficiency of this factor causes a bleeding disorder known as Von - Willebrand disease. Activation and adherence of the platelet results in the formation of a platelet plug. The surface of this plug is referred to as Platelet Factor 3.

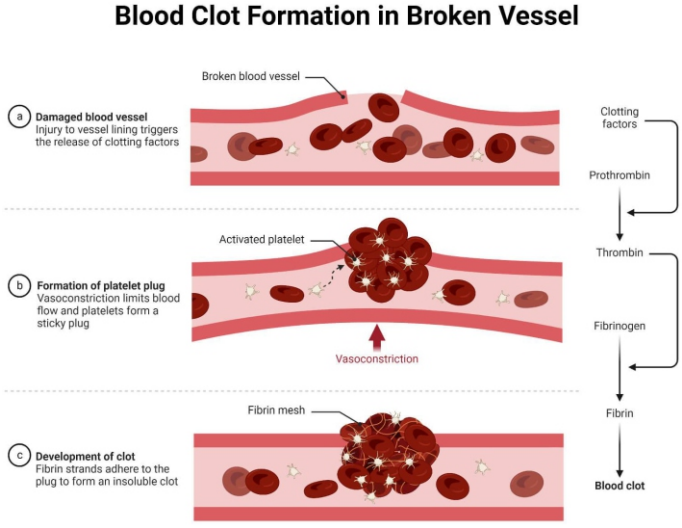


Fig: Diagram of platelet activation during vascular injury

COAGULATION SYSTEM

The Clotting mechanism is a result of a series of biochemical activity in which the enzymatic substances are converted from inactive to active proteins. These enzymatic proteins are called the clotting factors. These factors are present both in the plasma and in the platelets.

Given in the Table below is the Nomenclature and properties of these factors.

Characteristics of Clotting Factors and Associated Coagulapathies

Factor	Synonym	Site of Production	Half-life Disappearance	Minimum Hemostatic Level	Storage/ Stability	Inheritance	Functional Activity	Participation in Extrinsic and Intrinsic Pathways	Affected by Coumarin Drugs	Coagulapathies	
										Congenital	Acquired (Causes)
I	Fibrinogen	Liver	36-90 Hours	50-100 mg%	Stable	Autosomal Recessive / Autosomal Dominant	Substrate	Both Intrinsic and Extrinsic	No	Afibrinogenemia / Dysfibinogenemia	Severe Liver Disease, Intravascular Clotting Fibrinolysis
II	Prothrombin	Liver-Vitamin K Dependent	60-70 Hours	40% Concentration	Stable	Autosomal Recessive	Serine Protease	Both Intrinsic and Extrinsic	Yes	Prothrombin deficiency	Liver Disease, Vitamin K deficiency, Anticoagulation
III	Tissue Thrombo-plastin	Thromboplastic Activity Present in Most Tissues					Cofactor	Extrinsic	No		
IV	Calcium	Platelets					Cofactor	Both Intrinsic and Extrinsic			
V	Labile Factor	Liver	12-36 Hours	5-10% Concentration	Labile	Autosomal Recessive	Cofactor	Both Intrinsic and Extrinsic	No	Parahemophilia	Severe Liver Disease, Intravascular Clotting Fibrinolysis
VII	Stable Factor	Liver-Vitamin K Dependent	4-7 Hours	5-10% Concentration	Stable	Autosomal Recessive	Serine Protease	Extrinsic	Yes	Factor VII deficiency	Liver Disease, Vitamin K deficiency, Anticoagulation
VIII	Antihemo-philic Factor (AHF)	Possibly the Reticulo-endothetial System	10-12 Hours	30% Concentration	Labile	X-Linked Recessive Autosomal Dominant	Cofactor	Intrinsic	No	Hemophilia A Von Willebrand's Disease	Intravascular Clotting, Fibrinolysis
IX	Christmas Factor	Liver-Vitamin K Dependent	24 Hours	30% Concentration	Stable	X-Linked Recessive	Serine Protease	Intrinsic	Yes	Haemophilia B	Liver Disease, Vitamin K deficiency, Anticoagulation
X	Stuart-Prower Factor	Liver-Vitamin K Dependent	48 Hours	8-10% Concentration	Stable	Autosomal Recessive	Serine Protease	Both Intrinsic and Extrinsic	Yes	Stuart-Power Factor deficiency	Liver Disease, Vitamin K deficiency, Anticoagulation
XI	Plasma Thrombo-plastin Antecedent (PTA)		60 Hours	20-30% Concentration	Stable	Autosomal Recessive / Autosomal Dominant	Serine Protease	Intrinsic	No	PTA deficiency	
XII	Hageman Factor			0 %	Stable	Autosomal Recessive	Serine Protease	Intrinsic	No	Hageman Factor Deficiency	
XII	Fibrin Stabilizing Factor	No Information available	3-4 Hours	1% Concentration	Stable	Autosomal Recessive	Transmidase	Both Intrinsic and Extrinsic	No	FSF Deficiency	Liver Disease, Fibrinolysis

Factors are plasma proteins. They are denoted as roman numerals for e.g. Factor XII or XII'. In its activated form a factor is denoted with a suffix 'a' 'Factor XIIa or XIIa'. Some of these factors undergo only a modification and not activation and are denoted as Factor Vm with a suffix 'm'. In the above table if you notice the "Functional Activity" column some factors are labelled as cofactor and others as Serine Proteases.

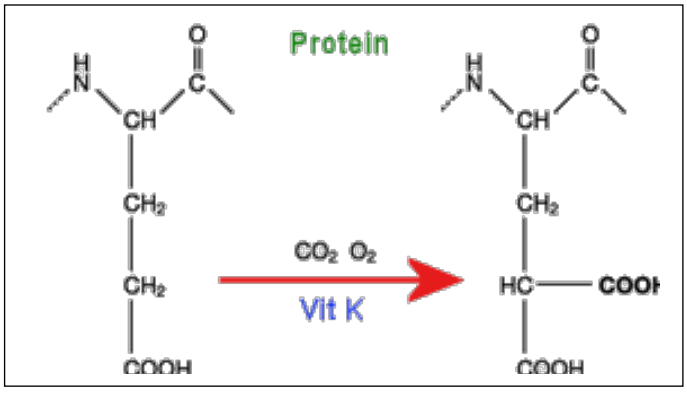
Cofactors: These include Factor V, Factor VIII, Tissue Factor (Factor III) and High Molecular Weight Kininogen. Cofactors accelerate the various enzymatic reactions in the clotting mechanism when they are in their activated form. e.g. Tissue Factor is a cofactor, which activates Factor VIIa.

Serine Proteases: These include Factor II, Factor VII, IX, X, XI, XII and Prekalikrein.

- Serine: They are Amino acids. All proteins are made of a series of amino acid chains joined by a peptide bond.
- Protease: They are a class of enzymes when activated break down the peptide bonds that join the amino acid (serine) chains in proteins.

e.g. Prothrombin is a serine protease which when activated cleaves Fibrinogen.

ROLE OF VITAMIN – K



The synthesis of Factors II, VII, IX, X and Proteins C and S are dependent on Vitamin - K. AThe N - Terminal end of these proteins has one Carboxyl group carries a negative charge COO^- (see diag.).

- Vitamin - K brings about oxidation of the N - Terminal end and adds a second Carboxyl group to this chain.
- This creates a double negative charge on these Factors.
- This enables them to bind more strongly to the positively charged Ca^{++}bound to PF3.
- During oral anticoagulant therapy the oxidation reaction which is synthesised by Vitamin - K is blocked. This blockage stops the carboxylation of Factor II, VII, IX, X, Protein C and S.

Hence Factors II, VII, IX, X, Protein C and S are also called Vitamin – K dependent Factors.

The coagulation cascade leads through two main pathways: the extrinsic pathway and the intrinsic pathway, which eventually merge into a common pathway.

- **Extrinsic Pathway:** Is so called because
  - It involves the tissues and is external to the microcirculation.
  - Uses the Factors released from the body tissues (e.g. Tissue Factor) and the enzymes found in circulating blood.
  - Tissue factor, with the help of calcium, activates Factor VII to Factor VIIa, which then activates Factor X to Factor Xa.

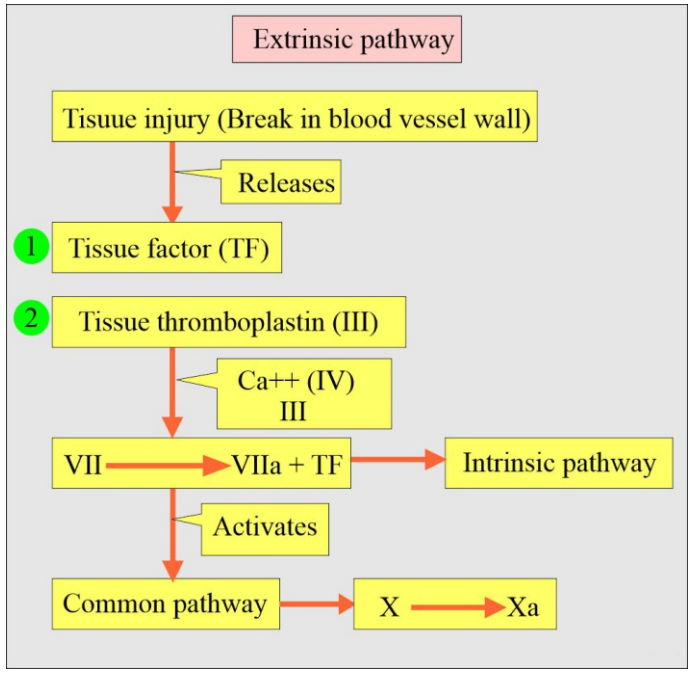


Fig: Diagram of the Extrinsic Pathway in vivo

- **Intrinsic Pathway:** Is so called because
  - It involves the contact activation within the microcirculation.
  - Uses the Factor and enzymes found in circulating blood.
  - A slower pathway (several minutes). Factor XII meets negatively charged surfaces (like exposed collagen). This leads to a series of activations involving Factor XI, Factor IX (Christmas factor), and Factor VIII (antihemophilic factor), culminating in the activation of Factor X to Factor Xa.

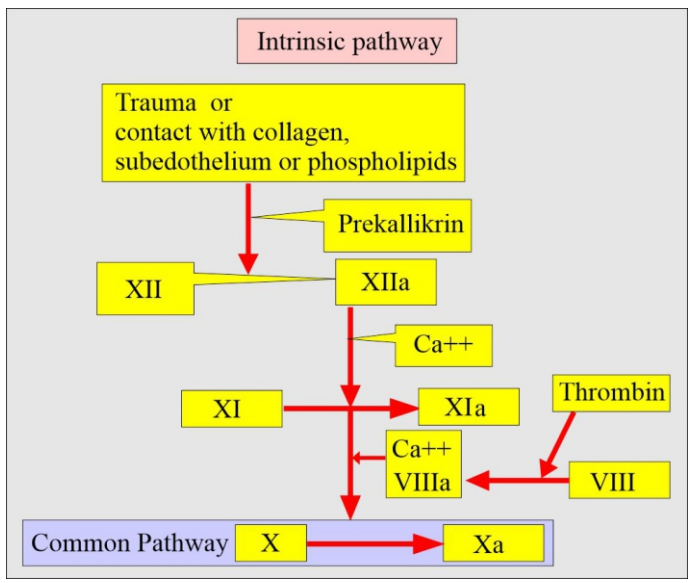


Fig: Diagram of the Intrinsic Pathway in vivo

- **Common Pathway:** Both the extrinsic and intrinsic pathways converge at the activation of Factor X to Factor Xa. Factor Xa, along with Factor V and calcium, converts inactive prothrombin (FII) into active thrombin (FIIa).

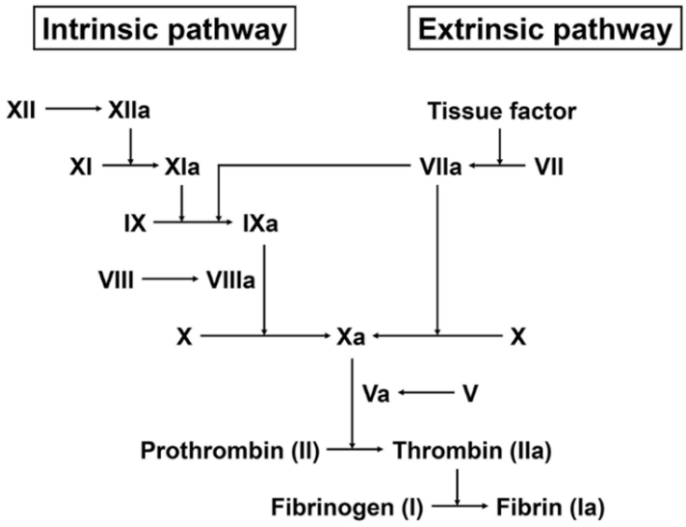


Fig: Diagram of the Common Pathway in vivo

- **Fibrin Formation:** Thrombin is a pivotal enzyme with multiple roles.
  1. Thrombin's primary function in clot formation is to convert soluble plasma protein fibrinogen (FI) into insoluble fibrin monomers. These fibrin monomers spontaneously polymerize to form a network of fibers that traps blood cells and platelets, creating a loose fibrin clot.
  2. Thrombin also activates Factor XIII to Factor XIIIa, which then stabilizes the fibrin clot by forming covalent bonds, making it more robust and insoluble. The resulting structure is known as a thrombus or blood clot.

Following the formation of the blood clot, two further processes ensure successful haemostasis:

- Clot Contraction: The clot begins to shrink, drawing the edges of the injured vessel closer.

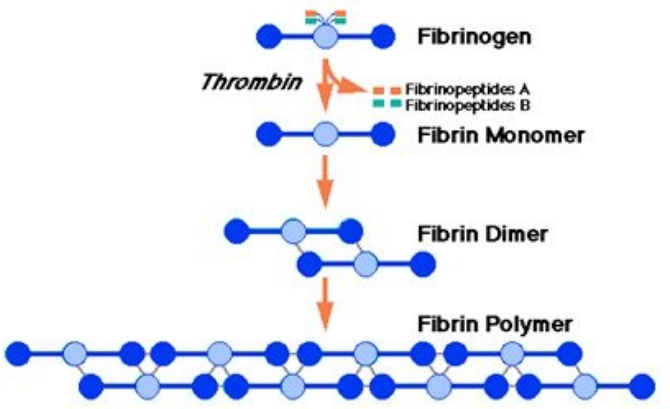


Fig: Diagram of Clot Formation Fibrinolysis

- **Fibrinolysis:** Once the tissue has healed, the fibrinolytic system is activated to systematically degrade the fibrin clot into smaller fragments, maintaining the fluidity of blood flow and preventing long-term vessel obstruction. Plasmin, activated from plasminogen by tissue plasminogen activator (tPA), is the key enzyme in this process. One important degradation product is the cross-linked D-dimer.

Key Elements for Normal Haemostasis: Successful haemostasis relies on a complex interaction between several components:

- Vessel Wall Integrity: The endothelial cells lining blood vessels maintain a non-thrombogenic surface but initiate clotting upon injury.
- Adequate Number of Platelets: Essential for forming the initial platelet plug.
- Proper Functioning Platelets: Their ability to adhere, activate, and aggregate is crucial.
- Adequate Levels of Clotting Factors: All factors in the intrinsic, extrinsic, and common pathways must be present and functional.
- Proper Function of Fibrinolytic Pathway: Ensures clot removal after healing.
- Anticoagulant Inhibitory Proteins: Such as Protein C, Protein S, and Antithrombin III, which regulate and inhibit the clotting mechanism to prevent excessive thrombosis.

Laboratory Tests in Haemostasis: To assess this complex system, various laboratory tests target specific phases and pathways of coagulation. PT, aPTT, and TT – Pillars of Coagulation Assessment.

PROTHROMBIN TIME (PT) AND INTERNATIONAL NORMALIZED RATIO (INR)

The Prothrombin Time (PT) is a crucial laboratory test that measures the activity of clotting factors involved in the extrinsic pathway and the common pathway of blood coagulation. It assesses the function of factors II (prothrombin), V, VII, X, and fibrinogen. Normal PT values typically range from 9 to 15 seconds.

Test Method: The PT test is performed on citrated plasma, which is blood treated with sodium citrate (3.2% or 0.11 mol/L) to prevent clotting by binding calcium. The ratio of blood to anticoagulant is critical, ideally 9:1. Both the patient's plasma and the calcium thromboplastin reagent are prewarmed to 37°C. The prewarmed thromboplastin (a combination of tissue factor and phospholipids) is then added to the plasma, and the time it takes for a fibrin clot to form is measured. Clot detection can be done visually or, more precisely, by instruments.

International Normalized Ratio (INR): Historically, PT results varied significantly between laboratories due to differences in thromboplastin reagents, instruments, and methodologies. To standardize reporting and allow for uniform oral anticoagulant therapy worldwide, the World Health Organization (WHO) introduced the International Normalized Ratio (INR) in 1984. The INR accounts for the varying sensitivity of different thromboplastins by calibrating each commercial reagent against an International Reference Preparation (IRP). The sensitivity is expressed as the International Sensitivity Index (ISI). **The INR is calculated as: INR = (Patient PT ratio)^ISI**. A PT ratio is obtained by dividing the patient's PT in seconds by the mean normal PT (MNPT) of the local laboratory. A lower ISI value (closer to 1.0) indicates a more sensitive thromboplastin, and the INR will be closer to the observed PT ratio.

Benefits of INR standardization:

- Helps apply uniform oral anticoagulant therapy based on internationally established therapeutic ranges (e.g., 2.0-3.0 for most warfarin patients).
- Factors influencing INR precision include the derivation of the MNPT (ideally from 20+ normal samples), the magnitude of the ISI difference from 1.0, and the clot detection method.
- Eliminates confusion in PT interpretation, as INR remains comparable despite changes in thromboplastin or equipment.
- Allows for comparison of PT results between different laboratories.
- Facilitates monitoring of patients who travel internationally.

Factors Influencing the INR

The variability in the responsiveness of the PT reagents is corrected through the ISI calibration, however three technical factors influence the INR:

1. Derivation of MNPT
2. Magnitude of difference in the ISI value of test thromboplastin and IRP (ISI = 1.0)
3. Method of clot detection employed during PT test.

MNPT

Ideally each laboratory must derive its own MNPT from 20 or more normal patients' sample for a given PT reagent and lot under use. This corrects within laboratory variables that influence PT results. For e.g. when a same patient's plasma is tested on three different days using the same PT reagent, but different control plasmas:

Reagent ISI 2.5	Test 1 at day 1	Test 2 at day 2	Test 3 at day 3
Patient Pt	16	16	16
Normal control plasma	11.5	10.4	12.3
Resulting INR	2.27	2.89	1.92

The reported INR varies due to the change in control time. It is therefore necessary to establish the MNPT for each PT lot under use. If the control time is greater than the mean normal range (MNPT), the PT ratio for any patient will be smaller, potentially leading to over coagulation. If the control time is lesser than MNPT the ratio for any patient PT will be greater, leading to under coagulation.

The MNPT for a particular laboratory using the same combination of reagent, instrument and methodology would remain constant resulting in consistent INR.

ISI value of PT used and method of clot detection

The INR loses precision when comparisons are made with thromboplastin with markedly different ISI values as against IRP's and different method of clot detection (manual, mechanical, optical).

The impact of imprecision increases with the increasing INR. This variability is diminished and within acceptable limits by universal use of responsive thromboplastin reagents, with an ISI up to 1.5

Clinical Applications of Prothrombin Time: A prolonged PT indicates a slower clotting time and suggests potential issues with the factors it measures. Its clinical utility is extensive:

- **Preoperative Screening:** PT is often included in presurgical screening to assess a patient's overall clotting ability and identify those at increased risk of bleeding during surgery.
- **Monitoring Oral Anticoagulant Therapy (Warfarin):** This is the most common application. Warfarin, a vitamin K antagonist, inhibits the synthesis of vitamin K-dependent clotting factors (Factors II, VII, IX, X). Monitoring PT/INR helps ensure the patient's dose is within the narrow therapeutic range, balancing the risk of bleeding (too high INR) and thrombosis (too low INR). Dysfunctional factors known as PIVKA's (Proteins Induced by Vitamin K Absence/Antagonism) also appear in plasma with warfarin therapy, and the varying sensitivity of thromboplastin reagents to PIVKA's can give confusing results.
- **Assessing Liver Function:** Since many clotting factors (including II, V, VII, X, fibrinogen) are produced in the liver, liver diseases (e.g., cirrhosis, hepatitis, liver failure) can impair their synthesis, leading to prolonged PT.
- **Diagnosing Bleeding Disorders:** Prolonged PT can help diagnose abnormalities in the extrinsic and common pathways.
- **Vitamin K Deficiency:** A prolonged PT can indicate a deficiency in vitamin K, which is essential for the synthesis of Factors II, VII, IX, and X.

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

The Activated Partial Thromboplastin Time (aPTT), often referred to as PTT, measures the activity of clotting factors of the intrinsic pathway and the common pathway of coagulation. Normal aPTT values typically range from 22 to 37 seconds.

Test Method: Like PT, aPTT is performed on citrated plasma. The aPTT reagents contain a phospholipid (acting as a platelet substitute to remove platelet number/function variability) and an activator (such as kaolin, ellagic acid, bentonite, celite, or colloidal silica). The activator ensures consistent activation of Factor XII, eliminating variability that might arise from contact with different glass surfaces. Plasma is incubated with the aPTT reagent at 37°C, followed by the addition of prewarmed calcium chloride to initiate clotting, and the time to clot formation is measured.

Clinical Applications: A prolonged aPTT indicates a deficiency in one or more factors in the intrinsic or common pathways, or the presence of an inhibitor.

- **Detecting Deficiencies in Intrinsic Pathway Factors:** Prolonged aPTT can indicate deficiencies of Factor XII, XI, IX, VIII, V, X, II, fibrinogen, prekallikrein, and high molecular weight kininogen (HMWK). This is crucial for diagnosing conditions like Hemophilia A (Factor VIII deficiency) and Hemophilia B (Factor IX deficiency).
- **Detecting Clotting Inhibitors:** The aPTT is sensitive to circulating anticoagulants, such as antibodies against specific clotting factors (e.g., Factor VIII inhibitors) or lupus anticoagulants. Mixing studies (patient plasma mixed with normal plasma) are used to differentiate factor deficiencies (which typically correct with normal plasma) from inhibitors (which do not correct or only partially correct).
- **Monitoring Heparin Therapy:** Heparin, an anticoagulant, primarily exerts its effect by combining with antithrombin III (AT-III) to form a potent antithrombin complex, mainly affecting the intrinsic pathway. The aPTT is the test of choice for monitoring heparin therapy, ensuring rapid anticoagulant effects are achieved while preventing excessive bleeding.
- **Other Applications:** aPTT can be used preoperative screening, and investigating unexplained bleeding or thrombotic events.

THROMBIN TIME (TT)

The Thrombin Time (TT) is a straightforward coagulation test that assesses the final step of the common pathway: the conversion of fibrinogen to fibrin. It provides a qualitative estimation of fibrinogen in a sample based on the time required for clotting and the quality of the clot formed.

Test Method: A known quantity and concentration of thrombin reagent is added to citrated plasma. The time taken for thrombin to cleave fibrinogen into fibrin monomers, which then polymerize into a clot, is measured.

Clinical Applications: A prolonged TT indicates an issue with fibrinogen availability or function, or the presence of inhibitors to thrombin.

- **Low Fibrinogen Levels (Hypofibrinogenemia or Afibrinogenemia):** This is a common cause of prolonged TT, seen in conditions like Disseminated Intravascular Coagulation (DIC) or, more rarely, congenital deficiencies.
- **Dysfibrinogenemia:** This refers to functionally defective fibrinogen,

where the levels may be normal, but the protein does not clot properly, leading to a prolonged TT. This can be congenital or acquired (e.g., in liver disease or neonates).

- **Presence of Fibrin Degradation Products (FDPs):** Increased concentrations of FDPs, which act as competitive inhibitors of fibrin polymerization, can prolong TT, as encountered in DIC or liver disease.
- **Heparin Interference:** Heparin, a potent anticoagulant, strongly interferes with the thrombin-fibrinogen reaction, significantly prolonging the TT. A normal TT can therefore be used to rule out the presence of therapeutic levels of heparin in a sample, especially when interpreting a prolonged aPTT.
- **Pre-Surgical Screening:** TT is considered essential alongside PT and aPTT for comprehensive presurgical screening, particularly for assessing the lower common pathway.

When the Thrombin Time is prolonged, further quantitative fibrinogen assay may be required to precisely ascertain the amount of fibrinogen present (normal range 200-400 mg/dL). Quantitative estimation is particularly important in obstetric cases (e.g., post-delivery, septic abortion). **Tests for quantitative fibrinogen estimation should ideally be insensitive to heparin.** Together, PT, aPTT, and TT provide a powerful diagnostic panel that helps clinicians pinpoint where in the complex coagulation cascade a problem lies, enabling targeted diagnosis and appropriate patient management.

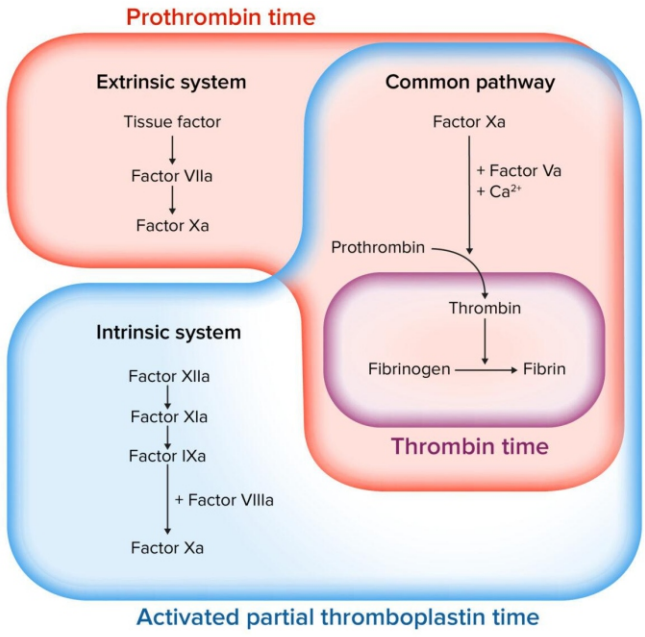


Fig: Diag of Coagulation tests detecting the factor deficiencies of different coagulation cascade

INTERPRETATION

DECODING THE CLOTTING CASCADE

Interpreting coagulation test results is an art as much as it is a science, requiring a thorough understanding of the clinical context, patient history, and the interplay between various laboratory parameters. Raw numerical values of PT, aPTT, and TT gain their true significance when viewed as part of a "**Coagulogram"** – a comprehensive assessment that, alongside platelet count and morphology, paints a broad picture of a patient's haemostatic condition.

The following Coagulogram Table summarizes how combined results from PT, aPTT, TT, and Platelet Count can lead to a specific clinical diagnosis:

TESTS				CLINICAL DIAGNOSIS
PT	APTT	TT	Platelet Count	
Normal	Normal	Normal	Normal	Disorder of platelet function, Factor XII deficiency Disorder of vascular Haemostasis Normal Haemostasis
Long	Normal	Normal	Normal	Factor VII deficiency Early oral anticoagulation
Normal	Long	Normal	Normal	Factors VIII, IX, XI, XII Prekallikrein HMWK deficiency, Von Willebrand disease Circulating anticoagulant
Long	Long	Normal	Normal	Vitamin K deficiency Oral anticoagulants Factors V, VII, X and II Deficiency
Long	Long	Long	Normal	Heparin Liver diseases Fibrinogen deficiency Hyperfibrinolysis
Normal	Normal	Normal	Low	Thrombocytopenia
Long	Long	Normal	Low	Massive transfusion Liver disease
Long	Long	Long	Low	DIC, Acute liver disease

Specific Interpretations:

- **Isolated Prolonged aPTT:** This is a classic pattern for deficiencies in factors of the intrinsic pathway (VIII, IX, XI, XII) or the presence of an intrinsic pathway inhibitor. To distinguish, a mixing study (patient plasma mixed with normal plasma) is performed.
  - **Correction:** If the aPTT corrects to normal, a factor deficiency is likely (e.g., Hemophilia A or B). Further testing with specific factor deficient plasmas will help identify the deficient factor.
  - **No Correction (or incomplete):** If the aPTT remains prolonged, it suggests the presence of an inhibitor (e.g., heparin, lupus anticoagulant, or specific factor inhibitors).
- **Prolonged Thrombin Time (TT):** An isolated prolonged TT suggests issues with fibrinogen (either low quantity or dysfunctional), the presence of heparin, or high levels of FDPs. If heparin is suspected, a normal TT can help rule it out as a cause for a prolonged aPTT.

TROUBLESHOOTING

NAVIGATING THE COMMON CHALLENGES AND SOLUTIONS IN COAGULATION TESTING

Even with state-of-the-art instruments and highly skilled personnel, coagulation testing is susceptible to various issues that can lead to inaccurate results. These "troubleshooting" scenarios often arise from problems in the pre-analytical, analytical, or post-analytical phases of testing. Understanding these variables and implementing robust quality assurance measures are paramount to generating reliable results for daily laboratory use. **The pre-analytical phase,** encompassing everything from patient preparation to sample collection and initial processing, is widely recognized as the most frequent source of errors in laboratory testing. This following table explains these variables and their effects on Coagulation testing. Blood sample collection is one of the most important aspect of coagulation testing. It is preferable that patients are not heavily exercised before collection. Fasting or only light non-fatty meals prior to blood collection provide samples with desirable lower opacity. Blood should be withdrawn with undue venous stasis or frothing onto a plastic syringe fitted with a short needle of 19-20 SWG. Blood should be transferred to the appropriate collection tube after detaching the needle from the syringe. As the coagulation tests are performed on plasma, Sodium citrate (3.2 % or 0.11 mol/l) is the anticoagulant of choice for collecting blood. The other anticoagulants EDTA, heparin and oxalate are unacceptable for routine coagulation procedures. Oxalate cannot be used because Factor V deteriorates rapidly in oxalated blood. EDTA cannot be used because it acts as a chelating agent and it inhibits the conversion of fibrinogen to fibrin. Heparin is unsuitable as a anticoagulant for coagulation studies because it inhibits thrombin. The ratio of blood to anticoagulant is 9:1 i.e., nine parts of freshly collected blood are mixed with one part of sodium citrate (3.2%). For occasional patients with haematocrit less than 20% or greater than 55% this ration must be readjusted to ensure valid results. The blood must be with mixed well immediately by inverting gently 5-6 times. The blood must be centrifuged immediately for 15 minutes at 1500g and plasma must be transferred in a clean test tube. It must be ensured that the plasma must be free from platelets. The reagents and samples must be brought to 37°C prior to testing wherever recommended. As some of the reagents are tissue extracts, they must be homogenised well before testing.

Following are the effects of preanalytical & analytical variables.

Variables	Effects
Smaller needle bore size (more than 22 SWG)	With smaller needle bore, blood remains in contact with metal surface for longer time which initiates platelet activation and therefore prolonged results. **Syringe needle bore size should be 19-22 SWG**.
High haematocrit (>55%)	False prolonged PT, aPTT, INR (reduced plasma volume → relative citrate excess, requires adjustment)
Tube sequence error (EDTA or heparin contamination)	Invalid / uninterpretable result; clotting inhibition
Short blood draw (less than 2.7 mL blood)	**Anticoagulant to blood ratio exceeds 1:9**, more citrate in sample consumes calcium in reagent leads to prolonged results
High blood draw (more than 2.7 mL blood)	Chelating activity of citrate will not be sufficient to bind calcium present in the sample, will lead to formation of clot therefore prolonged results
Failure to gently mix specimen 3-4 times immediately after collection	Blood clot form when anticoagulant and blood is not mixed rapidly, which lead to loss of fibrinogen and other factors and therefore prolonged results
Excessive and vigorous sample mixing	Haemolysis and platelet activation can cause activation of coagulation cascade
Sample storage without cap	Leads to loss of CO_2, which causes pH to increase, leading to prolongation of results
Frequent change in vacuum tubes	Different brands of vacuum tubes have different quality of plastics, sodium citrate conc., and mark labelling, which may have variable effects on test results.
Excess centrifugation	May destroy clotting factors due to heat generated during centrifugation
Under centrifugation	Would lead to presence of platelets in plasma which could cause activation of clotting mechanism, leading to erroneous results
Reagent is not mixed before testing	After few tests, change in ratio of reagent composition e.g. Activator and Cephaloplastin in aPTT or Calcium and Thromboplastin in PT, which leads to prolonged results

REPORTING PROTHTOMBIN TIME

A PT test result can be reported as:

1. *Time in seconds*

2. *As a ratio*
   PT result can be expressed as a Ratio R

   $$\text{Where R} = \frac{\text{Mean of patient plasma PT in seconds}}{\text{PT of MNPT in seconds}}$$

3. *As an index*
   PT result is sometimes expressed as an index

   $$\frac{\text{Control PT}}{\text{Patient PT}} \times 100$$

4. *Percentage Activity*
   Normal control plasma is diluted with physiologic saline. The Prothrombin time of each dilution is determined. A calibration time of PT against each dilution is plotted on log/log graph. The patient clotting activity can be interpolated by the PT value on the graph.

WHY IS STANDARDISATION OF PT RESULTS ESSENTIAL?

To bring uniformity in monitoring oral anticoagulant therapy. Thromboplastins based on their tissue origin and method of preparation vary markedly in their responsiveness to depression of vitamin K dependant factors and PIVKA's. Historically the PT results were reported in "seconds", or as percentage of coagulant activity or as ratio (PTR). Physicians use sufficient oral anticoagulant drugs to maintain a PTR at 1.5 - 2.0 the median value of the normal range. The problem with this approach is that it fails to take into account the variation in sensitivity or "responsiveness" of different PT reagents.

Prothrombin ratio (PTR)

For example.

For a given reduction in the plasma levels of clotting factors, more responsive PT reagents (A) results in a greater prolongation of PT in seconds as compared to an unresponsive PT reagent (B). Practically this translates into different PT times or ratios for the same patient sample, when tested using reagent A or B

Laboratory	Reagent	MNPT	Test	PTR	Therapeutic ratio
X	A	12.0	30	2.5	1.5 – 2.0
Y	B	12.0	18	1.5	1.5 – 2.0

This patient appears over anticoagulated when tested with reagent (A) and just about adequately anticoagulated when tested with reagent (B).

Percentage activity

Since the dilution of normal plasma (FNP) used for preparing the dilution curve, do not contain PIVKA's and thromboplastins vary in their sensitivity to PIVKA's this method of reporting results is not reliable. Therefore, the PT results reported using different reagents are not interchangeable between laboratories when the older system such as seconds, ratios or percentage activity are used to report PT results. A physician decides on the dose of anticoagulant to be administered based on the PT result. The physician is highly confused by the various methods of reporting results and is also unaware of which thromboplastin reagent is used by the laboratory and the effects of different types of thromboplastins on the PT results. Optimal dosage of oral anticoagulants is extremely important because if unnecessarily high doses of anticoagulants is administered the patient is being exposed to an unnecessary risk of bleeding. Low doses of anticoagulants lead to the risk of thrombosis. Since oral anticoagulant therapy is administered based on the PT results, it is extremely essential to standardize the reporting of PT test results. In view of the above discussed variations in reagent sensitivities, an attempt to standardize the PT results was initiated internationally. By mid 1970s, a few regulatory and advisory groups such as WHO (World Health Organization), ICTH (International Committee for Thrombosis and Haemostasis) and ICHS (International Committee for Standardization in Haematology) started work towards standardization of PT for monitoring oral anticoagulant therapy leading to the birth of INR system. The first IRP (International Reference Preparation) was developed by W.H.O. This primary reference reagent served as a standard for calibrating responsiveness/sensitivity of commercial thromboplastin reagents. Later various IRPs were made from thromboplastins derived from various animal tissues in use for manufacture of commercial thromboplastin reagents. Subsequently after the stocks of primary IRP's were exhausted the secondary IRP's have been made available.

Basis of calibration

The commercial preparations of thromboplastin (test) and the reference thromboplastin (IRP) of the same tissue origin are tested on the same plasma samples (which include normal plasmas, plasmas of donors on OAC in the stable phase containing PIVKA's). Log of PT of reference thromboplastin is plotted on Y-axis and the log of test PT is plotted on the X-axis respectively on a log/log graph. The slope of the calibration line indicates the sensitivity of the test thromboplastin in comparison to IRP. The slope is referred to as the "ISI" (International Sensitivity Index). The ISI thus indicates the sensitivity of the test thromboplastin in comparison to the reference preparation. When the calibration line has slope = 1.0, the test thromboplastin equates to the IRP in sensitivity and responsiveness. The lower the ISI (close to 1.0) more sensitive is the thromboplastin. This method of standardization of PT reagent sensitivity based on "ISI" was the first step towards eliminating reagent based variability in expression of PT results. Manufacturers assign ISI value for each lot of thromboplastins by WHO recommended methods to assist the laboratorians in calculation of the INR (International Normalized Ratio).

INR method of reporting results

The INR represents the PT ratio which would have been obtained for a particular patient sample as if the WHO reference thromboplastin itself (ISI = 1.0) had been used in the PT determination.

$$\text{INR} = R^{ISI}$$

$$\text{INR} = \left(\frac{\text{Patient PT in seconds}}{\text{Mean of normal range}}\right)^{ISI}$$

A PT ratio is obtained by dividing the patient PT in seconds by the Mean of normal range (MNPT). This ratio is then "normalized" by raising the results to the power of ISI of the PT reagent used. Lower the ISI of the reagent used, closer will be the INR to the observed PT ratio. When the ISI of the PT reagent used is 1.0 the INR = PT Ratio. The WHO along with the international committee on thrombosis has recommended the International Normalized Ratio (INR) as the basis for standardisation of

PT results worldwide. The introduction of INR facilitates application of uniform oral anticoagulant therapy on a worldwide scale, and most problems of intra-laboratory comparison are eliminated.

- The INR system helps alleviate confusion in the interpretation of PT results. Usually, laboratory changes like change in thromboplastin and/ or equipment's could go unnoticed by the attending physicians. The INR remains constant with such changes.
- INR system affords comparison of PT results between laboratories.
- INR system provides a more accurate and convenient means of monitoring patients who travel extensively.
- The INR therapeutic ranges for different clinical conditions are based on international collaborative studies. Use of standardized dosage reduces the risk of thrombotic episodes or secondary bleeding.

Factors Influencing the INR have been discussed earlier.

Limitations of INR

Although the ISI corrects for major differences in PT results between test systems (reagent/coagulometer combinations), persistent INR disagreement between results with different test systems is frequently observed. INR are based on an average result over a range of multiple patient samples, but single plasmas may still show marked INR differences when tested in parallel with different reagents. INR, although approximations, nevertheless, quantify and standardize the coumarin clotting defect better than simple PT ratios. Differences in INR between different laboratories on the same test samples (see **Table 1**) should not be regarded as an indictment of the WHO system, but as a demonstration of local variables in PT testing which were unrecognized before INR introduction.

Table 1 : Causes of INR differences

Incorrect prothrombin time ratios due to:
Pre-test variables: (sampling and blood collection problems)
Trisodium-citrate concentration, storage time or temperature, evacuated tubes, inadequate sample, variations in manual technique

Incorrect normal values from:
Non-use of MNPT, error in MNPT due to
(a) unrepresentative selection; (b) technical faults (see above);
(c) non-use of geometric mean
Incorrect ISI of local thromboplastin reagent/test system from:
Incorrect ISI provided by the manufacturer
Incorrect choice of IRP
Poor distribution of coumarin test samples
Inadequate numbers of test samples
Incorrect transformation of ratios to INR
Error in ISI calculation
Drift of ISI
Uncorrected instrument (coagulometer) effects
Lupus anticoagulants
Early days of warfarin therapy
Results over 4.5 INR
852 L. Poller

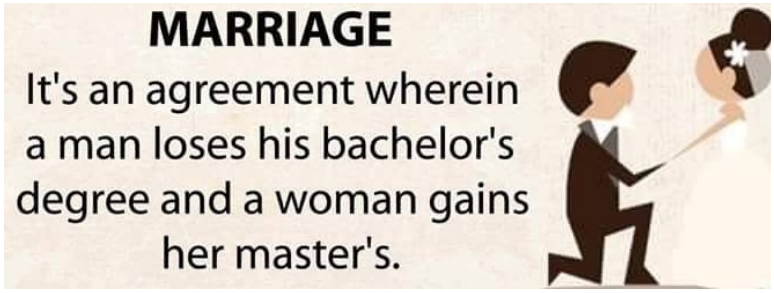
Coagulometers (automated or semiautomated instruments) have replaced the original manual PT technique in most countries. Several reports in recent years have shown that coagulometers have marked and unpredictable effects on INR. It has also been shown that low-ISI human thromboplastins are more affected by coagulometers than high-ISI rabbit preparations. This is largely due with the former to disproportionate shortening of the normal PT with alteration of the prothrombin ratio on which the INR depends. To correct this, local system (thromboplastin/coagulometer combination) ISI have been recommended which reduce the error although they do not fully correct the INR, largely due to the differences between individual instruments of the same make. Apart from these turbid, icteric, lipemic and grossly haemolysed samples may generate erroneous results. Clotting times of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and the time lag between the specimen collected and last dose. The PT may be shortened during acute inflammatory conditions which are accompanied by increase in fibrinogen levels and by agents such as antihistamines, butobarbital, phenobarbital, caffeine, oral contraceptives, and Vitamin K. The PT may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate, erythromycin, ethanol, tetracycline, aspirin, and anticoagulants such as warfarin and heparin.
Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

Quality Assurance as the Ultimate Troubleshooting Tool

At its core, robust quality assurance acts as the primary troubleshooting mechanism in haemostasis testing. By rigorously following IQC procedures, participating in EQA, ensuring proper staff training and competency, and maintaining meticulous documentation, laboratories can pre-emptively mitigate most sources of error. This systematic approach to quality guarantees that the intricate symphony of blood clotting is accurately measured and interpreted, leading to optimal patient outcomes and fostering continued interest in the fascinating world of haemostasis. The EQAS, operated by CMC Vellore is the most easily accessible and widely used external quality assessment scheme for coagulation parameters in our country.

BOUQUET

In Lighter Vein



Nokia falls =ground breaks

BlackBerry falls=screen breaks

Samsung falls= explodes

IPhone falls =owner dies

Wisdom Whispers

"Perfection is achieved, not when there is nothing more to add, but when there is nothing left to take away."

"Just stick with it. What seems so hard now will one day be your warmup."

"Starve your distractions, feed your focus."

"If you don't sacrifice for what you want, what you want becomes the sacrifice."

Brain Teasers

1. **What is the primary function of platelets in blood coagulation?**
   a) Transport oxygen
   b) Initiate clot formation
   c) Fight infections
   d) Carry nutrients

2. **Which plasma protein is converted into fibrin to form the mesh of a blood clot?**
   a) Albumin
   b) Globulin
   c) Fibrinogen
   d) Hemoglobin

3) **CHECK WHICH FOUR FACTORS ARE VITAMIN K DEPENDENT**
   a) II, VII, IX AND X
   b) III, V, 0
   c) XI, XII, XIII
   d) I, XIII,0, V

4) **WHAT TUBE IS USED FOR THE PROTHROMBIN TIME TEST**
   a) Light blue
   b) Yellow
   c) Pink
   d) Brown

ANSWER: 1.B, 2.C, 3.A, 4.A

EMPOWERING EVERY LAB TO PERFORM COAGULATION ASSAYS

Coagulation Range	Salient Feature
Uniplastin	• Liquid stable, Sensitive , Rabbit Brain Calcified Thromboplastin reagent for the Prothrombin Time • Responsive to F VII • Calibrated against WHO reference thromboplastin RBT 90 • Uniformity in INR-Most widely used PT reagent nationally • ISI value synergised on Hemostar XF coagulometer
Liquicelin-E	• 3 in 1 system for aPTT • Sensitive to heparin • Sensitive to LA • Responsive to factor VIII and Factor IX deficiencies
Fibroquant	• Reagent for quantitative estimation of Fibrinogen • 20 Test pack with 30 days reconstituted stability
LADS	• dRVVT for screening and confirmation of Lupus anticoagulants • LADS : 10T • LA Screen : 5T • LA Confirm : 5T
Tulip XL FDP	• Latex Agglutination Slide Test for D-dimer, a marker of Invivo thrombosis • To detect DIC, DVT, PE and Preeclampsia
Profact (3.2% buffered tri-sodium citrate)	• Arrests shift in pH due to the release of carbon dioxide (CO_2) during centifugation • Well preserves labile Factor-V and Factor-VIII leading accurate results of clot based assays
CONTROL PLASMAS (Suitable for PT, aPTT, TT and Fibrinogen assays)	• PLASMATROL H I : Level I Control Plasma • PLASMATROL H II : Level II Control Plasma • PLASMATROL H III : Level III Control Plasma • PLASMATROL R Reference Plasma
Hemostar XF	• Indigenized Haemostasis Analyzer • Optomechanical working principle • 50 programmable assay locations • Synerzised with Tulip's coagulation reagents • 2 Models available

Hemostar XF 1.0

Hemostar XF 2.0

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