1. PURPOSE
   1. To distinguish factor deficiencies from factor inhibitors in blood plasma samples.

1. PRINCIPLE
   1. The one‑stage prothrombin time test is used to determine deficiencies of clotting factor activity, either hereditary or acquired, in the extrinsic pathway. Since most of the factors depressed by oral anticoagulant drugs are in the extrinsic pathway, the prothrombin time test is the test of choice in controlling oral anticoagulant therapy.
   2. The Activated Partial Thromboplastin Time Mixing Study is indicated when the PTT is prolonged in the absence of heparin therapy. Deficiencies can be attributed to five common causes: Medication, Coagulation factor deficiencies, Non-specific (lupus type) anticoagulant, coagulation factor deficiencies associated with significant hemorrhage, and Specific factor inhibitors of which 15 percent of patients have severe factor VIII or IX deficiency develop alloantibodies that recognize the deficient factor when transfused.
   3. Hemostasis is the process which retains the blood within the vascular system. When there is injury to a blood vessel, the hemostatic process is designed to repair the break and arrest hemorrhage. The most immediate response to bleeding is vasoconstriction, which decreases the blood flow through the injured blood vessel. Platelets then clump together and adhere to the injured vessel in this area in order to form a plug and further inhibit bleeding. The coagulation factors present in the blood interact, forming a fibrin meshwork or clot, to stop the bleeding completely.
   4. Blood coagulation occurs as a series of step‑by‑step, bio-chemical reactions. The working hypothesis of blood coagulation can be divided into three stages: 1) the formation of thromboplastin, 2) the conversion of prothrombin to thrombin, and 3) the formation of an insoluble fibrin clot through the interaction of fibrinogen and thrombin. Further, this scheme is presumed to be applicable in either of two systems that bring about clot formation. The pathway used in the presence of tissue juices is referred to as the extrinsic system, whereas that used in the absence of tissue extracts is referred to as the intrinsic system.
   5. Tissue thromboplastin, in the presence of calcium, is an activator that initiates the extrinsic pathway of coagulation.
   6. By adding tissue thromboplastin to normal anticoagulated plasmas, the clotting mechanism is initiated and a solid gel clot will form within a specified period of time. If there is a deficiency of factor activity in the extrinsic pathway of coagulation, the time required for formation of the clot will be prolonged beyond that expected for normal plasma.
   7. A mixing study involves mixing an equal volume of the patient’s plasma and the control plasma and immediately performing an aPTT. If the prolonged aPTT fails to correct to the normal reference range, an inhibitor is likely present and evaluation of the type of inhibitor is indicated. If the aPTT corrects to the normal reference range, it may indicate a factor deficiency. It will be necessary to further incubate the diluted sample at 37°C for 90 minutes to determine if a time dependent inhibitor is present.
   8. The fresh citrated plasma employed provides all factors necessary for the intrinsic clotting mechanism except ionic calcium (removed by the citrate) and platelet factor (removed by centrifugation of the platelets). In the test system, brain lipids are added that replace the platelet phospholipid, calcium is restored, and an activator to enhance reproducibility is added to the plasma. The platelet substitute is referred to as a "partial" thromboplastin. The term "activated partial thromboplastin time" simply means that an activator (ellagic acid) is added to the thromboplastin. This surface‑activating substance produces maximum activation and in-creases the sensitivity and reproducibility of the test system.
2. **SPECIMEN AND WORKSHEET SPECIFICATIONS**
   1. Collection Tubes
      1. Becton Dickinson (B‑D) #366415 blue top tube containing 0.5 ml buffered 3.2% sodium citrate in a sterile, silicone‑coated tube.
         1. Approximate draw 4.5 ml blood ±10% to achieve a 9:1 blood to anticoagulant ratio.
      2. Becton Dickinson (B‑D) #36308 pediatric blue top tube containing 0.2 ml buffered 3.2% sodium citrate in a sterile, silicone coated tube.
         1. Approximate draw 2.7 ml ±10% to achieve a 9:1 blood to anticoagulant ratio.
   2. Establishing Proper Tube Fill
3. A minimum and maximum draw tube demographic is kept at the coagulation station against which each specimen is to be compared. Any specimen not falling within acceptable range is to be rejected (see **HEM10-001**,Rejection of Hematology Specimens Procedure).
4. "Short draw" tubes provide insufficient blood for the amount of anticoagulant and lead to prolonged results.
5. Overfilled tubes are unacceptable because insufficient anti-coagulation may occur, especially in severely anemic patients.
   1. Specimen handling
6. Stability
7. Coagulation testing is optimally performed within two (2) hours, but no longer than twenty-four hours, following collection. Once a specimen is uncapped, stability is (4) hours following collection.
8. Centrifugation
9. Centrifuge for 7 minutes at 5,500 rpm OR 10 minutes at 3,500 rpm (within 30 minutes of collection).This speed or centrifuge will yield platelet poor plasma.
10. Centrifuge STATs for 6 minutes at 4500 rpm using the EBA centrifuge. This speed or centrifuge will yield platelet poor plasma.
11. Removal of Plasma
12. Using a plastic transfer pipette, transfer the plasma into a plastic tube and cap. Transcribe the patient's name, medical record number or DOB, accession number, and date unto the plastic tube. Place tube in the freezer in Blood Bank. This is to be followed for samples not processed within 24 hours of collection. Samples may be frozen for 2 weeks; do rapid thaw and process within 1 hour.
13. Checking for Hemolysis and visible clots
14. Check plasma for visible clots and hemolysis; the presence of visibly pink plasma indicates RBC destruction, which may have occurred in vivo prior to or during or after filling the collection tube. (The presence of hemolysis strongly suggests the possibility of in vitro clots.) Append "HEM" to the result (hemolyzed).
15. Sample Storage
16. Room Temperature
    * + - 1. Centrifuged samples can be left up to 24 hours at room temperature.
17. Freezing
18. If the sample must be frozen and tested later, quick freezing of the plasma in small aliquots at ‑70oC is desirable to prevent formation of ice particles.
    * + - 1. **NOTE**: Frozen plasma should be rapidly thawed at 37oC before testing. (Factor VII and factor XI activities may increase with storage when frozen.)
    1. Patient Requirements
       1. A prolonged PT (≥ 3 seconds above normal range) is required for PT mixing study.
       2. A prolonged APTT (≥ 5 seconds above normal range) is required for APTT mixing study.
19. **Standards and Controls**
    1. A new range is established for each new lot of control material by repetitive analysis on both analyzers for one month, during which the manufacturers range is used.
    2. STA system control Ⓝ+Ⓟ (cat No. 00678) contains a vial of abnormal and normal control.
       1. Reconstitution
          1. Reconstitute each vial of control with exactly 1mL of distilled water.
          2. Allow the material to stand at room temperature for 30 minutes.
          3. Swirl each vial gently before use.
       2. Storage and Stability
          1. Unreconstituted vials are stable until the expiration date listed on the box label when stored at 2-8 oC.
          2. Once reconstituted both levels of controls are stable for 8 hours and is to be kept on board the analyzer.
       3. Loading on the analyzer
          1. Click Products then Loading Products or click the  icon to request the product drawer.
          2. Scan the barcode on the reagent bottle and press Enter **⮠**
          3. Select  and click confirm to close the drawer.
       4. Running and Reviewing QC
          1. Qc can be ordered from the Quality Control Menu
          2. All controls are monitored automatically by the Compact Max.
             1. Any controls outside the ± 2SD range will result in audible and visual alarms.
          3. Results can be reviewed in the individual QC files.
          4. All controls must be resulted on form HEM40-012/HEM40-013/HEM40-014/HEM40-015/HEM40-016 Form A Daily Qc on Stago, and in the Lab Information System.
          5. Control results are automatically filed on the STA Compact Max QC file
          6. All results for a 24 hour period will be converted to a “mean” value on the first run after midnight.
             1. This mean is used in the statistical data and is plotted on the Levi- Jennings chart as a daily mean.
          7. Print all the QC data points prior to the first run after midnight.
             1. Prior to midnight when the analyzer is not running any test s click the  icon or select Quality Controls and the Windows Methodologies List appears.
             2. Double click the PT test and click the QC Tables icon 
             3. Click the  icon.

Printout dialogue box will appear. Select print, then click confirm.

* + - * 1. Click the  icon to return to the QC Graph.
        2. Click **Next Level** and repeat process for other levels.
  1. Cryocheck Pooled Normal Plasma
     1. Product description
        1. Consists of a pool of normal citrated human plasma from a minimum of 20 healthy individuals, then buffered using HEPES buffer, aliquoted, and rapidly frozen.
     2. Source
        1. Precision BioLogic Inc.

Dartmouth, NS

* + 1. Availability
       1. Ordered when needed.
    2. Storage
       1. Unopened - store at -40o ‑ -80oC.
       2. Thawed - store at 2 o - 8 oC.
    3. Preparation
       1. Thaw each vial at 37 oC in water bath.
       2. Refer to thawing table in package insert for recommended thawing time based on aliquot size.
       3. Allow thawed plasma to acclimate to room temperature and invert gently prior to use.
    4. Stability
       1. Unopened - stable at refrigerator temperatures (-20 ‑ -80oC) for the dating period of the product.
       2. After thawing – stability at 2 o - 8 oC is up to 24 hours.

1. REAGENTS
   1. .Refer to individual procedure for reagents.
2. **Equipment and Materials**
   1. Equipment
      1. MLA or Volumetric pipette
      2. Centrifuge
   2. Materials
      1. Tips for volumetric pipette
      2. Cuvettes
      3. Printer paper
      4. Waste container
      5. Specimen Cup
      6. Transfer Pipette
3. **Instrumentation**
   1. Identification

|  |  |  |  |
| --- | --- | --- | --- |
| **Facility** | **Name** | **Serial #** | **“Live” Date** |
| **EMCP** | Compact Max 1 | SN5071531 | 3-15-2016 |
| Compact Max 2 | SN5071537 | 3-15-2016 |
| **EMC-EP** | Compact Max 3 | SN5061483 | 3-15-2016 |
| Start 4 | B932 | 3-15-2016 |

* 1. Parts information and Physical Requirements
     1. Refer to instrument reference manual
  2. Routine Maintenance
     1. Refer to The Stago operators manual regarding all daily, weekly, and as needed maintenance.
        1. Record all maintenance on Stago Compact Maintenance form HEM40-012/HEM40-013/HEM40-014/HEM40-015/HEM40-016 Form B Stago Maintenance
  3. Error Messages
     1. If there is not enough reagent(s) to run the test(s), the deficient reagent(s) and the amount of reagent necessary to complete the testing will appear in red.
     2. This deficiency will BLOCK the SAMPLE PIPETTING and the icon will appear on the bottom of the screen. When you receive this error you need add the necessary reagent(s).
        1. Respond to the error “New Tests are Delayed- Reactivate?” With N or NO.
        2. Add required reagents to the drawer and reactivate test(s).
  4. Emptying of waste
     1. Liquid Waste
        1. The Stago does not contain Sodium Azide and can be discarded in a dirty sink. The instrument utilizes emptied rinse containers as its liquid waste chamber. When replacing the rinse bottle also remove the waste container and dump the liquid waste down the drain of a dirty sink. The rinse bottle being replaced is now used to collect future liquid waste.
     2. Cuvettes
        1. When the cuvette waste is full remove the bag from inside the analyzer and dispose of in the sharps container. Replace the yellow biohazard liner of the cuvette waste container.

1. PROCEDURE DIRECTIONS

NOTE: Mixing Studies are only performed on 1st shift Monday through Friday.

1. Initial Run
   * 1. PT/APTT must be ordered along with its corresponding mixing study. Request order from physician if needed.
     2. If PT/APTT are normal and fail to meet the criteria for mixing study, cancel the test as “Test not Indicated”.
     3. Verify with ordering physician that the patient is not on anticoagulant.

**Note 1:**

Warfarin acts as an anticoagulant by inducing factor deficiencies (decreasing function of Factors II, VII, IX and X). Therefore, if a mixing study is performed on a patient receiving Warfarin, results of PT Mix will be consistent with factor deficiency.

**Note 2:**

Heparin inhibits Factors IIa, IXa, Xa, X19, XIa, and probably VIIa.

**Note 3:**

Check with Pharmacy to see if any thrombin inhibitors such as Lepirudin, Bivalirudin, and Argatroban are included in pharmacy treatments.

1. **Prolonged PTT Only**: Initial Run
2. Ensure all quality control has been completed before running patient sample.
3. Order and perform a Thrombin Time to rule out the possibility of heparin contamination. Refer to Thrombin Time procedure by the lab (reflex test approved by Medical Staff).
   1. Thrombin Time between 14.6-18.6 seconds indicates no heparin contamination. Perform mixing study.
   2. Thrombin Time > 18.6 seconds indicates the presence of heparin. Heparin explains the PTT prolongation. No further testing is necessary. Mixing study un interpretable, and credit Mixing Study for heparin & thrombin inhibitor contamination.
4. Immediate (1:1 Dilution)
   1. Pull a vial of “Normal Pooled Plasma” from the Blood Bank freezer, thaw, and remove immediately from the heat block. Perform PT and PTT on Normal Pool Plasma to make sure Pooled Plasma is with EMC normal posted range.
   2. Determine which test is prolonged: PT, APTT, or both.
   3. Mix 0.5 mL of patient’s plasma with 0.5 mL Cryocheck Normal Pooled Plasma in a plastic sample cup. Label sample cup (patient and control). Mix well and place in patient rack.
   4. Place 1mL Cryocheck Pooled Normal Plasma in a plastic sample cup and label tube (control) and place in patient rack.
   5. Place 1mL of the patient’s plasma in a plastic sample cup and label tube (patient) and place in patient rack.
   6. Place patient rack on analyzer.
   7. See individual coagulation procedures for manual sample processing without barcodes.
   8. Run PT and/or APTT on all three samples indicated (immediate Mix/0 Minute).

**NOTE: IF YOUR PATIENT’S BASELINE PT/PTT RESULTS ARE CRITICAL FOLLOW THE CRITICAL VALUE POLICY AND CALL THE CRITICAL RESULT.**

1. Incubated (90 min at 37°C)
   1. Incubate sample cups covered with Parafilm for 90 minutes at 37°C in the water bath.

.

* 1. After incubation, perform PT and/or PTT on all samples indicated using the manual sample processing method in coagulation procedures.

1. Resulting Mixing Study:
   1. Worksheet: AEMC COAG MAN
      1. Enter sample accession number.
      2. If mixing study not indicated for designated test, NA out result.
      3. Attach footnote by right clicking test box, covert result, alpha, and then click “SEE FOOTNOTE” to indicated Mixing Study test (i.e. APTT Mix or PT Mix).
      4. Right click SEE FOOTNOTE to add comment.
      5. Under the result comment tab, enter **MIXPTTEMP** (one word) for PT Mixing study AND/OR **MIXPTTTEMP** (one word) for PTT mixing study and click the F9 key to populate the template.
      6. Enter mixing study results within the template.
      7. To order the path review click Slide Review Interpretation box and enter a period. This will prompt you to the next window that pops up. Enter another period in this window and click OK. Click PERFORM, and then VERIFY.
      8. Deliver paperwork to designated pathologist.
2. **CALCULATIONS**
   1. Tests reported in singlet. The INR will not be reported when performing PT Mixing studies.
3. **QUALITY ASSURANCE**
   1. Tolerance Limits of Controls
      1. Acceptable limits for STAGO are calculated monthly via Stago Clarity peer comparison program.
      2. All control ranges are monitored by the STAGO. If any controls are outside the SD range, the STAGO will audibly and visually alarm the operator by FAILING and the result will show in red.
      3. Otherwise, the results can be found in the QC files. Control results are automatically filed in the STAG QC LOG.
      4. QC is also monitored in Cerner via ARE. Data points must be entered into the LIS, using ARE function. If a data point fails criteria, QC must be rerun and corrective action documented on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form B**.
   2. Out of Tolerance Controls
      1. When control time values fall outside of set limits:
         1. Repeat to verify.
         2. Recheck all instrument operations and volume of reagents onboard and ensure sufficient volume of sample, and confirm expiration time and date.
         3. Open and reconstitute a new vial of control material, making sure the Protocol water and not saline is used.
         4. If step #3 is still out of tolerance, notify supervisor and call Biomedical Engineering.
         5. Write comment in Action Log (form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form B**).
   3. Tolerance Limits For Acceptable Performance ‑‑ Patient Samples
      1. Any Prothrombin Time over 100 seconds or less than 9.5 seconds and any INR ≥10.0 should be checked for clots and reviewed.
      2. Any patient with a critical value of 5.0 or greater may be released after the INR is verified.
   4. Tolerance Limits of Temperature Controlled Areas
      1. Reagent Cooling System
      2. Maintain reagents at 25oC or lower. Instruments will Auto Stop when temperatures of controlled areas are out of range.
   5. Frequency of Assay
      1. Each Shift:
      2. The Normal and Abnormal Control must be assayed at least every 8 hours when patient testing is performed.
   6. Quality Control Program
      1. New Lot of Controls
         1. When a new lot of control is put in use utilize the assayed range over twenty runs to establish and or confirm assayed range.
      2. Failed QC
         1. Failed QC on the STAGO will cause a FAILED flag on the printout alerting staff of a problem which should result in a repeat of the failed control. If failed QC is acceptable on the basis of the Cerner range, still repeat failed control(s) on the analyzer. If controls continue to fail alert Lead Tech or Supervisor.
      3. Controls in Cerner
         1. Controls are released into LIS QC files for Westgard rules. All QC is released the same as a patient result in Cerner and **requires** technical review prior to release.
   7. INSTRUMENT CORRELATION
      1. The four STAGO instruments are correlated semiannually.
         1. Three patient samples are to be processed for correlation on each instrument and recorded on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form C** Semi-Annual Coagulation Instrument Correlations.
            1. PT’s are to correlate +/- 0.5 seconds.
            2. PTT’s are to correlate +/- 2.0 seconds or 5% whichever is
   8. Verification of platelet poor plasma
      1. The actual platelet concentration of the normally spun plasma used for coagulation testing is counted semiannually or when centrifuge is changed to confirm that it is platelet-poor.
         1. Obtain plasma from spun blue top tube.
         2. Run plasma on the Hematology analyzer.
         3. Print out and record on form **HEM40-003/HEM40-004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form D** Centrifuge Platelet Concentration Correlation.
         4. The platelet count of this platelet poor plasma must be less than 10,000
         5. Notify supervisor if outside acceptable limits and discontinue use of centrifuge.
4. **PROCEDURE NOTES**
   1. Lipemic Specimens
      1. When a specimen for prothrombin testing is visually lipemic, special attention must be given to the validity of results. Ensure the clot detection and not the optics is being used.
   2. Minimum and Maximum Times
      1. The minimum time for PTT testing is 16 seconds. Samples should be checked for clots and repeated prior to resulting as <16.
      2. The minimum time for PT testing is 0 seconds. Samples should be checked for clots and repeated prior to resulting as 0- 9.5.
      3. The maximum time on the ACL for PT testing is 320.0 seconds and will give a message of >100.0 seconds. Such a result should never be reported without confirmation.
      4. The maximum time on the ACL for PTT testing 400 seconds and will give a message of >240 seconds. Such a result should never be reported without confirmation.
      5. Repeat any terminal time on the ACL to rule out instrument failure or pipetting error.
         1. If no reportable result is obtained FAILED will appear in place of result.
   3. Hemolyzed and Icteric Specimens
      1. Hemolyzed or icteric plasmas may interfere with optical results from the analyzer. All clot curve results are to be reviewed and tech is to determine whether the result is acceptable.
   4. STAT Specimens
      1. All stat specimens are to be run and released within an hour of receipt. All stroke specimens are to be run and released within a half hour of receipt.
   5. Hematocrit greater than 55%
      1. When a patient has a hematocrit greater than 55%, the 9:1 ratio of blood to anticoagulant (3.2% sodium citrate) is not adequate and will result in inaccurate PT, APTT, and Fibrinogens. A specially prepared tube, based on the patient's hematocrit, must be used.
      2. Implementation
         1. According to the following formula, calculate the amount of 3.2% sodium citrate that is required:

Amount of citrate = 0.00185 x amt. blood to be drawn x (100 - Hematocrit)

**Below is an example of the calculation when a 2.7 ml tube is to be drawn**

Patient's hematocrit = 60%

You wish to draw 2.7 ml of blood

Amt. citrate = 0.00185 x 2.7 x (100-60)

Amt. citrate = 0.00185 x 2.7 x 40

Amt. citrate = 0.199 ml

* + 1. Open several blue top tubes and pool the sodium citrate in a small, plastic, capped tube.
    2. Pipette the required amount of sodium citrate into an empty red top tube (do not use a gel tube).
    3. Patient's blood must be **drawn by syringe** and the exact amount of blood used in the calculations must be added to the tube containing the measured amount of citrate.
    4. When this tube is received in Hematology Laboratory, handle as any other specimen for testing.
    5. Append the footnote “HCT” (HCT > 55%, SPECIAL TUBE REQUIRED) to the result.
  1. Additional Protocols for Coagulation Testing
     1. All new lot numbers and package inserts are checked prior to usage.
     2. Controls are run to verify acceptable results.
     3. No results are released if controls are outside of acceptable range. Document all out of range QC in both Cerner and Action Log (**HEM40-003/HEM40- 004/HEM40-005/HEM40-007/HEM40-009/HEM40-010 Form B**).
     4. Studies are run on new lot numbers of coagulation reagent to verify normal population range.
     5. A normal and abnormal control is to be run at least every 8 hours when results are being released and with each change of reagents. Techs must check QC is run and acceptable at the beginning of their shift or whenever QC is run. No patients are to be run when QC is being run. After QC is run and found to be acceptable then patient testing can be resumed.
     6. Questionable results are repeated.
     7. Specimens with short or very prolonged results are checked for clots or short draws.

1. **EXPECTED VALUES**
   1. Reference Ranges
      1. PT 11.8. – 14.3 seconds
      2. PTT 25.5 – 36.0 seconds
2. Variables Affecting Anticoagulation Dosage
   * 1. Plasma proteins
        1. Most of the oral anticoagulants are bound extensively to plasma proteins. Plasma binding has been shown to influence greatly the elimination of warfarin in humans; that is, the less extensive the binding, the more rapid the elimination. Since more than 97% of the material is bound, minor changes in the amount of binding can produce up to a fourfold difference in the rate at which the drug is eliminated.

.

* + 1. Liver receptor sites
       1. The liver is the principal site of metabolism of oral anti-coagulants. The drugs are reduced to warfarin alcohols by soluble enzymes of the hepatic and renal parenchyma. These alcohols show only slight anticoagulant activity. The warfarin molecule is a racemic mixture with two enantiometers. The S warfarin is the more potent isomer, but its rate of elimination is greater than that of the R warfarin. The kinetics of these compounds are made even more complicated because the metabolism of the isomers is different.
       2. Variations in affinity for receptor sites for the warfarin compounds also cause individual differences seen in humans. In 1964, a group of patients was reported who had genetic resistance to warfarin. A second group was reports in 1969. These patients required 90‑145 mg of warfarin per day to achieve therapeutic effects. (Most patients are satisfactorily maintained at a dose of 2 to 20 mg daily.) The inheritance pattern was believed to be autosomal dominant.
    2. Vitamin K
       1. Another interesting aspect of these patients was that they also had much higher vitamin‑K requirements than did normal subjects. It has been suggested that resistance to warfarin was secondary to an altered hepatic receptor.
       2. Variations of vitamin‑K availability can produce variations in vitamin‑K nutrition status. Patients have two sources of vitamin K: vitamin K1 absorbed from the diet seems to be the most important; however, vitamin K2 produced by intestinal bacteria has enabled people to maintain sufficient levels of the vitamin in the absence of the dietary vitamin K1. Thus, a diet free of vitamin K1 normally does not lead to changes in the PT unless the numbers of intestinal bacteria that produce vitamin K2 are simultaneously reduced by the use of oral antibiotics. In subjects who receive long-term therapy with oral anticoagulants, a diet deficient in vitamin K1 has been shown to prolong further the PT.
       3. The combination of vitamin K1‑free diet and oral antibiotics may be enough to prolong the PT because of the suppression of vitamin K‑dependent factors. Likewise, malabsorption states caused by intrinsic disease of the small intestine are known to produce increased sensitivity to oral anticoagulants or decreased amounts of the vitamin‑K factors.
    3. Liver disease
       1. Other pathologic and physiologic states may affect the response to oral anticoagulants. For instance, liver disease is known to affect vitamin K‑dependent factors by defective synthesis. Because of the inability to synthesize the clotting factors, patients with liver disease may be unable to reverse the prolonged PT when given vitamin K. Likewise, liver disease produced by congestive heart failure and the hepatic damage of viral hepatitis greatly increases sensitivity to oral anti-coagulants.
    4. Hyper metabolic states
       1. In hyper metabolic states, the turnover of vitamin K‑dependent factors is greatly increased. Thus, patients with fever or hyperthyroidism can show disappearance rates for factors II, VII, IX, and X that are three to four times normal. These states, however, do not affect the metabolism of the oral anticoagulants.
    5. Fluid changes
       1. Rapid changes of fluid compartments can greatly affect the synthesis of vitamin K‑dependent clotting factors. For instance, in patients who receive diuretic drugs, most of these effects are due to a decrease in the amount of congestion of the liver and thus increase the synthesis of the vitamin K‑dependent factors, leading to a decreased sensitivity to vitamin‑K inhibitors.
    6. Heparin
       1. Heparin prolongs the on-stage prothrombin time. Therefore, to obtain a valid prothrombin time when heparin and coumadin are given together, a period of at least 5 hours should elapse after the last intravenous dose and 24 hours after the last subcutaneous dose of heparin before blood is drawn.

1. BACK‑UP INSTRUMENTATION AND/OR METHODOLOGY
   1. EMC has two Stago instruments, which serve as backups for each other.
   2. EP has one Stago Compact Max instrument and One Start 4, , which serve as backups for each other
   3. In the event of a total system shutdown or instrumentation failure in which ECMP Stago Compacts are out of service, specimens must be transported to another facility (i.e. ECM-EP or Montgomery).
   4. In the event of a total system shutdown or instrumentation failure in which ECM-EP Stago analyzers are out of service specimens must be transported to another facility (i.e. ECMP or Montgomery).
2. **LIMITATIONS OF THE PROCEDURE**
3. Many commonly administered drugs affect PT results. (Example: coumadin, heparin and direct thrombin inhibitors).
4. STA® - Neoplastine® CI and CI+, both contain a specific inhibitor of heparin. The test is insensitive to unfractionated heparin levels up to 1 IU/ml and to low molecular weight heparin levels up to 1.5 anti-Xa IU/ml.
5. **REFERENCES**
6. STA**®** - Neoplastine**®** CI 5 ml (Cat. # 0605) or STA**®** - Neoplastine**®** CI 10 ml (Cat. # 0666), or

STA**®** - Neoplastine**®** CI PLUS, 5 ml (Cat # 00606) & STA**®** - Neoplastine**®** CI PLUS (Cat. # 0667) are used for Determination of Prothrombin Time (PT) by STA® Analyzers. Package insert for use in PT determinations.

STA**®** - Neoplastine**®** CI package insert 26332 06-Revised November 2011

STA**®** - Neoplastine**®** CI PLUS package insert 26336 08-Revised June 2012.

1. STA**®** - Coag Control Ⓝ+ABN (Cat. No. 00676): citrated control plasmas

normal and abnormal levels; or

STA**®** - System ControlⓃ+Ⓟ (Cat. No. 00678): Control Plasmas for Assays of

Coagulation Parameters on STA® Analyzers.

STA**®** - Coag Control Ⓝ+ABN package insert 23678 – Revised December 2009.

STA**®** - System ControlⓃ+Ⓟ package insert 26384 – Revised June 2011.

1. STA - Desorb U (Cat. No. 0975) Decontamination solution for STA**®** analyzer systems. Package insert #26265 – revised June 2011.
2. STA Compact Max® Operator’s Manual. 0931946 October 2012.
3. Woodhams B *et al*. Stability of Coagulation Proteins in Frozen Plasma. *Blood Coag Fibrinol.* 2001;12(4):229-236.
4. Clinical and Laboratory Standards Institute (CLSI). Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline- Fifth Edition. H21-A5 vol. 28 No. 5 or latest revision.
5. Clinical Laboratory Standards Institute. Collection and Processing of Blood Specimens for Testing Plasma Based Coagulation Assays and Molecular Hemostasis Assays:  Approved Guideline. Fifth Edition.  Wayne, PA: Clinical Laboratory Standards Institute; 2008. Document H3 – A6 or latest revision..

*For additional information, please refer to the manufacturer’s package inserts.*

**Approval signatures:**

|  |  |  |
| --- | --- | --- |
| **Date** | **Printed Name** | **Signature** |
| 3-4-2016 | David Hinkle, MT, ASCP  Hematology Supervisor |  |
| 3-4-2016 | Vanessa Rawlings  Elkins Park Supervisor |  |
| 3-4-2016 | Vivian Arguello, MD  Section Director of Hematology |  |
| 3-4-2016 | Nancy A. Young, MD, FCAP  Medical Director |  |

## Review History

|  |  |  |
| --- | --- | --- |
| **Date**  **Reviewed** | **Reviewed By** | **Revisions** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |