Laboratory:
Activated Partial Thromboplastin Time (aPTT)
Skills= 18 Points

Objectives:
The student should be able to:

1. Perform either manual or automated partial thromboplastin times on two (2) controls and one (1) patient within ± 2 SD of known values.
2. Accurately report results with 100% accuracy.
3. Accurately record quality control results with 100 % accuracy.
4. State the reference range for the APTT.
5. State the critical or panic value for the aPTT.
6. Interpret the results of routine APTT testing as abnormal or normal.
7. Explain the clinical significance of the Activated Partial Thromboplastin Time.
8. Recognize the optimal range for patients on therapeutic anticoagulants.
9. Evaluate APTT results and identify possible conditions present.
10. Describe the function(s) of heparin.
11. State the therapeutic range for a person on anticoagulant therapy.
12. State the type of sample used for aPTT testing.
13. Differentiate between unfractionated and low molecular weight heparin.

Materials:

1. 12 x 75 test tube
2. Oxford pipettes 100 µL
3. Pipette tips
4. Calcium chloride reagent, 0.025M
5. Partial thromboplastin : consists of phospholipids and a contact activator
6. Heat block at 37˚C
7. Test tube rack
8. Kimwipes or gauze
9. Stop watch
10. Controls Level I and III (normal and abnormal)
11. Patient citrated plasma specimen (platelet poor)

Sample:
Sodium citrate plasma is used for the Activated Partial Thromboplastin Time procedure. The plasma must be platelet poor. Centrifuged specimens are stable at room temperature for 4 hours. If testing cannot be completed within 4 hours, the plasma should be frozen**.**

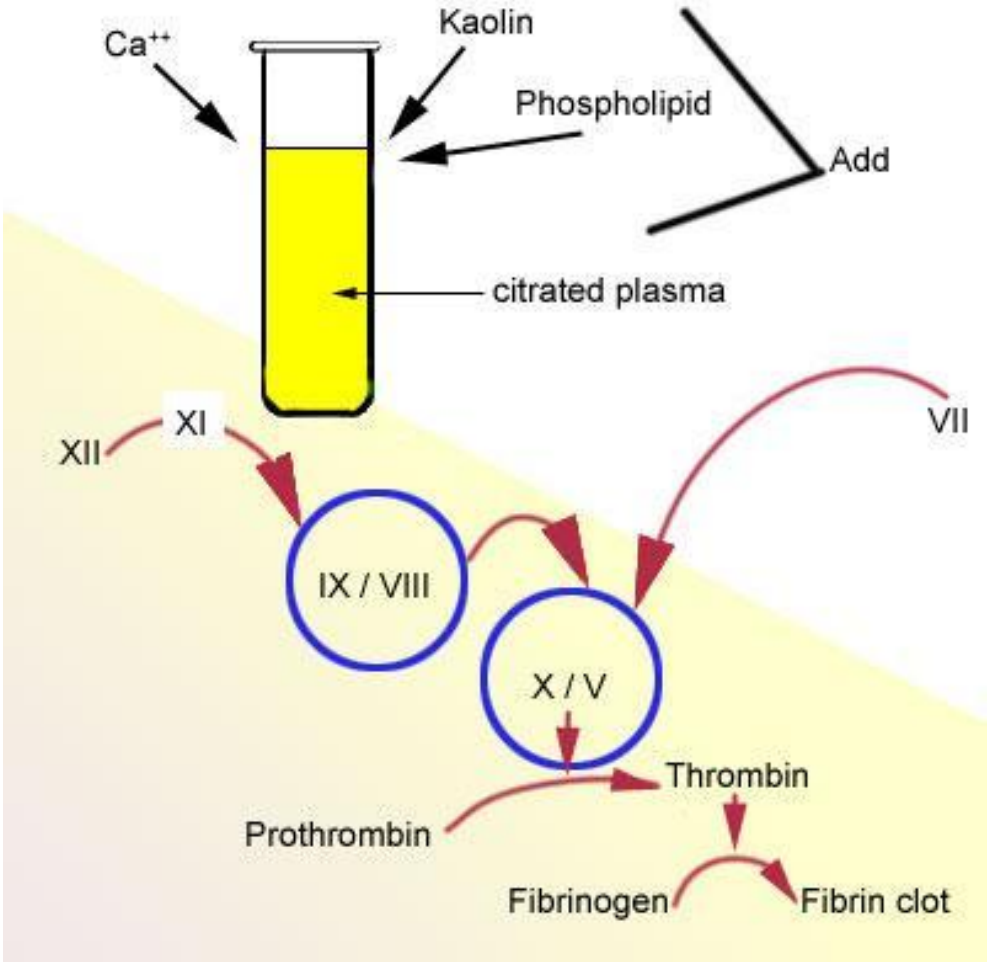
References:
Powers, Lawrence W., Diagnostic Hematology, pp. 481-482.
Brown, B. A., Hematology: Principles and Procedures, 5th edition, Chapter 5.

Principle:

The activated partial thromboplastin time (aPTT) is also known as the partial thromboplastin time (PTT). The PTT result (in seconds) is the time required for plasma to clot when maximal surface contact activation, optimal phospholipid, and calcium concentration are provided. Calcium is removed from the plasma of the test specimen during collection in a sodium citrate tube. Because calcium is a coagulation co-factor clotting is inhibited by calcium chelation. The specimen is centrifuged to obtain platelet-poor plasma (PPP) with all available coagulation factors except for calcium and platelets.

Under carefully controlled conditions and with properly prepared reagents, calcium, partial thromboplastin and an activator or agonist (such as kaolin, celite or ellagic acid) are added to the plasma to be tested. The partial thromboplastin reagent stimulates activated platelet surfaces by providing a phospholipid surface on which enzymatic reactions can occur. The activator (agonist) provides a negatively charged surface for activation of factor XII. After a specific incubation time of citrated plasma with the APTT reagent which allows for optimum activation of contact factors, calcium chloride is added. The calcium chloride activates the clotting cascade. The time required for the plasma to clot is the activated partial thromboplastin time. Clot formation can be detected by optical or electromechanical methods, using manual, semi-automated or automated devices.

PTT PRINCIPLE



Forms of Heparin

Unfractionated Heparin (UFH)

Unfractionated heparin is given to people to prevent blood clotting, when a rapid effect is desired. UFH is given through IV following acute thrombotic conditions such as Myocardial infarction or venous thromboembolism (VTE). Possible side effects of heparin use include Heparin Induced Thrombocytopenia (HIT) and Osteopenia.

Due to high patient to patient variability, frequent monitoring is required.

Mechanism of action-
UFH binds to inactive antithrombin (AT) which results in a conformational change of antithrombin making it "active". Once in its active form, antithrombin can bind Factor Xa to stop the conversion of prothrombin to thrombin, and/or directly inhibit the actions of thrombin.
In either case, fibrin formation is restricted, and clotting is prevented.

In general the therapeutic range for the PTT of a patient on heparin therapy should be **1½ to 2½** times normal.

Low Molecular Weight Heparin (LMWH)

LMWH is given via subcutaneous injection once or twice a day to prevent thrombosis in orthopedic surgery and acute medical conditions such as cancer and heart failure. LMWH is also used to treat VTE. LMWH provide better advantages in the management of thromboembolism and does **not** require the close monitoring associated with traditional heparin.

APTT is **NOT** used for LMWH monitoring.
LMWH is monitored by the Anti-Xa assay rather than the PTT.

Mechanism of action-
LMWH catalyzes the interaction between antithrombin (AT) and factor Xa. This results in inhibition of the formation of fibrin.

PTT Reference Range: (adults) 28.0-35.0 seconds
PTT Critical/Panic Value: ≥ 100 seconds

PTT Applications:

1. Monitor Unfractionated heparin (UFH) therapy.
2. Detect LACs (Lupus Anticogulant(s))
    a. LACs neutralize phospholipids in the PTT reagent
3. Detect congenital or acquired procoagulant deficiency
    a. The PTT measures functional activity of the intrinsic and common pathways. The PTT can evaluate for inherited or acquired deficiencies. The PTT primarily measures deficiencies in factors VIII, IX, XI, and XII, but can detect deficiencies of all factors **except** III and VII.

Clinical Significance:

A long partial thromboplastin time indicates a significant defect in at least one of the plasma procoagulants. When the partial thromboplastin time is used in combination with the prothrombin time, most procoagulant disorders can be classified. The PTT time is abnormal with deficiencies XII, XI, IX, VIII, PK, HK (intrinsic pathway) and X, V, II, I (common pathway)

Prolonged PT	Prolonged PTT	Potential Factor Deficiency
√		VII
√	√	X,V, II, I , Vitamin K deficiency, DIC, Liver Disease
	√	XII, XI, IX, VIII

Manual (Tilt tube)
Procedure:

1. Working in pairs, pre- warm a sufficient quantity of calcium chloride reagent to 37˚C for the number of tests to be performed.
2. For this assay, a minimum of 2 test tubes will be needed for each control. Label test tubes as “Normal Control 1, Normal Control 2,” for example.
3. Pipette 100 µL or 0.1 mL of normal control into each labeled test tube.
4. Into each test tube, add 100 µLor 0.1 mL of prewarmed, partial thromboplastin reagent.
5. Incubate the control/ partial thromboplastin mixture at 37ºC for a minimum of three (3) minutes.
6. Forcefully add in100 µL or 0.1mL of calcium chloride into the control/ partial thromboplastin mixture and start the stop watch simultaneously.
7. Mix the tube once, immediately after adding the calcium reagent. Allow the tube to remain in the heat block, approximately 20 seconds, mixing occasionally.
8. After 20 seconds, remove the tube from the heat block. Wipe off the outside of the tube with a Kimwipe. Gently tilt the tube back and forth, looking for a visible clot. If a clot is not seen, place the sample back in the heat block for a few more seconds, then check for a clot again.
9. As soon as a clot is observed, stop the stop watch immediately and record the time in seconds in the report form. Carry out 1 significant figure passed the decimal point. For example, if your result is 30.31, report as 30.3 seconds.
10. Repeat the procedure for a second run of control.
11. If the results from run 1 and run 2 are less than 40 seconds, and within $\pm$ 2 seconds of each other, the runs can be accepted. If the results from run 1 and run 2 are greater than 40 seconds, and within $\pm$3 of each other, the runs can be accepted. Once the acceptability of the runs has been determined, the two results will be averaged and report in the “PTT Avg” column on the report form with appropriate units.

12. If results are not within required limits, a third run should be performed and average the two results that match within acceptable limits. **Be sure and cross out any values you are not using for the final PTT average.** Write the PTT average of values in the "PTT Avg" column on the report form with appropriate units. **Include measurement units of seconds on report sheet.**
13. Repeat steps 2-12 for the abnormal control, as well as the patient sample. Remember to label the test tubes with appropriate labels to avoid a reporting error.

Cascade M-4
Procedure:

1. Turn on instrument and allow system to initialize.
2. Place appropriate volume of $CaCl_2$ reagent in 37˚C reagent well.
3. The screen will read "Test Menu: Select: Run Tests →" Press <ENTER>
4. The instrument is set to run each sample in duplicate. The next screen will read "Duplicate all same patient ID?" Select NO and press <ENTER>
5. Select channels to be run by pressing <1> for channels 1 and 2 or press <3> for channels 3 and 4 to be run in duplicate. Then press <ENTER>
6. Enter Patient ID or control ID
7. Select aPTT as test type
8. Add 100 µL plasma to cuvette in the first of the duplicate channels through the lid to start timer for equilibration
9. Wait 5 seconds add 100 µL plasma to cuvette in the second of the duplicate channels through the lid to start timer for equilibration
10. At prompt, mix and add 100 µL aPTT of reagent to cuvette of the first channel through lid to start timer for activation and repeat for the second channel.
11. At prompt, add 100 µL of pre-warmed $CaCl_2$ to cuvette through lid to start test for both channels.
12. Remove cuvette(s) and press "Enter" for next test.

KC-1 Delta
Procedure:

1. Working in pairs, allow aPTT reagent to warm to room temperature.
2. Place calcium chloride reagent into a 15mm test tube in reagent well to keep warm. Do not fill above well. Allow $CaCl_2$ to sit in reagent well for at least 15 minutes. 100 µL of $CaCl_2$ is required for **each** test to be performed.
3. Place univettes in incubation wells.
4. Pipette 100 µL of sample OR control into bottom of cuvette opposite the ball, without touching the side or bottom of cuvette. Incubate sample for a minimum of 30 seconds, not to exceed 180 seconds. Discard tip.
5. To display available testing modes, hit #2.
6. Aspirate 100 µL of prewarmed, aPTT reagent into the pipette tip and dispense on the opposite side then the ball.
7. Select the PTT mode by pressing button #3. The time will start to count down.
8. Aspirate 100 µL of $CaCl_2$ into pipette tip
9. Once timer hits zero, and you see "START", press #3.
10. Once you see "P/M" displayed dispense $CaCl_2$ reagent and press #4.
11. The timer will stop automatically when the clotting begins and the ball is pulled away from its position.
12. Record data on report sheet.

13. Remove cuvette from the test well and discard into biohazard trash.
14. Position the next cuvette into the test well. Press button #2 to proceed with the next sample.
15. Repeat steps 4-11.

Procedure Note:
The technician should confirm the acceptability of the runs PRIOR to calculating the PTT average. Please see acceptability note under the Manual (tilt tube) method, steps 11-12.

Quality Control:

Quality control materials (normal/Level 1 and abnormal/Level 3) with established control limits should be run. Controls are tested at the beginning of each testing day, followed by testing during each subsequent shift or with each batch of assays. Additionally, quality controls should be performed with any part or reagent change as well as noticeable shifts or trends in the QC data. Adherence to good laboratory practice and careful following of the recommended procedures will result in clinically accurate and reproducible results. **Once reagents and controls are reconstituted, put today's date/time, the expiration date/ time and technologist's initial on the bottle.**

Sources of Error/Troubleshooting:

1. Associated with specimen
    a. Inappropriate ratio of anticoagulant to blood
    b. Failure to correct citrate volume if hematocrit > 55%
    c. Clotted, hemolyzed or lipemic samples
    d. Lack of PPP (<10,000 platelets per µL)
    e. Delay in testing or processing
    f. Inappropriate storage
2. Associated with storage
    a. Incorrect preparation of reagents
    b. Failure to properly store reagents
    c. Use of reagents beyond reconstituted stability time or expiration date
    d. Contaminated reagents
3. Associated with procedure
    a. Incorrect temperature
    b. Incorrect incubation times (↑ incubation time=↓PTT due to contact activation and > 5min heating will result in loss of heat-labile factor V)
    c. Incorrect volumes of sample, reagents or both

Lab #: Partial Thromboplastin Time
Results Form

Name_____
Date_____
Points= 18

**** Include measurement units**

CONTROLS	Lot and Expiration date	Run 1	Run 2	Run 3	PTT Avg.	Is the Control In? Y/N
Normal control						
Abnormal control						

Patient Name & ID	Run 1	Run 2	Run 3	PTT Avg.	Within PTT Recommended Range?

Name_____
Date_____

Lab#: Activated Partial Thromboplastin
Study Questions
Points= 20

1. What coagulation factor deficiencies may be detected using the PTT as a screening test? (4 pts)

2. What is the reference range of the PTT? (1 pt)

3. What therapeutic anticoagulant is most often monitored using the PTT? (Be specific) (1 pt)

    B. What is the mechanism of action of the anticoagulant therapeutic listed above. (1 pt)

    C. If a patient is on this therapeutic anticoagulant, how many times normal do we want the PTT results to be? (1 pt)

    D. Name the derivative of the therapeutic anticoagulant listed above that is more frequently used today and requires less monitoring than its larger (older) version. (1pt)

4. What two (2) reagents are used in performing the PTT? (1 pt)

    B. Explain the purpose of each reagent used. (1 pt)

5. Which coagulation pathway is monitored by the PTT test that is NOT monitored by the PT? (1 pt)

   B. What initiates this coagulation pathway? (1 pt)

6. What factor deficiencies can be detected by using the PTT but will not affect the PT? (2 pts)

7. What would be the PTT result in a patient with hemophilia A?(1 pt)

8. What would be the PTT result in a patient with Factor VII deficiency and **WHY**? (2 pts)

9. Name TWO (2) potential sources of error when performing the PTT test. (2 pts)