I. Tests for Secondary Hemostasis/Coagulation

A. Thrombin Time (TT) or Thrombin clotting time (TCT)

Mainly a qualitative test but is affected by quantity.

Principle: The thrombin clotting time is proportional to the fibrinogen concentration of the plasma.

● Measures the time required for thrombin (reagent) to convert fibrinogen to fibrin.

ONLY fibrinogen (factor I) is measured. ● NOT affected by deficiencies of any factors above fibrinogen since it bypasses ALL reactions (including factor II).

Procedure: Add thrombin reagent to patient citrated plasma and time for clot formation. Compare patient clotting time to normal control and report clotting time in seconds.

\[
\text{excess thrombin (reagent)} \\
\downarrow \\
\text{fibrinogen} \quad \rightarrow \quad \text{fibrin clot (thrombin clotting time)}
\]

Example: TT Control = 14.0 secs
Patient A clotting time = 15.0 secs…normal thrombin time.
Patient B clotting time = 25.0 secs…abnormal thrombin time.

Interpretation: Abnormal/prolonged thrombin times are due to delayed fibrinogen conversion to fibrin which may be caused by:

1. A low/decreased fibrinogen level (concentration <100 mg/dl).
2. Presence of heparin in the patient's plasma since heparin (with AT) neutralizes the thrombin reagent.
   a. Presence of heparin may be due to contamination (line draw with insufficient discard) or the patient is on heparin anticoagulant therapy.
   b. The thrombin time is the most sensitive test for detecting heparin action but the APTT is the most practical test for monitoring heparin therapy.
3. Presence of significant levels of degradation products (which are normally not detectable in circulation).
   a. Fibrin or fibrinogen degradation fragments [Y and D] inhibit the spontaneous fibrin monomer polymerization needed to form a fibrin clot. Therefore, FDP's delay (prolong) the thrombin clotting time.
   b. Degradation fragments [X and E] inhibit the thrombin reagent.
4. Dysfibrinogenemia - functionally abnormal fibrinogen.

NOTE: The thrombin clotting time evaluates both secondary hemostasis AND fibrinolysis.

B. Fibrinogen Level

Principle: Adaptation of the thrombin time. A quantitative technique used to measure the concentration of functional fibrinogen present in the plasma. ● Measures the time required for thrombin (reagent) to convert fibrinogen to fibrin.

Most procedures: The patient citrated plasma is diluted to remove interfering substances (i.e. heparin). Add thrombin reagent to diluted plasma and time for clot formation.

Interpretation: The thrombin clotting time (seconds) is converted to fibrinogen concentration in mg/dl using a standard curve set up with calibration standards of known fibrinogen concentration.
Example: Patient thrombin clotting time of 12.5 seconds → 220 mg/dl. Normal fibrinogen level: 150-450 mg/dl. Fibrinogen controls must be within range to report.

The thrombin clotting time is inversely proportional to the fibrinogen concentration of the plasma (i.e. a high fibrinogen concentration = a short thrombin clotting time).

C. Mixing Study
Principle: ● A mixing study is done to differentiate a factor deficiency from a circulating inhibitor (VIII or IX inhibitor, Lupus inhibitor, heparin…but not coumarin).

Performed when in vitro PT or APTT results are abnormal and patient is on no anticoagulant therapy.

Procedure:
1. Repeat abnormal PT and/or APTT using part patient plasma and part normal pooled plasma = MIXING study (1:1 mix). Normal fresh plasma contains all factors.
2. If the mixing study is now normal, the result “corrected” and indicates the patient has a factor deficiency which was corrected by the addition of normal plasma. (No inhibitor present). A factor assay can be done to quantitate.
3. If the 1:1 mix result does not correct to normal (remains prolonged) this is indicative of an inhibitor.

Significance:
The four causes of non-correction of a mixing study (in order of prevalence) are: heparin, Lupus inhibitor, factor VIII inhibitor, or factor IX inhibitor. To be discussed in greater detail.
D. Factor Assays

Principle: Measures the ability of the patient's plasma to correct PT or APTT results obtained with plasma known to be factor deficient. The % of factor activity is reported.

- Performed to verify a suspected factor deficiency or to monitor the level of a deficient factor. PT and APTT screening tests are performed first (identify cascade defect) AND the mixing study shows correction (an inhibitor is not present).

Method:
1. Perform PT or APTT depending on suspected deficient factor.
   (PTT - VIII, IX, XI, XII; PT - II, V, VII, X)
2. Using known factor deficient plasma + patient plasma → time for clot formation.
3. Look at degree of correction by comparing patient clotting time with normal standard clotting time (using standard curve). Normal standard plasma = 100% activity.
4. Degree of correction = % activity of patient's factor.

Significance:
Normal reference range is 50-150% or ≥ 50% factor activity.
Example: a Factor VIII level of 45% is abnormal.

- Factor activity must be less than about 25-30% to obtain prolonged screening test results...sensitivity of PT/APTT tests.

Normal PT & PTT and factor activity levels

<table>
<thead>
<tr>
<th>AGE: 3M</th>
<th>SEX: F</th>
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<tbody>
<tr>
<td>INTER NORMAL RATIO PTT</td>
<td>1.0</td>
</tr>
<tr>
<td>FACTOR V (5) ASSAY</td>
<td>89</td>
</tr>
<tr>
<td>FACTOR VII (7) ASSAY</td>
<td>100</td>
</tr>
</tbody>
</table>

Child with liver disease

<table>
<thead>
<tr>
<th>AGE: 6M</th>
<th>SEX: M</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT &amp; PTT PROTIME</td>
<td>94.4*</td>
</tr>
<tr>
<td>INTER NORMAL RATIO PTT</td>
<td>&gt;14.0</td>
</tr>
<tr>
<td>FACTOR V (5) ASSAY</td>
<td>16*</td>
</tr>
<tr>
<td>FACTOR VII (7) ASSAY</td>
<td>1*</td>
</tr>
</tbody>
</table>

E. Urea Solubility Test/5 M Urea Test

Principle: Test for detecting Factor XIII deficiency which is the fibrin stabilizing factor...patients with this deficiency cannot form a cross-linked insoluble fibrin clot.

- This is the ONLY test available → screening tests (PT/APTT) DO NOT measure the stable fibrin clot so will be normal when factor XIII is deficient.

Method: 5M urea + patient clot.

Normal - it takes >24 hours for clot to dissolve in 5M urea.
Abnormal - it takes only a few hours for clot to dissolve which indicates a factor XIII deficiency.
II. Tests for Fibrinolysis

As a potent clot lysing enzyme, plasmin can attack the fibrin clot as well as fibrinogen. Plasmin's action on fibrinogen produces fibrinogen degradation products. Plasmin's action on the fibrin clot leads exclusively to the generation of derivatives of cross-linked fibrin containing the **D-dimer** (DXD). In the process of fibrinogen or fibrin degradation, fragments X, Y, D, and E are produced.

The FDP detection and D-dimer tests are quantitative immunoassays that use antibody coated latex particles to detect elevated plasma levels of degradation products. *The D-dimer is the test of choice.*

A. **FDP Detection Test** - specimen used is serum drawn in special **FDP tube**.

Principle: Latex particles coated with polyclonal antibody to D and E fragments will clump in the presence of *either* fibrin OR fibrinogen degradation (split) products.

Interpretation: ● The FDP test detects D and E fragments produced from the action of plasmin on fibrinogen, fibrin, or fibrin monomers.

Normal: <10 ug/mL…negative  Abnormal: >10 ug/mL…positive

Significance: The detection of fibrin or fibrinogen degradation products indicates the presence of excessive fibrinolytic activity (due to uncontrolled plasmin). *Normally, degradation products are undetectable* in circulation.

The presence of degradation products in circulation impairs normal hemostasis: interfere with polymerization of fibrin monomers [Y and D], interfere with platelet aggregation [all] and inhibit thrombin [X and E].

B. **D-dimer test** - specimen is citrated plasma.

Principle: Done to distinguish between the degradation products of fibrin versus fibrinogen. Latex particles coated with a monoclonal antibody specific to the D-dimer fragment from the degradation of cross-linked fibrin will clump in the presence of fibrin degradation products (manual method).

Interpretation: ● The D-dimer test detects **ONLY** cross-linked fibrin degradation products (contain DXD). Fibrinogen degradation products are **NOT** detected.

Normal: D-dimer < 150 ng/mL…negative  [Dimer reporting units need standardization]

Significance: Fibrin derivatives in plasma containing D-dimer are a specific marker of fibrinolysis found in both DIC and thrombotic events. A diagnosis of DIC or PE is unlikely if the D-dimer test is negative. A positive D-dimer indicates that both the clotting and fibrinolytic systems were activated.

C. **Thrombin Time** - prolonged by the presence of significant levels of fibrinogen or fibrin degradation products - already discussed. (TT evaluates both coagulation and fibrinolysis).