REVIEW OF COAGULATION

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LEARNING OBJECTIVES

1. Describe the specimen type used for coagulation studies
2. Understand the classic coagulation pathways.
3. Describe the set-up of the PT, PTT, fibrinogen and thrombin time assays
4. Discuss Factor activity Assays
5. Revise platelet physiology and the role of platelets in hemostasis
6. Describe and understand methods of platelet function testing in the clinical laboratory
Specimen for Hemostasis Studies

- Whole blood drawn into a tube containing liquid 3.2% sodium citrate (109 mM) at a ratio of 9 parts blood and 1 part anticoagulant.
Specimen for *Coagulation* studies

1) Citrated, platelet-poor plasma
2) Platelet count < 10,000 mm$^3$
3) Clotting is initiated by adding calcium chloride back to specimen – to overcome effects of citrate
CALCIUM CHLORIDE

Phospholipid

37°C

COAGULATION
**Tissue damage, release of tissue factor**

**Extrinsic Pathway**
- Tissue Factor, Factor VII

**Intrinsic Pathway**
- Factor XII, XI, IX, VIII

**Common Pathway**
- Factor V, X, Prothrombin (Factor II)

**THROMBIN**

**Fibrinogen**

**Fibrin**

*Change in charge/surface properties of vessel lining*
Extrinsic Pathway
PROTHROMBIN TIME (PT)

Intrinsic Pathway
aPTT

Common Pathway
PT, aPTT

THROMBIN

Fibrinogen
Thrombin Time

Fibrin
How long can we keep the specimen?

- **PT** - 24 hours and must be at room temperature
- **PTT** - only 4 hours and only if refrigerated at 4°C
- Frozen plasma → months
Add **Thromboplastin**, calcium chloride, then start timer.
International Normalized Ratio (INR)

- \( \text{INR} = \left( \frac{\text{PT}_{\text{specimen}}}{\text{MNPT}} \right)^{\text{ISI}} \)

\( \text{ISI} = \text{International Sensitivity Index; } \)

\( \text{MNPT} = \text{Mean Normal Prothrombin Time: Geometric Mean of Population PT} \)
Activated Partial Thromboplastin Time

Add Phospholipid & Silica

Incubate 4 min

Add calcium chloride
Start timer

Clot formation
Stop Timer

aPTT
Add high concentration of thrombin

Time in seconds

Stop Timer

Clot formation

Diluted Plasma

Plasma

Fibrinogen mg/dL

Fibrinogen
Thrombin Time

Add Low concentration of thrombin

PLASMA (UNDILUTED)

Time in seconds

Clot formation
Stop Timer
• **PT**
  – Sensitive to FVII & X, V, II
  – Surrogate for warfarin therapy.
  – Sensitive to Vitamin K Deficiency

• **PTT**
  – Sensitive to FXII, XI, IX, VIII & X, V, II
  – Surrogate for unfractionated heparin therapy

• **TT**
  – Very sensitive to UFH, Direct thrombin inhibitors
  – Sensitive to low fibrinogen.
**Mixing Study**

- If PT or PTT are prolonged, perform a "1:1 Mixing Study"

1. "Correction"
2. "No correction"
Examples

Range

• aPTT is 60 seconds

Reference

23 – 38 s

Corrected

• 1:1 Mix is 35 seconds

• 1:1 Mix is 49 seconds

NOT CORRECTED
Measurement of Clotting in the Laboratory
1) Photo-optical detection.

2) Mechanical.

3) Chromogenic.
4) **Electrochemical:**

- Utilizes a synthetic substrate for thrombin.
- When this substrate is cleaved by thrombin, an electrically charged molecule is released.
- Detected by current flow ("amperometric").
- Used by iSTAT POC coagulation testing.
Photo-optical Detection.

End of clotting

Transmittance of light

PT & PTT

Time
Viscosity-based Detection System
Factor Activity Assays
Factor Activity

In order to do a Factor assay you need:-

• A standard or calibrator with an exact known or defined Factor VIII (or IX etc.) factor activity concentration

• A commercial factor-depleted plasma – lacking the exact factor you need to measure
Patient Specimen

VIII-Deficient plasma
Mix Together

The FVIII in the subsequent PTT assay is derived entirely from the patient.
ONE-STAGE FACTOR ASSAYS

STEP 1 – SPECIMEN DILUTION

1 part specimen + 9 parts diluent = 1:10 dilution
STEP 1 – SPECIMEN DILUTION

1:10 dilution

1:20 dilution

1:40 dilution
STEP 2 – MIXING ASSAY

Diluted specimen

Single-factor deficient plasma
STEP 3 – Initiate clotting

Perform PT or PTT
One-stage factor assays

- PTT: Factors XII, XI, IX, VIII
- PT: Factors VII, X, II, V
- A calibration curve is created, plotting TIME (seconds) as a function of FACTOR ACTIVITY.
- By definition, the 1:10 dilution has the activity defined by the manufacturers.
Platelets
• Normal Platelet count
  150- 450,000/µL
• Lifespan: 8 - 10 days
• Mean platelet volume:
  7- 9 fL
• Mean platelet diameter: 2-3 µM
Resting Platelet - Disc Shaped

Spherical Activated Platelet

Resting  
Activated
Adhesion – “plugging the hole”
vWF anchors platelets to subendothelial collagen
Platelets release various agents which produce platelet aggregation.
3) Aggregation

Formation of hemostatic platelet plug
Thromboxane $A_2$
Platelets provide certain phospholipids that are essential for the coagulation process.
ACTIVATED PLATELET

X

IXa
VIIla

Xa
Va

PROTHROMBIN

THROMBIN
PLATELET FUNCTION TESTING
Tests of Platelet function

• 1) **Bleeding time**: a controlled incision is produced in the skin and bleeding commences. The time taken until bleeding ceases is measured.

• 2) **Platelet function analyser or PFA**.

• 3) **Platelet Aggregometer**.
PFA-100

Uses 0.8 mL of whole blood (Citrated; blue top)
“Closure time” is the assay end-point.

Collagen and epinephrine or ADP.

150 micron aperture

200 micron capillary
## PFA-100 Closure Times

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<tr>
<th></th>
<th>NSAID</th>
<th>vWD</th>
<th>Inherited Platelet Disorders</th>
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<tr>
<td><strong>EPINEPHRINE</strong></td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>(1&lt;sup&gt;ST&lt;/sup&gt; Cartridge)</td>
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<td><strong>ADP</strong></td>
<td>Normal</td>
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<tr>
<td>(2&lt;sup&gt;ND&lt;/sup&gt; Cartridge)</td>
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Anemia and/or Thrombocytopenia → ↑↑↑ closure times!
Platelet Aggregometer
Add agonist

Light Source

Cuvette

Transmitted Light

Platelet-Rich plasma

Light Detector

Professional Practice in Clinical Chemistry
An AC voltage in the millivolt range is established across 2 electrodes.
↑↑ Resistance (ohms)
End
Self-Assessment Question #1

A specimen is drawn from a patient with an unexplained bleeding tendency. The PT is entirely within normal limits, but the PTT is very prolonged. A PTT 1:1 “mix” corrects completely.

a) the patient has a deficiency of factor VII.

b) **the patient has a deficiency of factor VIII or IX.**

c) the prolonged PTT reflects a platelet abnormality.

d) the patient lacks fibrinogen.

e) The patient is deficient in factor II or V or X.

Explanation: The corrected 1:1 mix indicates a factor deficiency (rather than an inhibitor) and one that affects only the PTT and not the PT. A factor VII deficiency would prolong the PT only, while a lack of II, V, X or fibrinogen would affect both the PT and PTT.
Self-Assessment Question #2

A specimen for PT & PTT (sodium citrate is the additive) is noted to be less than half full with the required amount of blood. You would advise the medical technologist to:

a) run the specimen anyway and report the result.

b) run the specimen and divide the result by 2.

c) Incubate the specimen for twice as long.

d) **Reject the specimen and request a redraw.**

e) Reject the specimen and request an EDTA tube.

Explanation: Specimens for coagulation testing cannot be analyzed if the tube is inadequately filled. The specimen will be overcitrated and even overdiluted. Incubating specimens for longer or re-calculating cannot solve the problem.
Self-Assessment Question #3

Which of the following does *NOT* describe a known action/function of platelets:

a) adhesion and wound plugging

b) aggregation

c) *synthesis of factor VIII*

d) release of ADP, serotonin and thromboxane

e) providing an active surface for coagulation factors

Explanation: Platelets are not known to synthesize factor VIII. All the other functions are associated with platelets. Platelets do contain factor V and von Willebrand factor (vWF).