Laboratory: Mixing Study
Skills= 52.9 Points

Objectives:

The student should be able to:

1. Perform either manual, semi-automated, or automated protimes on two (2) controls and one (1) patient within ± 2 SD of known values.
2. Perform either manual, semi-automated, or automated partial thromboplastin time on two (2) controls and one (1) patient within ± 2 SD of known values.
3. Accurately report results with 100% accuracy.
4. Accurately record quality control results with 100% accuracy.
5. State the reference range for the PT.
6. State the reference range for the aPTT.
7. Interpret the results of routine PT and aPTT testing as abnormal or normal.
8. Evaluate PT and aPTT results and identify the condition present.
9. State the therapeutic range for a person on anticoagulant therapy.
10. State the type of sample used for PT, aPTT, and mixing study testing.

Materials:

1. 12 x 75 test tube
2. Pipettes: 100 µL and 200 µL
3. Pipette tips
4. Plastic transfer pipettes (2)
5. Calcium chloride/tissue thromboplastin (PT reagent)
6. Calcium chloride reagent, 0.025M (aPTT reagent)
7. Partial thromboplastin : consists of phospholipids and a contact activator (aPTT reagent)
8. Heat block at 37˚C
9. Test tube rack
10. Stop watch/timer
11. Controls Level I and III (normal and abnormal)
12. Patient citrated plasma specimen (platelet poor)
13. Normal plasma- pooled citrated plasma
14. Gloves
15. KC- 1 Delta or Cascade M-4 Instrument

Sample:

Sodium citrate plasma is used for the Protime, the Activated Partial Thromboplastin Time, and the mixing study procedures. 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:

When a prolonged PT and/or aPTT is recorded for a patient that is not taking any anticoagulant therapy, a factor deficiency or inhibitor may be present. To establish which condition may be the cause, a mixing study or circulating inhibitor screen may be ordered. To determine if a factor deficiency exists, “normal pooled plasma” is combined in equal quantities with the patient’s plasma and the PT and/or aPTT is repeated. The normal pooled plasma provides the missing factor(s) in the patient plasma. Patient samples must have at least 50% factor activity to produce a normal PT or aPTT. If the mixture of samples (pooled plasma + patient sample) results in a fully corrected clotting time, the patient is confirmed to have a factor deficiency.

If the mixture of samples (pooled plasma + patient sample) results in a partial correction or no correction at all, the patient may have an inhibitor. Inhibitors should be suspected if the PT and/or aPTT is abnormal but the patient has no clinical symptoms or bleeding. The most common inhibitors are Lupus-type inhibitors, antiphospholipid antibodies and Factor Eight inhibitors. These inhibitors indicate that an autoantibody is present. This antibody is not only binding the patient's factors, but the factors in the normal plasma that was added to the mixture as well. Specific factor inhibitors are IgG immunoglobulins directed against coagulation factors. Specific inhibitors arise in severe congenital factor deficiencies in response to factor concentrate treatment. Antifactor VIII is the most common.
The abnormal antibody reacts mildly in vivo with thrombotic events, but reacts stronger in vitro by binding to the phospholipids in the reagents used for coagulation testing. Commercially prepared reagents are available that do not contain the phospholipids that trigger this situation.

Heparin contamination may also act as an inhibitor.

Deficiency or Inhibitor	Immediate PT/ aPTT Post Mixing Study	Mixing Study Following 2 hr. incubation
Lupus like inhibitor	No correction	No correction
Factor deficiency	Correction	Correction
Factor VIII Inhibitor	Correction	No correction

Notes:

The clotting times tend to increase with time and incubation due to the loss of labile factors, especially factors five and eight. Therefore, it is important to compare the results of the patient’s diluted sample with the result from the normal pooled plasma.

Procedure:

1. Prepare the Normal Plasma: Obtain 2-4 coagulation specimens in Na-Citrate from individuals who are NOT on anticoagulant therapy and whose tube has been filled to the proper level. The pooled sample must have a PT and/or aPTT run on it as a reportable result and to confirm its normal status. Instructor will provide the normal pooled plasma.
2. Run quality control (normal/abnormal) in duplicate for PT. Record results, including units, on report form. Compare your QC results to the QC reference range that you have noted in the chart. Note whether the results are within the stated QC reference range by answering “yes” or “no”.
3. Perform a PT on the patient sample.
   a. If the result is normal, report the results in the “neat” line in the form and add the following “PT normal, mixing studies not performed.”
   b. If the result is abnormal, report the results, including units, in the “neat” line in the form and continue to step #6.
4. Run quality control(normal/abnormal) in duplicate for aPTT. Record results on report form, including units. Compare your QC results to the QC reference range that you have noted in the chart. Note whether the results are within the stated QC reference range by answering “yes” or “no”.
5. Perform an aPTT on the patient sample.
   a. If the result is normal, report the results in the “neat” line in the form and add the following “PTT normal, mixing studies not performed.”
   b. If the result is abnormal, report the results, including units, in the “neat” line in the form and continue to step #6.
   c. Compare your patient results to the patient reference range that you have noted in the chart. Note whether the results are within the stated patient reference range by answering “yes” or “no”.
6. Mix an aliquot of patient sample with an equal amount of Normal Plasma (1:1).
   a. In a 12 x 75 test tube, aspirate 200 µL of the patient specimen and 200 µL of the normal pooled plasma.
7. Run a PT OR an aPTT on the aliquot, depending on which test was abnormal. Report your results, including units, in the “mix” line in the form. Repeat the test on a second aliquot.
8. Interpret the results of the mixing study. If the mixture corrects to within the reference interval, there is a coagulation factor deficiency. If the mixture fails to correct and the patient is not experiencing any bleeding, an inhibitor is the issue.
9. Circle whether the mixing study was normal or whether it demonstrated the presence of a factor deficiency or the presence of an inhibitor.

NOTE: Duplicate results:

<40 sec: must be within ±2 seconds or repeat

>40 sec: must be within ±3 seconds or repeat

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: Mixing Study Results Form
Points= 52.5

Name:_____ **Date:_____**

**** Include measurement units ***

PT		Run 1	Run 2	Run 3	Final Result (AVG.)		Is QC/ Patient Within Range? Yes or No?
Normal control	Lot #: Exp.:					QC Reference Range:	
Abnormal control	Lot #: Exp.:					QC Reference Range:	
Patient (neat)	Name: ID #:					Patient Reference Range:	
Patient (mix)	Name: ID #:					Patient Reference Range:	

aPTT		Run 1	Run 2	Run 3	Final Result (AVG.)		Is QC/ Patient Within Range? Yes or No?
Normal control	Lot #: Exp.:					QC Reference Range:	
Abnormal control	Lot #: Exp.:					QC Reference Range:	
Patient (neat)	Name: ID #:					Patient Reference Range:	
Patient (mix)	Name: ID #:					Patient Reference Range:	

Interpretation: Circle One	Normal	Factor Deficiency	Inhibitor

Grading Rubric:
Each item listed is worth = 0.5 points:

- QC Lot/Expiration
- Patient Name/Identifier
- Results: Patient/QC/Avg
- Units: Patient/QC/Avg
- Addressed reference range: Patient/QC
- Addressed interpretation

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