

TRAINING UPDATE

Lab Location: GEC, SGAH & WAH
Department: Coag

Date Distributed: 3/13/2014
Due Date: 4/14/2014
Implementation: 4/15/2014

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

**Activated Partial Thromboplastin Time (APTT)
GEC / SGAH / WAH.G02 v3**

Description of change(s):

Major change is addition of instructions for preparing a collection tube when the patient's hematocrit is greater than 55 (appendices A&B). Techs are responsible to prepare the collection tube and inform phleb of volume of blood to be collected.

Section	Reason
3.1	Add reference to Appendices
3.2	Update tube volumes, add opened container storage
4.2	Change storage temp for 0.025M CaCl ₂
10.5	Add instruction for Hct >55, revise action for <Mmix
19	Add Appendix A: Instructions for Preparing Collection Tube for Hematocrit >55%, and B: Phlebotomist Instructions for Blood Collection

This revised SOP will be implemented on April 15, 2014

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 3)

Technical SOP

Title	Activated Partial Thromboplastin Time (APTT)	
Prepared by	Ashkan Chini	Date: 3/10/2011
Owner	Robert SanLuis	Date: 3/10/2011

Laboratory Approval		Local Effective Date:
Print Name and Title	Signature	Date
<i>Refer to the electronic signature page for approval and approval dates.</i>		

Review		
Print Name	Signature	Date

TABLE OF CONTENTS

1. Test Information.....3
 2. Analytical Principle4
 3. Specimen Requirements.....4
 4. Reagents5
 5. Calibrators/Standards6
 6. Quality Control6
 7. Equipment And Supplies8
 8. Procedure9
 9. Calculations.....10
 10. Reporting Results And Repeat Criteria.....10
 11. Expected Values.....11
 12. Clinical Significance.....11
 13. Procedure Notes11
 14. Limitations Of Method12
 15. Safety12
 16. Related Documents13
 17. References.....13
 18. Revision History13
 19. Addenda14

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
APTT	Clot based assay / STA Compact	PTT1

Synonym/Abbreviation
Partial Thromboplastin Time

Department
Coagulation

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2. ANALYTICAL PRINCIPLE

STA[®] PTT A 5 involves the recalcification time of plasma in the presence of a standardized amount of platelet substitute and a specific activator. This procedure minimizes test variables by standardizing the contact activation and optimizes the concentration of platelet-like phospholipids. The time of clot formation is measured on the STA Compact. The STA Compact is a fully automated coagulation instrument, which uses an electromagnetic mechanical clot detection system. The STA Compact monitors the oscillation of a steel ball within the cuvette with the reagents and plasma. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting plasma may be used for samples to be analyzed by this method. Vacutainer tube must be filled to the line to ensure the proper ratio of blood to anticoagulant.
Special Collection Procedures	If hematocrit >55%, refer to appendices A and B for collection instructions.
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred -Other Acceptable	Whole Blood (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum - Minimum - Optimum - Minimum	2.7 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 2.4 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.
Stability & Storage Requirements	Room Temperature: 4 hours (opened, vacuum broken) (20 ± 5° C) 24 hours (unopened, vacuum intact) 2 hours (if on heparin therapy)
	Refrigerated: Not recommended
	Frozen plasma: 1 month
Specimen preparation	Centrifuge whole blood for specified time /speed documented on each centrifuge for preparing platelet-poor plasma.
Unacceptable Specimens	Specimens that are unlabeled, improperly labeled, or those

Criteria	
& Actions to Take	that do not meet the stated criteria are unacceptable. Clotted or under-filled tubes are not accepted. Request a recollection and credit the test with the appropriate LIS English text code for “test not performed” message.
Compromising Physical Characteristics	Moderate to gross hemolysis. Reject sample and request a recollection. Credit the test with appropriate LIS English text code HMM (Specimen moderately hemolyzed) or HMT (Specimen markedly hemolyzed) Lipemia: Acceptable Icterus: Acceptable
Other Considerations	None

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering “SAFETY” for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
STA [®] PTT A 5	Diagnostic Stago (REF 00595)
STA [®] 0.025M CaCl ₂	Diagnostic Stago (REF 00367)
Pure Reagent Grade water	Millipore or NERL Diagnostics (Cat. # 0015)

4.2 Reagent Preparations and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent 1	PTT Automate
Container	Manufacturer supplied vial
Storage	2-8° C
Stability	Stable until the expiration date on the box label. Once reconstituted: <ul style="list-style-type: none"> • It remains stable for 24 hours on the STA[®] Compact. • It remains stable for 7 days at 2-8° C in its original capped vial.

Form revised 3/31/00

Preparation	Reconstitute each vial with 5 mL of Reagent Grade water. Allow the reconstituted material to stand at room temperature (18-25° C) for 30 minutes. Mix vigorously by turning the vial upside down, 5 – 10 times and vortex on low for 5 seconds before loading, to obtain a homogeneous solution and install the perforated plastic cap on the vial.
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Reagent 2	0.025M CaCl₂
Container	Manufacturer supplied vial
Storage	2 - 25° C
Stability	The solution in intact bottles is stable until the expiration date indicated on the box label, when stored at 2 - 25° C. After opening, it remains stable for: <ul style="list-style-type: none"> • 24 hours at 37° C • 3 days on STA[®] Compact
Preparation	If the solution is refrigerated, allow it to stand at room temperature (18-25° C) for 30 minutes before use. Do not install either on STA [®] - Reducer or a perforated cap on the solution bottle if it is to be used on analyzers of the STA [®] line.

Reagent 3	NERL Reagent Grade water
Container	Manufacturer supplied vial
Storage	Room temperature.
Stability	Stable 30 days after opening.
Preparation	Ready to use.

5. CALIBRATORS/STANDARDS

No calibration of the system is necessary for performing an APTT.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
STA [®] Coag control	Diagnostic Stago (REF 00676)

6.2 Control Preparations and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) time of reconstitution, (5) expiration date and time, (6) initials of tech, and (7) any special storage instructions; check for visible signs of degradation.

Control	Coag Controls N and ABN
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Form revised 3/3/10

Preparation	Reconstitute each vial of Reagent 1 or 2 with exactly 1 mL of Reagent Grade water. Allow the reconstituted material to stand at room temperature for 30 minutes. Then, swirl the vial gently before use.
Storage/Stability	2-8° C The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8° C. Once reconstituted, Reagents 1 and 2 remain stable for 8 hours on analyzers of the STA [®] line.

1. After the reconstitution period, request the product drawer to open through the MAIN MENU under LOADING and bar code the controls. Place the controls into the appropriate drawer.
2. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.
3. All control ranges are monitored automatically by the STA[®] Compact. If an controls are outside the ± 2 SD range, the instrument will audibly and visually alarm the operator. Otherwise, the results can be found in the individual QC files. Control results are automatically filed in the STA[®] Compact QC file. All results for a 24-hour period are converted to a “mean” value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean.
4. To print all the QC data points for the APTT test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** \leftarrow Cursor to the APTT test and press **Enter** \leftarrow to view the Levy-Jennings chart. Press **F1** to view the results in tabular form. Press **F6**, select **Execute** then press **Enter** \leftarrow to print the individual values under current controls. Press ESC key to exit (back to graph). Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.

6.3 Frequency

Both controls are run at the beginning of each shift and every 4 hours after and with the change of any reagent used in test performance.

6.4 Tolerance Limits

Step	Action
1	The established QC ranges are in the QC file of the STA Compact. The quality control results from the instrument are transmitted to the LIS and can be viewed in the OEM function. Any out-of-range QC results will be flagged by the LIS.
2	If all controls are within QC parameters all sample results can be reported.
3	Rejected runs must be effectively addressed by corrective action. Steps

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Step	Action
	taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed. Supervisor may override rejection of partial or complete runs only with detailed documentation that follows criteria that is approved by the Medical Director.
4	Corrective action documentation must include the following: QC rule(s) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information. See the QC/QA SOP “QC Responsibilities” for more detail.
5	If the assay is down and results will not be reported in the scheduled turnaround time, clients will be notified of the situation.

6.5 Review Patient Data

Technologist must review each result print-out for error messages. Refer to the STA[®] Compact system manual “Error messages” section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform
 STA[®] Compact – Analyzer

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7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge calibrated for preparing platelet-poor plasma

7.3 Supplies

- Cuvette Roll – Diagnostic Stago
- STA – brass adaptors
- Plastic micro cups
- Plastic transfer pipettes

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection is required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	Instrument Set-up Protocol
1	At the beginning of each shift, verify the instrument temperatures and availability of the cuvettes and cleaner solution by accessing the system status screen from the main bar.
2	Record the temperatures on the maintenance sheet. If the reagent arm 2, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patients being run.
3	Be sure there is an adequate supply of reagents in the analyzer.
4	Load cuvettes and cleaner/wash solution on the analyzer.

8.2	Analytical Procedure
1	Refer to the START-UP procedure for the STA® Compact before running patient specimens on the STA® Compact at the start of each shift.
2	Request Quality Control: Through MAIN MENU under CAL. /CONTROL, select QUALITY CONTROL and press Enter ← Cursor to the APTT test. Select APTT by pressing F1 and then F10 then enter the Access Code Enter ← to run QC.
3	Load patients' samples: Access the sample drawer(s) through the MAIN MENU under LOADING select Sample then press Enter ←. After the drawer opens, identify the type of specimen, such as micro sample, (press F8), or stat, (press F12). Identify the sample by bar-coding or manually entering on the keyboard the patient identification number and then placing the specimen into the drawer.
4	In MANUAL MODE, the operator must order the test(s) from the selection menu or from the recorded profiles. Cursor to the test or Recorded Profile and press Enter ← to select. When all tests are ordered, press F10 to save.

8.2	Analytical Procedure
5	In AUTO MODE, the STA [®] /STA [®] Compact will automatically order the test(s) selected in the AUTO MODE profile.
6	If TELELOADING is selected as the AUTO MODE profile, the STA [®] /STA [®] Compact will query the host computer and download the test(s) as well as assign the status (i.e. stat).
7	As soon as the sample drawer closes, the TEST STATUS screen will appear. If there is not enough reagent(s) to run the test(s), the suspect reagent(s) will appear in red with the amount of depletion. This depletion of reagent will BLOCK the SAMPLE PIPETTING. When this occurs, add the necessary reagent(s) to run the samples by responding N (NO) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?' Reagents can then be loaded in the drawer. By responding Y (YES) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?', the instrument will continue to perform all tests for which there is sufficient reagent (i.e. while waiting for reagents to stabilize after reconstitution)
8	All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the system status menu.
9	For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and press enter. This will display the FILE PROCESSING screen. Follow the options on the left-hand side of the screen (i.e. F3 - rerun test).

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

Results are rounded to the nearest whole number.

10.3 Units of Measure

Seconds

10.4 Clinically Reportable Range (CRR)

20 - 180 seconds

10.5 Repeat Criteria and Resulting

The printout from the STA Compact is reviewed for repeat criteria and samples are repeated if needed. Results will be transmitted to the LIS and released using the OEM function.

IF the result is ...	THEN...
> 80 seconds	Check for clots, but there is no need to repeat. Be sure the specimen is not under-filled or over-filled, then check the Hematocrit (HCT) result. If the HCT is greater than 55%, refer to appendices A and B for special tube preparation.
> Mmax	Check for clots, Repeat, Report results as >180 seconds and call the results.

< Mmin	Check for clots, Repeat. If same result is obtained, cancel with appropriate LIS English test code and request a recollection.
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Note: All patient results are resulted with the following comment:

Using the current lot of reagent, a PTT value of 65-110 seconds compares to Anti Xa values of 0.3-0.7 IU/mL. In patients in whom there is an apparent heparin resistance, a heparin level by an Anti Xa method is available.

11. EXPECTED VALUES

11.1 Reference Ranges

23 – 37 seconds

11.2 Critical Values

> 80 seconds

Note: The critical value for PTT is > 80 seconds, however, if a patient has a result between 80 seconds and 110 seconds, and that patient is on the heparin therapy list, then that result does **not** have to be called. Document in the computer that the result is consistent with the patient's history (use LIS code "HIS"). All PTT results >110 seconds will be called.

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

The activated partial Thromboplastin time (APTT) is a general coagulation-screening test of the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II, and fibrinogen). The main application of the APTT is to detect congenital and acquired deficiencies of these factors and to monitor the heparin therapy. The APTT is more sensitive and reproducible than other screening tests, such as plasma recalcification and partial thromboplastin time, due to the control of contact activation and platelet phospholipid concentration.

A prolongation of the APTT is encountered in the following situations. Congenital deficiencies of factors VIII, IX, XI, and XII, Liver diseases, consumptive coagulopathy, circulating anticoagulants, during heparin or oral anti coagulant therapy.

If an APTT monitored factor deficiency is present, immediate and incubated mixing studies will correct to normal. If an inhibitor is present, the immediate result may correct, but the incubated result will be abnormal.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

The STA uses electromechanical clot detection for the APTT test; therefore lipemia and icterus do not interfere with the APTT result. These findings should be reported with the result.

New lot of PTT-Automate: If **RATIO** is selected as CALIBRATION MODE in page 2 of test setup, the operator must enter the REFERENCE TIME before the STA® Compact will allow QC to be run. Through the MAIN MENU select CAL/CONTROL. Select CALIBRATION, press **Enter** ↓ Cursor to the APTT test, press **Enter** ← Press ESC key for options. Select MODIFY REFERENCE TIME/RANGE press **Enter** ← Type in your Access Code press **Enter** ↓ Enter reference time and save with **F10**. This is NOT necessary if the Calibration is set to **RAW** mode in test set-up (page 2).

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

20 – 180 seconds

14.2 Precision

Different plasmas were used for the intra-assay and inter-assay reproducibility studies on the STA®.

Sample	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
	Sample 1	Sample 2	Sample 3	Sample 4
n	21	21	10	10
mean (seconds)	30.8	52.7	31.6	54.7
SD (seconds)	0.20	0.53	0.44	1.46
CV (%)	0.7	1.0	1.4	2.7

14.3 Interfering Substances

- Many commonly administered drugs may affect the results obtained in APTT testing. (Example: heparin and coumadin).
- When monitoring heparin therapy, any release of platelet factor 4 (PF4) which is a potent inhibitor of heparin, represents a potential source of error.
- A congenital or acquired inhibitor may prolong the APTT.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Any release of platelet factor 4 (PF4), which is a potent heparin inhibitor, will lead to false APTT values. Careful and adequate centrifugation is essential, the higher the level of residual platelets, the greater the risk of PF4 release.

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Laboratory Safety Manual
3. Material Safety Data Sheets (MSDS)
4. **Hemolysis, Icteria and Lipemia Interference** (Lab policy)
5. Repeat Testing Requirement (Lab policy)
6. **Critical Values** (Lab policy)
7. **STA Compact Operating Instructions**, Coagulation procedure
8. Verification of Platelet Poor Plasma, Coagulation procedure
9. Current package insert for STA[®] PTT-A

17. REFERENCES

1. STA[®] PTT-A Determination of Activated Partial Thromboplastin Time (APTT) Package insert for use in APTT determinations. 24528 - Revised 05/2011.
2. STA[®]-Coag Control N + ABN (REF 00676): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA[®], Revised December 2009.
3. STA[®] Compact Operators Manual. STA[®] DSI-TSD-SM August 2004, STA[®] DSI-TSD-US April 2003, and V1.3 revised February 2003.
4. Reagents for STA[®] Compact Line, Reconstitution and Handling Information, revised 02/20/2009.
5. STA[®] 0.025 M CaCl₂ Solution for coagulation Tests Package Insert. Stago Diagnostics. Revised 05/2011
6. Quest Diagnostics Nichols Institute in Chantilly, VA. SOP ID QDHE716 Version 3.1, Coagulation Specimen Collection and Handling in 3.2% Sodium Citrate Blue Topped Tubes.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G005.006		

000	7/14/11	10.5	Change to match critical value	L Barrett	N Cacciabeve
000	7/14/11	11.2	Change in critical value	L Barrett	N Cacciabeve
000	7/14/11	15	Update to approved format	L Barrett	N Cacciabeve
001	2/15/12	10.5	Edited the result comment	A Chini	N Cacciabeve
001	2/15/12	11.3	Removed SGAH specific preop value	A Chini	N Cacciabeve
002	2/26/14	3.1	Add reference to Appendices	A. Chini	R SanLuis
002	2/26/14	3.2	Update tube volumes, add opened container storage	A. Chini	R SanLuis
002	2/26/14	4.2	Change storage temp for 0.025M CaCl ₂	A. Chini	R SanLuis
002	2/26/14	10.5	Add instruction for Hct >55, revise action for <Mmix	A. Chini	R SanLuis
002	2/26/14	16	Update titles	L Barrett	R SanLuis
002	2/26/14	17	Add references 5 and 6	A. Chini	R SanLuis
002	2/26/14	19	Add Appendix A and B	A. Chini	R SanLuis
002	2/26/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis

19. ADDENDA

- A. Instructions for Preparing Collection Tube for Hematocrit >55%
- B. Phlebotomist Instructions for Blood Collection

Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.

When a prolonged coagulation result is obtained, check the specimen for a clot first.

If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.

If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable. The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:

Anticoagulant in mL = $[(100 - \text{HCT}) / (595 - \text{HCT})] \times \text{Volume of blood}$

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = $[(100 - 70) / (595 - 70)] \times 2.7 = 0.15 \text{ mL or } 150 \text{ uL}$
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = $[(100 - 70) / (595 - 70)] \times 1.8 = 0.1 \text{ mL or } 100 \text{ uL}$
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = $[(100 - 60) / (595 - 60)] \times 2.7 = 0.2 \text{ mL or } 200 \text{ uL}$
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = $[(100 - 60) / (595 - 60)] \times 1.8 = 0.13 \text{ mL or } 130 \text{ uL}$
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.
13. Release the tourniquet. Ask the patient to open hand.

14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture site. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. **DO NOT FORCE** blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. **DO NOT** disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code.
22. Transport specimen to the Lab.