1. **PURPOSE**
	1. To determine deficiencies of clotting factor activity, either hereditary or acquired, in the extrinsic pathway.
2. **Principal**
	1. STA**®** - Neoplastine**®** CI 5 & 10 ml (Cat. # 0605 & Cat. # 0666) and STA**®** - Neoplastine**®** CI PLUS 5 & 10 ml (Cat # 0606 & Cat. # 0667) are reagents used for Prothrombin times (PT) and extrinsic factor assays on the STA Compact Max®. A mixture of thromboplastin is added to citrated plasma and the time for clot formation is determined. The STA Compact Max® is a fully automated coagulation instrument which uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette containing the thromboplastin and plasma is monitored by the STA Compact Max®. When the oscillation of the steel ball is slowed by clot formation, the sensor determines the time in seconds. The PT is a basic coagulation screening test for the activity of the extrinsic pathway (factors I (fibrinogen), II, V, VII, and X).
	2. A prolonged PT has been observed in the following clinical disease states: congenital or acquired deficiencies of Factor II, V, VII, X, or fibrinogen. The PT may also be prolonged in liver disease, treatment with vitamin K antagonists, disorders of metabolism of vitamin K, fibrinolysis, and DIC.
	3. The PT is also used to monitor Warfarin therapy because of its sensitivity to variations in the concentration of the Vitamin-K dependent factors II, VII and X. Because of the variations in the PT results with different thromboplastin reagents and instruments, it is recommended (by the World Health Organization) that PT results be converted to an International Normalized Ratio (INR). The INR corresponds to the value of the ratio of the patient’s PT and the geometric mean PT of the normal reference population raised to the ISI (International Sensitivity Index) power:



* 1. The ISI value of a thromboplastin reagent is determined by performing PTs on normal plasmas and Coumadin-treated patient plasmas with the *thromboplastin* reagent and the WHO reference thromboplastin and plotting the corresponding linear regression. The slope of this regression curve of the matched pairs is the ISI that is assigned for the thromboplastin by the manufacturer. The ISI value for STA**®** - Neoplastine**®** CI 5 & 10 ml and STA**®** - Neoplastine**®** CI PLUS 5 & 10 ml is determined using a secondary reference standard of rabbit brain thromboplastin on STA**®** analyzers. The resulting ISI value is provided by the manufacturer for each lot of reagent and is also contained within the barcode of the reagent for use on the STA**®** line ofStago analyzers. The geometric mean is calculated from the data collected for the PT reference range at the customer site.
1. **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
		1. 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).
			1. "Short draw" tubes provide insufficient blood for the amount of anticoagulant and lead to prolonged results.
			2. Overfilled tubes are unacceptable because insufficient anti-coagulation may occur, especially in severely anemic patients.
	3. Specimen handling
		1. Stability
			1. 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.
		2. Centrifugation
			1. 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.
			2. Centrifuge STATs for 6 minutes at 4500 rpm using the EBA centrifuge. This speed or centrifuge will yield platelet poor plasma.
		3. Removal of Plasma
			1. 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.
		4. Checking for Hemolysis and visible clots
			1. 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).
		5. Sample Storage
			1. Room Temperature
				1. Centrifuged samples can be left up to 24 hours at room temperature.
		6. Freezing
			1. 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.)
2. **Quality Control**
	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 N+P (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. **Reagents**
	1. All new reagent package inserts are reviewed by the Supervisor, Lead Technologist, or designee and compared with the current lot numbers to ensure no changes have been made. The current package insert is signed and dated with the date the new reagents are put into use
		1. Reagent 1: STA - Neoplastine CI Plus [Cat. # 667]: Lyophilized thromboplastin prepared from fresh rabbit cerebral tissue. The reagent contains a specific inhibitor of heparin; thus, times obtained will not be affected by heparin at therapeutic levels.

* + 1. Reagent 2 5 or 10ml solvent ready for use to reconstitute reagent 1
			1. Reconstitution of Reagent 1 with Reagent 2
				1. Transfer the entire contents of one vial of Reagent 2 into one vial of Reagent 1.
				2. Let sit for 30 minutes at room temperature. Then swirl gently
				3. Add magnetic stir bar to the vial.
				4. Place new STA reducer in vial.
				5. Install the perforated cap.
		2. STA-Desorb U
			1. Decontaminating solution for use with the STA**®** line of instruments. Install a new STA**®** - maxi reducer (REF 00801) and the perforated cap on a freshly opened bottle before loading in the reagent drawer+
		3. STA**®** - Cleaner Solution
			1. Washing aqueous solution used on the STA line of instruments. Sufficient STA cleaner solution must be loaded to operate the analyzer.
		4. Stability
			1. Each Reagent Requires an Open Date/Time and Expiration Date/Time to be placed on vial
				1. Unreconstituted vials are stable until the expiration date listed on the box label when stored at 2-8 oC.
				2. Reagent 1 reconstituted without reducer in its original capped vial is stable for 8 days at 2-8 oC.
				3. Reagent 1 reconstituted with reducer is stable for 48hours on board the Stago Compact MAX.
				4. Desorb U is stable for 5 days on STA Compact Max analyzer
		5. Load Reagents
			1. Click Products, then loading products or click the  icon to open the product drawer.
			2. Scan the barcode on the reagent bottle and press Enter **⮠**
			3. Place the reagent into a stirring position in the product drawer
			4. Select  and click confirm to close the drawer.
		6. Source

|  |
| --- |
| Diagnostica Stago, Inc. |
| Five Century Drive |
| Parsippany, NJ 07054 |

* + 1. Availability
			1. Reagents are shipped on a standing order
			2. Lot numbers are shared between EMCP and EMC-EP in case of shortage call other site to borrow.
1. **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
2. **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**
	* 1. **Running Quality Control**
			1. Click theicon or click Quality Controls and the Methodologies list appears.
			2. Select the check box for PT and click the  icon.
			3. Type the access code and click Confirm.
			4. A yellow triangle  is displayed to the right of the methodology showing the selected Quality Control is running.
		2. **Running Patient Samples.**
			1. Click Patient Analyses
			2. Click loading samples or the  icon.
			3. After the drawer opens, identify the type of specimen by clicking the box
				1. micro-sample
				2. Stat (urgent) by clicking the box.
			4. Identify the sample by scanning the bar-code or manually entering the ID using the keyboard.
			5. Place the specimen into the drawer.
				1. In AUTO MODE, the STA CompactMax® will automatically order the test(s) selected in the AUTO MODE profile.
				2. In MANUAL MODE, the operator must order the test(s) from the Selection menu, or from the Recorded Profile(s). Double click each methodology or Profile, click **Confirm**.
			6. As soon as the sample drawer closes, the ANALYSIS STATUS screen will appear.
			7. All patient results are displayed on the **TEST PANEL** screen and automatically print out and transmit if selected on the **SYSTEM** menu.
			8. For results in question that need operator intervention, cursor to the identification number in the **TEST PANEL** screen and double click.
				1. This will display the **PATIENT REPORT FORM** screen for the specific sample. Follow the options on the bottom of the screen (i.e. Confirm, Re-run, Delete, Add test). Click the  icon to save the changes.
				2. Click the icon to return to the **TEST PANEL** screen.
2. **Calculations**
	1. All instrument measurements and calculations are performed by the analyzer.
	2. The International Normalized Ratio (INR) is automatically calculated by the STA Compact Max® when INR is selected as a reporting unit.



* 1. The INR (see formula) is used to standardize PT values around the world to the World Health Organization (WHO) reference thromboplastin.
	2. The International Sensitivity Index (ISI) is the value determined by the manufacturer for a specific lot number of thromboplastin as compared to the WHO thromboplastin reference standard. It is used as an exponential power for calculating INR by the laboratory performing a PT using that specific thromboplastin lot number with a specific instrument. It is stored in the **CALIB** screen for **PT.**
	3. The PT Geometric Mean (***Reference Time***) is the statistically calculated value based on the reference population for PT reagent lot, reported in seconds. It must be manually entered in the **CALIBRATION** screen for PT (see NOTES at the end of this protocol).
	4. Patient’s PT is the measured PT in seconds for the reagent.
1. **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 10.0 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.
	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.
2. 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.
	2. Minimum and Maximum Times
		1. The minimum time for PT testing is 10.0 seconds on the analyzer. Samples should be checked for clots and repeated prior to resulting <10.0 seconds in Cerner.
		2. The maximum time on the STAGO for PT testing is 100.0 seconds on the analyzer and will give a message of >100.0 seconds in Cerner. Such a result should never be reported without confirmation.
			1. **Note**: The instrument is able to report PT results up to 100 seconds.
				1. Repeat any maximum time on the STAGO to rule out instrument failure or pipetting error.
				2. **NOTE**: If no reportable result is obtained VMIN OR VMAX will appear in place of result.
	3. 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.
	4. 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.
			1. Implementation
			2. 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. INR – Specific Protocols
		1. The INR calculation is checked under the following circumstances:
			1. Change in lot number of the PT reagent
			2. Establishment of a new PT reference range with a change in the geometric mean
			3. Semi-Annually
		2. The INR calculation confirms proper calculation by the instrument. Choose a sample from each analyzer within the INR range of 2.0 – 3.0. Record on form **HEM40-003 Form F**.
		3. Determine INR by the following method:
			1. Definitions
				1. INR = R ISI
				2. Where R = Patient PT

 Mean Normal PT

* + - * 1. ISI is the International Sensitivity Index of the reagent/instrument
				2. .

***NOTE:*** *Regardless of the method used to validate the manual and automated calculation, the two results should be the same. There may be instances where the rounding method used differs in the calculations, resulting in a minimal difference (0.1). If any discrepancies are identified, notify supervisor immediately*.

* + 1. An INR chart (**HEM40-003 Attachment A**) for each PT Reagent Lot in use is posted between instruments in Hematology. The ISI and seconds change with every lot number.
	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. INR calculations are verified by manual method semi-annually.
		7. Questionable results are repeated.
		8. Specimens with short or very prolonged results are checked for clots or short draws.
1. **EXPECTED VALUES**
	1. Reference Ranges
		1. PT: 11.8 to14.3 seconds
		2. INR: 1.2 to 1.4
		3. Reference range established upon initial instrument validation, and evaluated with each subsequent lot change.
	2. Critical Values
		1. Any INR > 5.0 will be called. Call and append message using codded comment “CALL” (Refer to procedure **AD02-004.07** Critical Values/ Diagnosis).
	3. Therapeutic INR Anticoagulation

Indication INR

Prophylaxis of venous thrombosis

(High-risk surgery)

Treatment of venous thrombosis

2.5 – 3.5

Treatment of pulmonary embolism

Prevention of systemic embolism

Tissue heart valves

Acute myocardial infarction

Valvular heart disease

Atrial fibrillation

--------------------------------------------------------

Mechanical prosthetic valves

Recurrent systemic embolism

3.0 – 4.0

* 1. Variables Affecting Anticoagulation Dosage

Because the therapeutic limits of anticoagulant therapy with warfarin‑type compounds are very narrow, the pharmacokinetic aspects of variations of the therapy play an important role in keeping the patient safely anticoagulated without the risk of hemorrhagic problems. Many variables have to be considered before arriving at the correct dosage for each patient.

* + 1. Factor half‑life.
			1. The effect of warfarin‑type compounds on synthesis of vitamin‑K clotting factors II, VII, IX, and X is fairly prompt. As measured by the prothrombin time (PT), however, there is a marked delay relative to the plasma concentrations of warfarin. This is because warfarin compounds inhibit the synthesis of clotting factors II, VII, IX, and X by the liver, but have no effect on their normal catabolic rates. Thus each clotting factor disappears at a rate determined by its relative half‑life, factor VII being the first to go. Were this the only problem, we could easily make adjustments; many other variations, however, affect the way persons respond to oral anticoagulants.
		2. 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.
		3. 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 reported 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.
		4. 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.
		5. 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.
		6. 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.
		7. 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.
		8. Heparin
			1. Heparin prolongs the one‑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.
		9. Factors increasing effect of oral anticoagulants.

|  |  |
| --- | --- |
| Endogenous  | Congestive heart failure |
|  | Fever |
|  | Hepatic disorders |
|  | Poor nutrition |
|  | Steatorrhea |
|  | Visceral carcinoma, especially pancreas |

|  |  |
| --- | --- |
| Exogenous | Acetaminophen (Tylenol) ‑ slight effect |
|  | Alcohol |
|  | Allopurinol (Zyloprim) |
|  | P‑Aminosalicylic acid (PAS) |
|  | Anabolic agents |
|  | Adroyd |
|  | Methandrostenolone |
|  | Norethandrolone |

|  |  |
| --- | --- |
| Exogenous Continued | Stanolone |
|  | Antibiotic therapy |
|  | Antilipemic agents: Clofibrate(Atomid‑S), d‑thyroxine Aventyl |
|  | Blood transfusion |
|  | Cathartics |
|  | Cincophen |
|  | Dextrothyronine (Choloxin) |
|  | Diazoxide |
|  | Dietary deficiencies; low protein,Vitamin C, choline, cystine |
|  | Drugs altering hematopoietic element quinine, quinidine, cytotoxic agents |
|  | Enzymes: bromelains and papain |
|  | Ethacrynic acid |
|  | Glucagon |
|  | Hepatotoxins: carbon tetrachloride |
|  | Mefenamic acid (Ponstel) |
|  | 6‑Mercaptopurine (Purinethol) |
|  | Methylthiouracil (Methiacil) |
|  | Monoamine oxidase inhibitors |
|  | Nalidixic acid |
|  | Oxyphenbutazone (Tandearil) |
|  | Phenylbutazone (Butazolidin) |
|  | Phenyramidol (Analexin) |
|  | Prolonged hot weather |
|  | Prolonged narcotic therapy |
|  | Radioactive compounds |
|  | Reserpine |
|  | Salicylates in excess of 1 gm/day |
|  | Sulfinpyrazone |
|  | Sulfonamides |
|  | Sulfonylureas |

* + 1. Factors decreasing effect of oral anticoagulants

|  |  |
| --- | --- |
| Endogenous | Diabetes mellitus |
|  | Edema |
|  | Hyperlipemia |
|  | Lactation |

|  |  |
| --- | --- |
| Exogenous | Antacids |
|  | Antihistamines |
|  | Ascorbic acid (vitamin C) |
|  | Cholestyramine |
|  | Corticosteroids |
|  | Diet rich in vitamin K |
|  | Diphenylhydantoin (Dilantin) |
|  | Diuretics (mercurials and some thiazides) |
|  | Ethchloroynol (Placidyl) |
|  | Grisefulvin |
|  | Haloperidol (Haldol) |
|  | Mineral oil |
|  | Oral contraceptives |
|  | Sedatives and hypnotics |
|  | Barbiturates |
|  | Amobarbital |
|  | Heptabarbital |
|  | Phenobarbital |
|  | Secobarbital |
|  | Glutethimide (Doriden) |
|  | Xanthines |

* + 1. Oral anticoagulants increase effect of
			1. Adrenalin
			2. Diabinese
			3. Diphenylhydantoin (Dilantin)
			4. Orinase
			5. Phenobarbital
			6. Sulfonamides
		2. Agents with uncertain effect
			1. Chloral hydrate ‑‑ may be a significant effect in either direction
			2. Indomethacin (Indocin) ‑‑ probably no effect
			3. Meprobamate ‑‑ probably no effect
			4. Methylphenidate (Ritalin)
			5. Tetracycline ‑‑ may decrease effect of anticoagulant
		3. Agents with no recognized effect
			1. Chlordiazepoxide hydrochloride
			2. Chlorothiazide
			3. Diazepam
1. REPORTING OF RESULTS
	1. Worksheet
		1. AEMC Stago
	2. Phoned Results
		1. If the INR result is > 5.0, result is called. Call is to be documented according to Critical Value Protocol. (Refer to [**AD02.004**](file:///C%3A%5CUsers%5CHinkleDa%5CAppData%5CLocal%5CADMINISTRATIVE%20AND%20QUALITY%20PROGRAM%5CADM-01-21%20CRITICAL%20VALUE%20REPORTING%20--.doc) Critical Values/ Diagnoses).
	3. Reportable Range:
		1. Prothrombin Time 10.0 - 100.0 seconds
		2. INR 0.9 – 10.0

**Note:** An interpretative comment is reported out with every INR patient result for the use of evaluating patients on stabilized oral anticoagulant therapy.

 Numerical results outside of the reportable ranges are to be resulted as less than (<) or greater than (>) the values listed above the reportable range. Any results above the linearity (<Min or >Max) must repeated to confirm results and checked for clots.

* 1. Release of results
		1. Results will print from the analyzer once completed.
		2. Review printout for any errors before resulting.
		3. Results are entered in function ARE (Accession Result Entry), enter Accession number from print out and worksheet AEMC Stago and click ENTER.
		4. Review results against print out. If results do not cross over, enter results from print out for each test performed, review and VERIFY.
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**
	1. Many commonly administered drugs affect PT results. (Example: coumadin, heparin and direct thrombin inhibitors).
	2. 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.
3. **REFERENCES**
4. 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, ASCPHematology Supervisor |  |
| 3-4-2016 | Vanessa RawlingsElkins Park Supervisor |  |
| 3-4-2016 | Vivian Arguello, MDSection Director of Hematology |  |
| 3-4-2016 | Nancy A. Young, MD, FCAPMedical Director |  |

## Review History

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