In the name of God

Laboratory Evaluation of Haemostatic Disorders

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IBTO Coagulation Lab
Laboratory Evaluation of Haemostatic Disorders

Coagulation lab has a vital role in the diagnosis and management of patients with familial and acquired hemorrhagic and thrombotic disorders
Important diagnostic and therapeutic decisions are based on the results of coagulation tests.
No single test is suitable for laboratory evaluation of blood coagulation.
Coagulation phase:
- PT
- aPTT
- TT
- Fibrinogen
- Mixing Studies

Vascular & PLT phases:
- BT
- PLT Count
- PFA-100
Specific Tests

- Factor Assays (VIII, IX)
- Inhibitor Screening
- Platelet Aggregometry
- F XIII assay
Bleeding Time:

- Ivy Method
- Template Method
Bleeding Time:

- Sensitivity of Ivy’s method:
  100% for GT
  85% for BSS
  68% for PLT secretion defect
  63% for vWD

Haemophilia (2008), 14(Suppl. 3), 93-103
Recommendation
(UKHcDO 2004)

BT **DOES NOT** have a role of screening test for vWD.
Preliminary Investigations

**PFA-100 (Closure Time)**

Simulates primary hemostasis in the high shear stress environment.

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Preliminary Investigations

InVivo Haemostasis

- Endothelium
- Collagen
- vWF
- Platelet
- Erythrocyte

PFA-100®

- 147µm Aperture
- Membrane
- Collagen
- FLOW
  - Shear Rate
  - 5000 - 6000 s⁻¹
- Capillary
  - 200 µm

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BT and PFA 100

“have low sensitivity in mild primary”

“haemostatic defect”

Haemophilia (2008), 14(Suppl. 3), 93-103
Prothrombin Time

Tissue factor induced coagulation time

• Since 1935, Quick
• Most common coagulation test
• Originally thought to measure Prothrombin
• Depend on I, II, V, X and VII
• Monitoring anti vitamin K therapy (AVK)
Prothrombin Time

End point

Manual technique:

- Difficult standardize and not widely used
- Duplicate
- Test should be repeated IF duplicate discrepancy

>5% from mean WHO 98
>10% from mean NCCLS 2004
Prothrombin Time

*Standardized manual tilt tube technique*

**3 Tilts through 90° every 5 seconds**

Tilt 3 times every 5 seconds

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WHO/LAB/98.3  
WFH lab science committee
Prothrombin Time

**Expression of results**

- Seconds along with *normal reference interval*
- INR
- Ratio
Prothrombin Time

Reference Interval

Should always be established by each lab.
(literature and manufacture’s information should only be used as a guide)

Should be verified with any change

OR

At least **ONCE A YEAR**
Prothrombin Time

• **Reference interval**

  • At least 20 healthy adults (50-100 healthy subject is recommended)

  • Men and women (not pregnant, not taking OCP)

  • Wide range of age (20-80 yrs)
In 1983 for correction of this variation in responsiveness of reagents, WHO introduced INR system of reporting PT results

\[\text{INR} = (\text{PT Ratio})\text{ISI}\]

INR is a calculated value dependent International Sensitivity Index

\text{ISI}: \text{Responsiveness of the PT reagent.\nDetermined by comparing the commercial PT reagent to a WHO standard.}
Thromboplastin

**Tissue Factor** (Specific Protein for initiating extrinsic system of Coagulation) +

**Phospholipid** (necessary for surface assembly of the coagulation complexes) +

**Calcium ions** (for correct orientation and binding of complexes)

Tissue extraction  Human-Derived
Tissue Culture   Animal Derived
Genetic technology Genetic technologies

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Prothrombin Time

International sensitivity index (ISI)

- More sensitive reagents have lower ISI

- PT based on low ISI reagent: More precise and wider therapeutic interval
Prothrombin Time

International sensitivity index (ISI)

- CAP/WHO 1998: 0.9-1.7
- NCCLS 2004 (CLSI): ISI<1.5
Patient samples have shorter clotting times when less responsive reagents are used and longer CT With more responsive reagents.
Activated partial thromboplastin time

Contact Activation

Intrinsic

XII
Pre-K
XI
HMWK
IX
PL
VIII

Extrinsic

Partial Thromboplastin Time

Common

PL
X
V
Prothrombin
Fibrinogen

Fibrin

Prothrombine Time

Stypven Time

Thrombin Time

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**aPTT**

**activator**
- Kolin
- Celite
- Ellagic acid
- Silica

**Partial Thromboplastin**
Lack the apoprotein of the complete tissue thromboplastin (animal tissue/vegetable source)
aPTT

- Screening for intrinsic clotting defects
- Detection of lupus anticoagulant
- Monitoring of heparin administration
aPTT reagent/instrument combination

Coagulation defect

- Sensitive to 0.35 - 0.40 u/ml deficiency of F VIII, IX, XI (NCCLS 2004 ≤ 30%)

Inhibitors

- Responsiveness to therapeutic range of UFH (0.3-0.7 iU/ml)
- Sensitive to LAC
aPTT

- Total concentration of PL and FA
- Amount of activator
- Length of incubation
Reporting of results

Seconds along with reference interval

RI = MNaPTT ± 2 SD
Deficiency or circulating anticoagulant

aPTT

PP

NP

Mix PP + NP
aPTT

Deficiency or circulating anticoagulant

PP

NP

Mix PP + NP

37°-120° Incubation

aPTT
<table>
<thead>
<tr>
<th>Table 122-1 Laboratory Evaluation of Abnormalities of Coagulation Protein: aPTT and PT Screening Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long aPTT, Normal PT</strong></td>
</tr>
<tr>
<td><strong>Associated with Bleeding</strong></td>
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<tr>
<td>Factor VIII (male only)</td>
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<tr>
<td>Factor IX (male only)</td>
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<tr>
<td>Factor XI</td>
</tr>
<tr>
<td>Antibodies to factor VIII</td>
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<tr>
<td>Amyloid adsorbed factor IX</td>
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<td></td>
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<td></td>
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<tr>
<td>Factor XII</td>
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<tr>
<td>Prekallikrein</td>
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<tr>
<td>High-molecular-weight kininogen</td>
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<tr>
<td>Lupus anticoagulant</td>
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<tr>
<td><strong>Single Factor Deficient</strong></td>
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<tr>
<td>Perform specific inhibitor assay</td>
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<tr>
<td>Bleeding Disorders in Which the Results of Primary Screening Tests May Be Normal</td>
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<tr>
<td>--------------------------------------------------------------------------------</td>
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<tr>
<td>von Willebrand disease</td>
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<td>Mild inherited coagulation disorders, particularly factor XI deficiency</td>
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<td>Heterozygous carriers of inherited coagulation disorders</td>
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<tr>
<td>Factor XIII (fibrin-stabilizing factor) deficiency</td>
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<tr>
<td>Some forms of dysfibrinogenemia</td>
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<td>Disordered platelet function, particularly deficient release reaction; Scott syndrome</td>
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<tr>
<td>Hereditary hemorrhagic telangiectasia</td>
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<td>Allergic and other vascular purpuras</td>
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<tr>
<td>$\alpha_2$-plasmin inhibitor deficiency</td>
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<tr>
<td>Elevated levels of plasminogen activator</td>
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</tbody>
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Thank You For Your Attention