Practical - Hemostasis

Purpose: to maintain blood in a fluid, clot free state in normal vessel and to stop bleeding at the site of injury

Stages/Sequence of hemostasis :
1. **Primary hemostasis** :
   - end point = platelet plug/clot
   - takes 3-5 min
   - Structures involved:
     a. Vascular wall- vasoconstriction of damaged vessel, vasodilatation of collateral vessels; endothelial cells
     b. Platelets:
       - Activation of platelets via stimulators such as thrombin
       - Adhesion of platelets to subendothelium via interaction between GPIb and von Willebrand Factor (VWF)
       - Release of platelet granule products in order to recruit more platelets to the injured site
       - Aggregation of platelets via interaction between GPIIb/IIIa (αIIbβ3) and fibrinogen to form the initial plug
     c. Hemodynamic factors
2. **Secondary hemostasis**: 2 end points - consolidated fibrin-platelet clot (thrombus)
                                          - clot retraction
   - takes 5-10 minutes
   - Coagulation proteins work together to generate thrombin
   - Thrombin converts fibrinogen to fibrin
   - Fibrin consolidates the platelet plug produced in primary hemostasis → a thrombus (secondary hemostatic plug)
   - Factors involved:
     - Plasma proteins – clotting factors
     - Calcium
3. **Fibrinolysis**: end point = plasmin dissolves the thrombus and the establishment of a normal circulation

Exploring the primary hemostasis:
   1) **Bleeding time**
   2) **Capillary fragility test**
   3) **Platelet count**

1) **Bleeding time** – test the integrity of primary hemostasis in terms of interaction between blood vessel and platelets. There are two commonly used methods to perform this test: in the Ivy method, a blood pressure cuff is placed on the upper arm and inflated to 40 mm Hg, then a lancet or scalpel blade is used to make a stab wound on the underside of the forearm; for the Duke method a prick on the ear lobe is used. The stab wound is swabbed each 30 seconds until the bleeding stops. The time it takes for the bleeding to stop is called bleeding time.
Normal value: 2-4 min. Abnormal: Bleeding time is longer when the normal function of platelets is impaired, or there are a lower-than-normal number of platelets in the blood (severe thrombocytopenia, platelet dysfunction, vascular defects, Von Willebrand's disease).

2) **Capillary fragility test (Rumpel – Leede test, positive pressure test):** generally evaluate the fragility of the capillary walls, or the deficiency of blood platelets (thrombocytopenia).A blood pressure cuff is used to create pressure to the arm, so that the venous return is stopped. The increase in hydrostatic pressure will cause capillary rupture and will produce petechiae (small, purplish areas on the skin). After 5 minutes the pressure is relieved and the petechiae are counted in a 2 inch area and classified by a scoring system as follows:
Normal: +   3-10
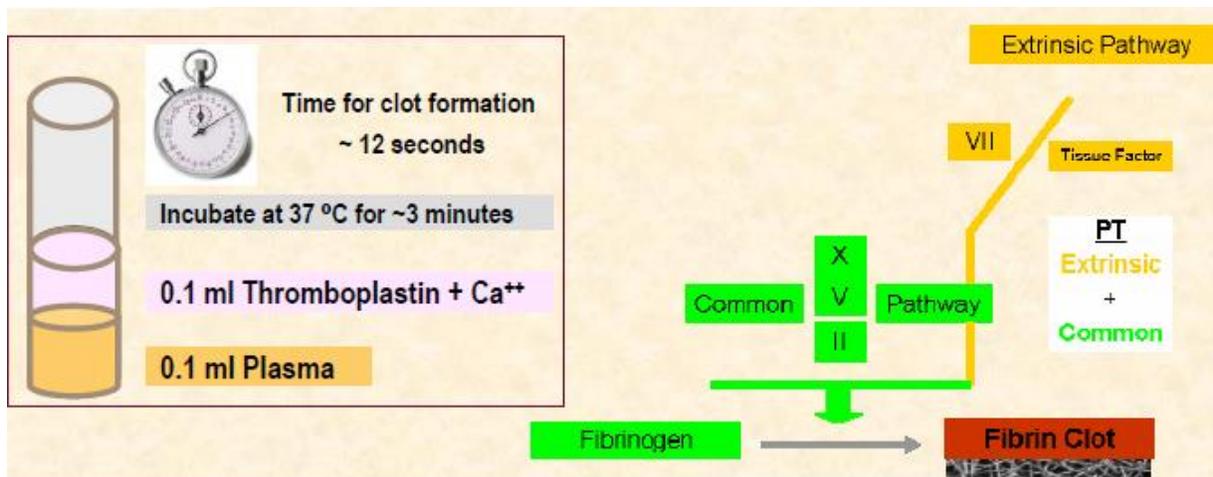   + + 11-20;   +++   21-50;   ++++   >50

3) **Platelet count :** It is done in the same manner as the white cell count, except for the counting itself which is done as for red blood cells. Spontaneous bleeding is possible if there are less than 20,000/μL. Count does not have anything to do with functionality of the platelets.
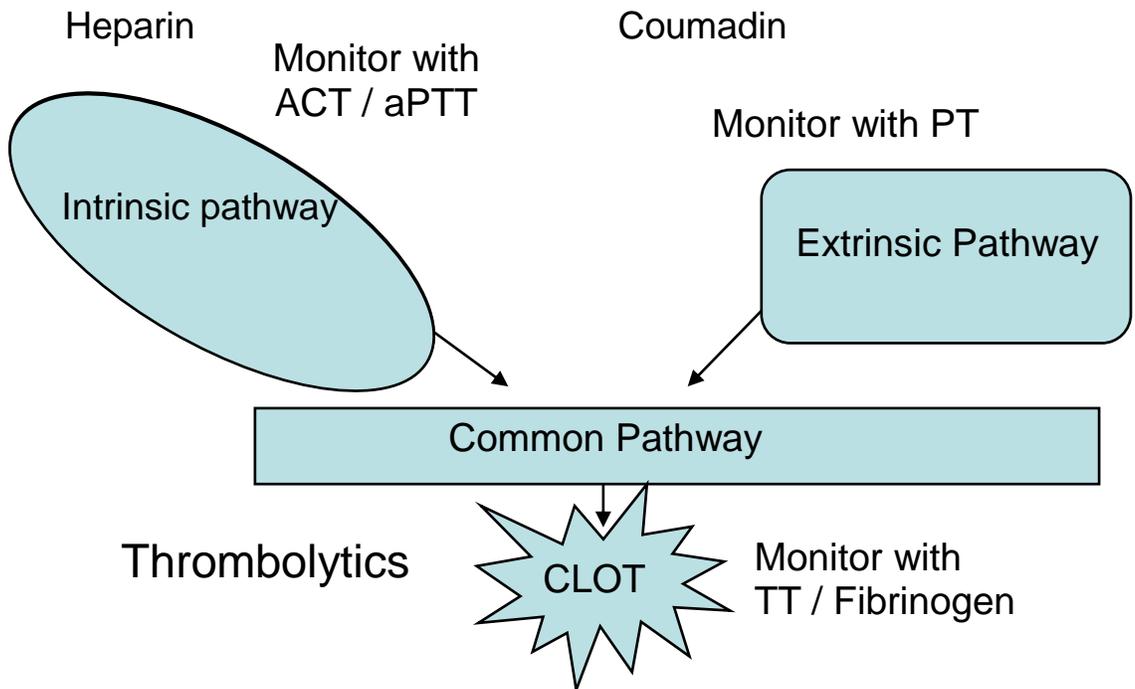   Normal value: 150.000 – 400.000/μl

Exploring the secondary hemostasis:

1) Clotting time test

2) Howell Gram test

3) Partial thromboplastin time (PTT)

4) Prothrombin time (Quick method-PT/QT)

1) **Clotting time test** explores the *intrinsic pathway* by measuring the time needed for a sample of blood to clot on a glass slide.
   Normal values= 6-8 minutes

2) **Howell Gram test** explores the *extrinsic pathway*; for this we add $CaCl_2$ to citrate plasma (with high amount of platelets) and start monitoring the time needed to clot.
   Normal values= 60 -120s

3) **Partial thromboplastin time (PTT)** Tests the intrinsic and common pathway. To citrate plasma (with low amount of platelets) we add $Ca Cl_2$, kefalin and start monitoring the time needed to clot. It is affected by heparin. Can be effected by coumadin at supra-therapeutic levels due to effects on the common pathway.
   Normal values =60-110s

4) **Prothrombin time (Quick method-PT/QT)** Tests the extrinsic and common pathways; to citrate plasma (with high amount of platelets) we add $Ca Cl_2$, thromboplastin and start monitoring the time needed to clot. Elevated in patients with liver disease and abnormalities in vitamin K sensitive factors. The International Normalized Ratio (INR) is used to compensate for differences in thromboplastin reagents used for Coumadin therapy.
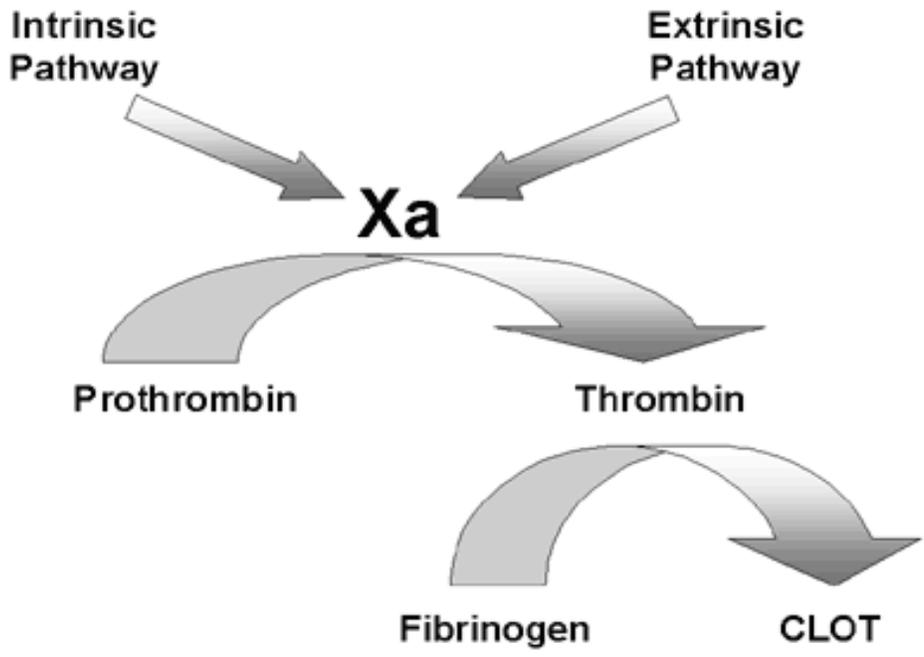   **Normal values = 12-15s**

<u>Coagulation monitoring</u>

Heparin   Coumadin

Monitor with
ACT / aPTT

Monitor with PT

Intrinsic pathway

Extrinsic Pathway

Common Pathway

Thrombolytics   CLOT   Monitor with
TT / Fibrinogen

LABORATORY TEST	COMPONENTS MEASURED	NORMAL VALUES
Bleeding time	platelet function vascular integrity	2 - 4 mins
PT	I, II, V, VII, IX, X	12 - 15 sec
PTT	I, II, V, VIII, IX, X, XI, XII	60 -110 sec

Intrinsic
Pathway

Extrinsic
Pathway

Xa

Prothrombin   Thrombin

Fibrinogen   CLOT

Intrinsic Pathway

HMWK

XII → XIIa
K ← PK

HMWK

XIIa

HMWK

XI → XIa

Ca++

IX → IXa

Extrinsic Pathway

VIIa

VIIIa
Ca++
PL

X

TF
Ca++

X

Common Pathway

Xa

Va
Ca++
PL

II → IIa

Fibrinogen → Fibrin

XIIIa

Fibrin (crosslinked)

■ (Zymogen)	Zymogen
● (Protease)	Protease
TF	Tissue factor
PL	Phospholipids
PK	Prekallikrein
K	Kallikrein
HMWK	HMW Kininogen