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ACL TOP Analyzer Maintenance Procedure

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Updated by S.DeMarinis, MT. (ASCP)

Revised and Adopted on 12/5/22 by Lab Director: J. Mills Barbeau MD

Annual Review:

Reviewed by Medical Director	Date	Reviewed by Medical Director	Date

Revisions:

Bristol – MARI 1180 Hope Street Bristol, RI		**East Greenwich CC** 1454 S. County Trail East Greenwich, RI
Miriam Hospital 164 Summit Avenue Providence, RI	**Newport Hospital** 11 Friendship Street Newport, RI	**Rhode Island Hospital** 593 Eddy Street Providence, RI

Purpose

To ensure proper operation of the ACL TOP Analyzers, the following daily, weekly and monthly maintenance procedures will be performed by the laboratory staff.

Annual and Semi-Annual Maintenance is performed by authorized Service personnel every 6 months (including syringe tip replacement at 6 month intervals). Documentation of these maintenance procedures is stored in database of each analyzer.

Principle

- The following maintenance procedures will be performed on the ACL TOP 300, 500 and 700 instruments. Procedures must be performed daily, weekly and monthly for optimal performance of instruments. Documentation of maintenance is stored in analyzer database.
- Technologists have a unique log on for analyzer operation and all testing of specimens and quality control. Operator Identification is maintained throughout analyzer use. Technologists update their operator identification at the start and end of their working shift.

- Review of maintenance procedures is performed monthly and recorded by tech specialist(s) on documentation of review form (included in this procedure) and stored in Maintenance Review log book located in Coagulation lab stat area along with instrument service reports.

Reagents

HemosIL Cleaning Solution (Clean A Solution: Acid:), 0.1N HCL, Instrumentation Laboratory
Ready to use, in original container. This solution cleans analyzer probes. Stable until manufacturer's expiration when stored or is use at room temperature in original container. Cleaning Solution (Clean A) must remain on instrument at all times;

Replace Cleaning Solution as needed, when analyzer sends alarm signal (red exclamation point), indicating level has reached 25 ml. Analyzer will self prime after replacement

HemosIL Critical Care Cleaning Agent (Clean B Solution: Base), Sodium Hypochlorite, Instrumentation Laboratory

Caution: Corrosive material. This concentrated solution is used to perform enhanced cleaning of probes and must be removed from instrument immediately after daily cleaning procedure. Store with cap in glass vial. Stable until manufacturer's expiration date.

HemosIL Rinse Solution, Instrumentation Laboratory
Ready to use in original container. Stable until manufacturer's expiration when stored or is use at room temperature in original container.
Replace daily and as needed, when instrument sends alarm signal (red exclamation point) indicating level has reached 600ml. Analyzer will self prime after replacement.

Daily Maintenance Procedure : ACP TOP: Day shift

Daily Maintenance includes the following procedures:

- Enhanced Cleaning of Probes
- Prepare and load new vial of "Dilute Clean B' Solution
- Change Factor Diluent
- Empty Liquid waste and cuvette waste

A. Perform "Enhanced Clean for All Probes"

1. Purpose ; Daily deep cleaning of sample and reagent probes:

   Load one full 10ml vial of **Critical Care Cleaning Agent** (Clean B) into each instrument location as follows:

ACL TOP 300
Load 2 vials:
One vial in D1 +
One vial in R1 (or R2 or R3 or R4)

2. Select: Menu Bar > System> Maintenance

   Select: Enhanced Clean for all Probes> Perform (running man with wrench icon)

   When task is complete, comment window will appear:
   Enter your initials in comment Window >select OK

3**. Immediately remove all vials of Critical Care Cleaning Agent** (Clean B) **from instrument**. Cap tightly and store at room temperature. Failure to remove this concentrated cleaning solution will cause corrosion of instrument.

B. Prepare 1:8 "Dilute Clean B Solution" for continual use on the instrument

1. To prepare 1:8 Dilute Clean B Solution:

   Add 2.0 ml HemosIL Critical Care Cleaning Agent (B) + 14.0 ml distilled water into 20 ml glass vial with "Dilute Clean B' barcode label.

2. Remove expired Dilute Clean B solutions from instrument and discard.

3. Load two vials freshly prepared Dilute Clean B Solution onto each instrument as follows: To prevent carryover of dilute clean B solution into other reagents during instrument initializing process:

USE ONLY DESINATED VIAL POSITION OF THE APPROPRIATE RACK.

ACL TOP 300
One vial in position 6 of row R4

4. Update Maintenance records as follows:
   From Screen
   Select >Daily Maintenance
   Select> "Make Fresh Clean B Diluted"> Perform (running man with wrench icon)
   Enter your initials in comment Window >select OK.

5. Diluted Clean B Solution must remain on instrument at all times for reagent probe cleaning to occur during test procedures. Instrument will not run in absence of "Dilute Clean B solution".

C. **Change Factor Diluent:**

1. Remove the white plastic barcoded vial that contains Factor Diluent solution.

2. Discard remaining diluent, rinse vial with distilled water, and allow to thoroughly dry.

3. Fill a clean/dry white plastic barcoded vial with at least 10 ml Factor Diluent solution.

4. Load into Diluent Rack as follows:

ACL TOP 300
One vial in Row D1*

*Additional vials of factor diluent may be added for special coagulation tests as needed.

5. Update Maintenance record as follows:

From Screen > Daily Maintenance;
Select> Change Factor Diluent> Perform (running man with wrench icon)
Enter your initials in comment Window >select OK.

D. Liquid Waste Container; Day Shift and as needed

1. The 10 liter water container holds the fluid waste that is pumped from the accumulator.

2. Empty this container each day, and as needed during the day, when alarm warning sounds. If allowed to fill completely during testing, analyzer will perform emergency stop, testing will suspend and operator must empty waste container to allow testing to resume.

3. Discard liquid waste in sink, **rinsing with copious amounts of water to prevent build up of Sodium Azide;** Allow tap water to run 2 minutes or more, to completely flush rinse liquid through the drain.

4. Replace container and cap; check tubing for large air bubbles, blockages or kinks and remove if necessary.

5. Empty waste while instrument is idle.

6. If liquid waste is emptied while analyzer is running, the container must be replaced within 3 miuntes of removal or analyzer will initiate "emergency stop" and "Recovery" must be performed to resume operation.

E. Empty Cuvette Waste: Midnight shift and as needed

1. Cuvette drawer may be emptied any time; even while instrument is running.

2. Remove the cuvette waste drawer and discard used cuvettes in biohazard waste bin as needed.

3. Instrument will signal with alarm when waste drawer is nearly full.

4. Instrument will place itself into "Controlled stop" and operation will pause if drawer is completely full;

5. Change while instrument is idle or running.

6. If instrument is running, replace cuvette drawer within 3 minutes of removal, or analyzer will "emergency stop" and a"Recovery" must be performed to resume operation.

Maintenance As Needed: Replace Clean A and Rinse Solution

1. Important: Replace Clean A and Rinse Solution ONLY when a Material Error alarm has occurred.

2. Change these solutions ONLY when status of fluids is indicated as an alarm "red exclamation point". Automatic priming of solutions to remove air bubbles from the rinse lines occurs only after container is changed when status light is "red".

- Amber warning alarm indicates that Clean A has reached <75 ml; Rinse has reached 1000 ml.
- Red warning alarm indicates that Clean A has reached 25 ml ; Rinse 600 ml.

Weekly Maintenance Procedures

A. Clean Cuvette Waste Drawer

1. Select Menu Bar> System> Maintenance

   Select Clean Cuvette Waste Drawer> Perform (running man with wrench icon)

2. Remove the used cuvette waste drawer liner and replace with a clean liner. Select >OK.

3. Enter your initials in Comment window ; Select>OK.

4. Discard the used cuvettes into the biohazard waste bin. Use a scoopful of powdered Buell laboratory cleaner to wash the waste drawer liner. Allow to dry at room temperature. Replace with clean liner and resume operation.

B. Clean Deep Wash and Cup Area

1. From Menu Bar Select> System> Maintenance

2. Select> "Clean Deep Wash and Clean Cup Area" > Perform (running man with wrench icon)

3. Open the Sample area cover.

4. Using a LINT free cotton swab, clean the deep wash and clean cup as follows:

5. Rinse both areas with a maximum of 10ml distilled water to remove debris. Use a transfer pipet or small 10 cc syringe with tubing to rinse areas.

6. Using LINT free cotton swab, thoroughly dry the cover and the metal ring of the wash deck around the deep wash well clean cup.

7. Select OK at the prompt to indicate that these maintenance procedures have been performed. Open the cover(s), perform the maintenance work, close the covers(s) once again and press OK.

8. Enter your initials in the Comment window> select OK.

9. Wait: The instrument will initialize by checking volumes of reagents, setting probes and equilibrating temperatures, before it can be used for testing.

Monthly Maintenance Procedures

The following maintenance procedures will be performed on the ACL TOP instruments.

- Database Back up Procedure
- Computer Shutdown Procedure

  Refer to procedures for Database Backup, create a backup CD in this manual.

Annual and Semi-Annual Maintenance

Preventive maintenance is to be performed by authorized Service personnel every 6 months (including syringe tip replacement at 6 month intervals).

References:

ACL TOP Instrument Training Manual (2010).

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Normal Reference, Reportable Results and Critical Values

Prepared by: S.DeMarinis MT, (ASCP)
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Adopted on 12/9/22 by Lab Director Dr. J. Mills Barbeau MD

Annual Review:

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Normal Reference, Reportable Results and Critical Values

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Clinical Significance:

The following parameters have been established as guidelines for reporting (highest/lowest acceptable) values for PT, PTT, and D-Dimer tests.

These values are also located in the LIS Help screen for each test. The following limitations are provided for each test.

Method:

See "Critical Results" procedure for complete instructions for contacting clinical staff regarding PTT, INR, critical results.

Procedure		**Normal Reference**	**Highest Reportable Value**	**Lowest Reportable Value**	**Critical Value**
PT	PT	10.0-13.0 sec	>100.0 sec	<5.0 sec	Not Applicable See (INR)
INR	INR	0.8-1.2	>12.0	<0.5	>5.0 **Call all Results**
PTT	PTT	24.0-37.0 sec	>200 sec	<8.0 sec	>135.0 sec **Call all Results**
D-dimer	DDIM1	0-300 ng/ ml	>60,000 ng/ ml	21 ng/ml	Not Applicable
Ddimer ER Patient	DVTD1	Cut off PE/DVT <230 ng/ml	>60,000 ng/ ml	21 ng/ml	Not Applicable

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APTT
Activated Partial Thromboplastin Time

Prepared by: S. DeMarinis MT (ASCP)

Adopted on 12/5/22 by Lab Director Dr. J. Mills Barbeau JMBarbeau MD

Annual Review:

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APTT Activated Partial Thromboplastin Time

Clinical Significance:

The Activated Partial Thromboplastin Time (APTT) is a recommended parameter for the monitoring of the anticoagulant effects of Heparin, the presence of inhibitors, especially the Lupus Inhibitor and deficiencies of XII, XI, IX, VIII, X, II, I. A prolonged APTT and PT may be an indicator of early liver malfunction, Disseminated Intravascular Coagulation, and single or multiple factor deficiencies.

Principle:

The APTT (APTT/PTT Screening test) evaluates the function of the intrinsic and common pathways of the coagulation cascade. The HemosIL reagent SynthASil is a liquid buffered reagent that contains synthetic phospholipid and a non-settling colloidal silica for optimal activation of contact phase of coagulation. After incubation at 37 degrees C, addition of Calcium Chloride initiates clotting. SynthASil is sensitive to deficiencies of Factors XII, XI, IX, VIII, X, II, I, the anticoagulant effects of heparin and the presence of inhibitors, especially the Lupus Inhibitor.

Verification of the a no endpoint APTT-SS clotting time is performed on the ACL TOP instrument using APTT-SS(E) extended time clot detection and review clot signature, followed by repeat testing using the "short test. The "Short PT" test uses a reduced instrument blank time (5 sec), to distinguish a no endpoint PTT (>200sec) from an unusually short PTT (<8 sec)

Verification of APTT results that are accompanied by error codes and/or unacceptable clot signatures will be verified using the STArt mechanical detection instrument. (Procedure for performing APTT on this instrument is found in the STArt Procedure manual)

Method:

Clot Detection Method: In the Activated Partial Thromboplastin test, the addition of APTT activator reagent (negatively charged silica and synthetic phospholipids) to patient plasma in the presence of calcium ions (Calcium Chloride reagent), initiates the activation of the intrinsic (clotting) pathway. The results in the conversion of fibrinogen to fibrin, with the formation of a solid gel (clot). The solid gel (clot) is detected by the change in absorbance of light detected by the analzyer, and is reported as the Activated partial Thromboplastin Time (APTT) in seconds.

Equipment:

APTT Activated Partial Thromboplastin Time

ACL TOP Family Analyzer: 300, 550, 500, 750, 700 series

Specimen:

3.2 % Citrated platelet poor plasma. Stability is 4 hours at 24-27 degrees. Specimens are maintained at 15 degrees while stored on the instrument. Upon test completion, specimens should be removed from the instrument and stored at room temperature.

Specimens which cannot be assayed within 4 hours of collection should be centrifuged 20 minutes/3500 rpm and frozen at -70 C. Frozen plasma samples should be rapidly thawed at 37^{o}C while gently mixing. After thawing the assay must be performed within 2 hours.

Reagents:

HemosIL SynthASil APTT Reagent (Instrumentation Laboratory)

Ready to use. Each vial must be equilibrated at 15-25 degrees C for at least 15 minutes before use. Mix well (by inversion 3-4 times) just before placing reagent on the instrument to ensure homogeneity.

Stability: Once opened, the reagent is stable for 3 days at 15 degrees C when store in the original vial, on the instrument or 30 days when stored at 2-8 degrees C. Remove reagent from the instrument only when it has expired, its quantity is insufficient to perform testing or quality control failure due to reagent has been demonstrated.

HemosIL SynthASil Calcium Chloride (Instrumentation Laboratory)

Ready to use. This reagent is specifically matched to the kit lot number for optimum performance. Do not substitute any other calcium chloride reagent or lot number.

Stability: Opened reagent is stable for 30 days at 2-30 degrees C.

HemosIL Normal Control, Assayed (Instrumentation Laboratory)

Reconstitute with 1.0 ml deionized water using automatic pipet. Allow to stand for 30 minutes at room temperature to assure complete hydration. Swirl gently to mix before use. Do not shake. Stability is 24 hours when stored at 15 degrees C on the analyzer in rack D1.

HemosIL High Abnormal Control, Assayed (Instrumentation Laboratory)

Reconstitute with 1.0 ml deionized water using automatic pipet. Allow to stand for 30 minutes at room temperature to assure complete hydration. Swirl gently to mix before use. Do not shake. Stability is 24 hours stored on the analyzer in rack D1.

Clean B Diluted Solution:

1: 8 dilution Critical Care Cleaning Agent (Sodium Hypocholorite), (Instrumentation Laboratory)

Prepare Daily: 2.0 ml Critical Care Cleaning Agent + 14.0ml distilled water in appropriate glass vial. Stability is 24 hours at 15 degrees C while on the instrument.

Must remain on instrument reagent rack in order for all assays to run.

APTT Activated Partial Thromboplastin Time

Reagents/Controls	ACL TOP 300/500/550 Location	ACL TOP 700/750 Location
Controls Nl, Low, High	D1	D1, D2
HemosIL SynthASil APTT Reagent	R1, R2, or R 3	R1, R2, R3
HemosIL SynthASil Calcium Chloride	R1, R2 or R 3	R5, R6
Clean B Solution: (1:8 Dilution)	One vial in position 6 of R4	One vial in position 6 of R1 AND One vial in position 6 of R6

Test Procedure: APTT-SS

1. Load reagents into appropriate locations onto ACL analyzer (see chart above). Testing will only occur if reagents/controls and cleaning solutions are on board instrument locations and quantities.

2. When testing begins, the instrument sample probe pipets 0.05 ml of control (or specimen) into a cuvette and then 0.05 ml of Synthesil reagent warms it to 37 +/- 1 degrees C, then 0.05ml Calcium Chloride is added by the reagent probe, into the cuvette and timing for clot detection simultaneously begins.

3. APTT-SS is performed in single determination testing; Results are reported in sec, to the nearest whole number (ie 123.0).

4. APTT-SS clot detection occurs for 120 seconds. Results (sec) are displayed on test screen and downloaded by the LIS. Results are auto-verified by the LIS, or manually by the technologist, as defined by the limits established by this laboratory.

4. Repeat testing is automatically performed by the instrument when APTT-SS > 120 sec.

5. APTT-(E): APTT-SS Extended test
If a clot endpoint is not detected within 120 second detection time, APTT-SS is automatically repeated by the instrument with extended clot endpoint detection time up to 400 sec; Instrument screen displays APTT-(E) when extended test is in progress

6. Verification of APTT-SS no endpoint clotting time is performed as described in this procedure using evaluation of “Extended APT”T-SS and “Short APTT” clotting times and review of clot signature.

Quality Control:

1. HemosIL Normal and High Abnormal Controls are assayed prior to any patient specimens and will be assayed automatically by the instrument every 8 hours, as needed.

2. Controls must fall within the ranges specified by the laboratory. The normal control and abnormal control must fall within established limits in order to report patient results.

3. Controls that fail to fall within the specified ranges will be automatically repeated by the instrument when specimens are assayed.

4. Controls are assayed automatically whenever new reagent is placed on the instrument.

- New vials controls and/or reagent should NOT be placed in same locations(s) as vials currently in use. Different location is a signal to analyzer that the vials are freshly made ,reagent and QC will be run automatically.

- Replace SynthAsIL and CaCl2 reagent or controls when "low reagent"notification alarm occurs.

5. Controls are assayed by the technologist performing the testing and are tracked by the instrument database via technologist log on procedure.

To order QC: APTT-SS

1. From Menu Bar

- Select: QC > Results List
- Double click (left side of screen) on any test in the test selection column
- Select Material/Tests Definition Tree
- Select the check box next to controls for test: **APTT-SS**
- Select "Program QC" (running man icon) to start analysis

  - To remove all previously selected test requests:
    - From the Menu Bar: Select Action
    - Hover on "QC", scroll down to locate "Clear QC selections"
    - Click on "Clear QC Selections"

2. All levels of Control for the selected test will be automatically assayed using single determination testing.

3. QC results are displayed on instrument test screen and may also be printed by the instrument.
4. QC Failure alarm will sound and icon will appear in status area of the instrument screen in the event of any failed control.

- To view/repeat QC: Click on the alarm icon at bottom of screen;
- Click on message to view message & determine the test and level of failed control
- Select "QC Results List"
- Clear previous selections
- Select the individual level of control to be repeated
- Select Program QC (running man) icon to start test

5. Patient specimens may be assayed, when control values fall within established limits.

To assay Patient Specimens:

1. Load patient specimens into appropriate sample racks (see below). Be sure entire barcode (top to bottom) is facing outward.

2. Tubes with blue hema-guard vacutainer caps on MUST be run in BLUE tabbed, Closed Tube System rack. The instrument has cap-piercing ability for this type of tube.

3. FAILURE TO USE BLUE TABBED RACK FOR CAPPED TUBES WILL RESULT IN PROBE DAMAGE, ASPIRATION ERRORS OR INSTRUMENT FAILURE.

4. Un-capped vacutainer specimen tubes and plasma in pour tubes may be run in appropriate BLACK open tube (OTS) rack as follows:

Tube	**Rack**	**Cap**
Capped Blue top vacutainer 4.5, 3.2 ml, 2.7 ml	Blue Closed Tube System (CTS) Sample Rack	CAP ON
Uncapped Vacutainer tube **or sample cup**	Yellow rack: Black Open Tube System (OTS) Sample Rack:	**CAP OFF**
12x75 mm polypropylene tube	Yellow rack: Open Tube System (OTS) **Sample Rack with Adapter**	**CAP OFF**

5. Load racks into Specimen rows (S tracks):
   - Barcodes on specimen tubes will be scanned and the appropriate test will be performed by the instrument.
   - Specimens that are not identified will be displayed on screen with "?" symbol. Remove rack, reposition or reprint specimen/label and insert into instrument.

6. Testing is performed in single determination;
   - Clot endpoint is determined when all four parts of clot signature are present
   - Delta Absorbance (mAbs) (change in Optical Density) for clot formation is APTT-SS $\geq$10
     - (View the y-axis of clot signature screen for actual values)

   - Vertical line indicating calculation of endpoint is present
   - No error flags are present: ie SE, HE, RE, CE, RW

7. **Automatic APTT-SS repeat testing** with extended endpoint detection APTT-SS(E) up to 400 seconds is performed by the instrument in the event of

   - APTT-SS with no result within 120 sec clot detection time

8. . APTT-SS (E) "FAILED" result message indicates **a clot was not detected during 400 second detection time;**

Results may be due to:
- APTT-SS result that has an endpoint >120 sec and <400 sec
- APTT-SS result is >400 and/or has no endpoint
- APTT-SS result that is unusually short (< 8 sec) and occurred during the instrument blank time

9. "FAILED" results must be verified by the following procedure to determine if APTT-SS is a no endpoint clot or an unusually short clot.

Verification of "Failed" APTT-SS Result

1. APTT-SS "FAILED" result message: indicates **a clot was not detected within 120 second detection time;**

   APTT-SS Extended "FAILED" test result indicates that a clot was not detected within 400 sec extended

2. To confirm the No Endpoint APTT, and differentiate it from unusually short clotting time, the instrument will automatically perform the "Short APTT" test on the ACL TOP

- During the "Short PTT" test, the instrument uses 10 measurements4/sec in the first few seconds of testing to search for the development of an unusually short APTT clot (ie 8 sec).

3. "FAILED" s (seconds) result may be accompanied by
" CE", Coagulation Error Flag
"RE" Result error Flag

4. To differentiate a No Endpoint APTT-Ss result from an unusually short clot APTT, be sure there are no other instrument error flags and then view the clot signature for this specimen.
Review the APTT-SS Extended and "Short PTT" Clot Signatures:

- Click on sample Extended result
- Select the Details icon (magnifying glass with test tube),
- Select the More details icon (Magnifying glass with multiple test tubes)

5. Interpret/Evaluate the Clot Signature
   - Look for presence or absence of the 4 stages of clotting reaction (blue line):
     Baseline, acceleration, deceleration, end

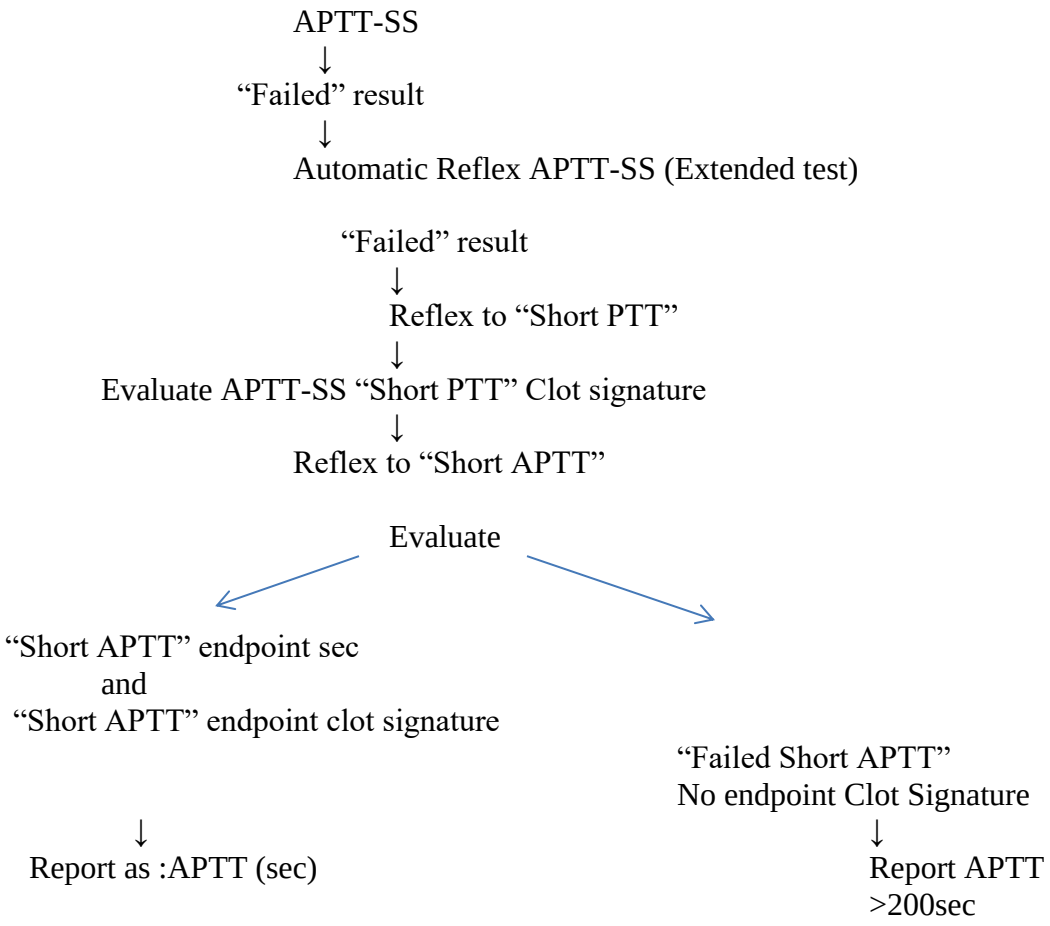
- Look for presence or absence of red vertical line detecting the clotting endpoint for clot signature
  A valid clot signature must be present to release results.

6. **A VALID No endpoint APTT will have the following characteristics:**

- "Failed" APTT result within 400.0 sec testing
- CE (Coagulation Error) flag
- Lack of 1 or more stages of a complete clot signature
- No red vertical line to determine endpoint for the clot signature.

7.Report APTT-SS as >200 if no clotting endpoint is detected The parameters for a valid clot and the clot signature are acceptable and consistent with no endpoint parameters. See attached examples of acceptable clot signatures included with this procedure.

8.Report APTT-SS in sec if unusually short APTT endpoint is detected



9. For all short APTT's, critical APTT and no endpoint APTT clotting times, check to be sure there is no clot in the plasma or red cell mass of the specimen by inserting 2 applicator sticks down to the bottom of the specimen and withdrawing them slowly. Perform this step using a plexiglass shield or goggles to protect from splatter or spray.

10. Examples of valid endpoint and No endpoint clot signatures can be found at the end of this procedure.

Data/Error Flags:

1. Under normal conditions, when samples are displayed on screen with no data flags, patient results may be reported.

2. Error Flags are displayed in capital letters on sample list/results screen indicating that an error
If a test and/or sample has multiple flags/error codes, the error with the highest priority is displayed with both capital letters underlined to indicate there are more flags.
3. All applicable flags and codes can be viewed, along with detailed explanation, by clicking on error code and then viewing explanation in "Details".
Common Error Flags:

- Coagulation Error (CE)
- Coagulation Warning (CW)*
- Sampling Error (SE)
- Mechanical error (ME)
- Hardware error(HE)

4 Each flag, material alarm and instrument alarm has its own numeric code number. View details to read the exact description type of error, and suggested action to resolve.

***CW Error: Do not report results in presence of a CW (Coagulation Warning). This message indicates the presence of an unusual clot signature. ACL TOP results may be reported if the test is repeated on the STArt instrument and the results are +/- 10% of TOP results.**

***The STArt mechanical clot detection process confirms that the TOP clot signature is valid.**
(See APTT Procedure in STArt instrument Manual.)

5. Interferences:
Gross hemolysis, bilirubin or lipemia rarely interfere with clot detection by the ACL TOP. In a rare instance, a clotting endpoint may not be detected if the instrument fails to detect a significant delta value (change in optical density) or a clot signature that meets the instrument parameters for determining a clot endpoint. **Delta Optical Density for clot formation APTT-SS ≥10.**

Testing may be repeated on the STArt instrument to obtain a result when interference prevents clot detection by the ACL TOP analyzer. See STArt procedure.

. Lipemia: In rare instances, excessive lipemia may interfere with testing. Specimens from patients receiving TPN (nutritional supplements) may be re-drawn when TPN infusion does not interfere with specimen collect time.

Specimens that cannot be re-drawn may have some lipids removed by high speed-ultra-centrifugation as follows.

a. Ultra centrifugation can be performed in the chemistry area: Chemistry tech will prepare 0.5 ml ultracentrifuged plasma-place into cup, use a tab yellow rack onto ACL TOP and repeat testing,
b. The supernatant (top) layer is discarded and the plasma should be removed to pour off tube.
c. If interference persists, perform testing on STArt (mechanical detection) insturment. If unable to obtain a result, report result as ".SEE NOTE" with Unable To Report (UTR) footnote as appropriate explanation in the LIS. Obtain repeat specimen for testing.

APTT Results:

1. The PTT is reported in time in seconds, rounded to the nearest whole number.

2. Reportable Results:

Auto-verification of PTT results occurs ONLY when

- Patient results are normal or abnormal, but do not fall within the limits established for critical values
- Quality control for the specified assays falls within limits established by the laboratory
- Results are **not** accompanied by clot detection error codes, such as critical value/panic message, short clot, no endpoint clot, or absurd result
- Results are **not** accompanied by analyzer sample error message, sample processing error or analyzer maintenance or failure message.

- PTT results that are auto-verified by the LIS are accompanied by the tech name "I/AUT".

(APTT-SS) Reportable Results: PTT
PTT (seconds)
Normal Reference Interval: 24-37 sec
Lowest Reportable PTT < 8 sec
Highest Reportable PTT > 200 sec
Critical Result: > 135

4. **No Endpoint Clot: Report the PTT as >200 seconds,**
This is a critical result, notify the nurse or doctor caring for the patient and report the results in the LIS with the appropriate critical result footnote.

5. **Unusually Short PTT: Report PTT as < 8 seconds if a short clot** that occurs has been detected:

- Ensure Specimen is not clotted or QNS; Check for clot

These parameters indicate that a "short clot" < 8 seconds occurred during the instrument blank time after Calcium Chloride reagent was added to test sample.

6**. UTR: Report PTT results as UTR (Unable To Report)** if any of the following criteria apply to the specimen:

If any of the following criteria apply to the specimen do not report results; request a new specimen for repeat testing:

- Gross hemolysis, icterus or lipemia that causes unreproducible results and/or interference with clot formation
- Unable to obtain reproducible results (+/- 10%) with duplicate tests
- Interference observed in clot signature and/or unable to obtain reproducible result on (+/- 10%).
- Unable to obtain reproducible results detected using ACL TOP and STArt Instrument (ie STArt has endpoint PT, ACL TOP has no endpoint detected)

References:

ACL TOP Operator's Manual, Instrumentation Laboratory (2010).

Package Inserts, HemosIL SynthASil (6/2017), HemosIL Normal (4/2018), Abnormal High Controls, 6/2017.

Clinical and Laboratory Standards Institute (CLSI). Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis asssys– 5th Edition; Approved Guideline. NCCLS document H21-A5, 2008

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Prothrombin Time (PT/INR)

Prepared by: S.DeMarinis MT, ASC)
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Adopted on 12/5/22 by Laboratory Director Dr. J. Mills Barbeau JMB MD

Annual Review:

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Clinical Significance:

The Prothrombin Time/INR (International Normalized Ratio) is a recommended parameter for monitoring oral anticoagulant (Coumadin) therapy and is an indicator of early liver malfunction, Disseminated Intravascular Coagulation, and single of multiple factor deficiencies of VII, X, V and II.

Principle:

The Prothrombin Time tests the function of the extrinsic and common pathways of the coagulation cascade. The reagent used in the ACL TOP Prothrombin Time (PT-RP) assay RecombiPlasTin 2G, is a high sensitivity thromboplastin reagent based on recombinant human tissue factor (RTF).

The thromboplastin reagent included in RecombiPlasTin 2G, is a liposomal preparation that contains human Recombinant Tissue Factor re-lipidated in a synthetic phospholipid blend combined with calcium chloride, buffer and a preservative. This reagent is formulated to be insensitive to therapeutic levels of heparin, does not contain any contaminating coagulation factors and is sensitive to factor deficiencies VII, X, V, II detected by the Prothrombin time test.

RecombiPlastinTin 2G has an ISI that is approximately 1.0 and is more sensitive than conventional rabbit brain reagents. The Prothrombin Time/INR (International Normalized Ratio) is a recommended parameter for monitoring oral anticoagulant (Coumadin) therapy and is an indicator of early liver malfunction.

Verification of the a no endpoint PT-RP clotting time is performed on the ACL TOP instrument using PT-RP (E) extended time clot detection and review clot signature, followed by repeat testing using the "short PT-RP test. The "Short PT" test uses a reduced instrument blank time (5 sec), to distinguish a no endpoint PT (>100 sec) from an unusually short PT (5 sec).

Method:

Clot Detection Method: In the Prothrombin Time test, the addition of tissue thromboplastin to patient plasma in the presence of calcium ions (RecombiplasTin 2G reagent), initiates the activation of the extrinic (clotting) pathway. The results in the conversion of fibrinogen to fibrin, with the formation of a solid gel (clot). The solid gel (clot) is detected by the change in absorbance of light detected by the analzyer, and is reported as the Prothrombin Time in seconds.

Equipment:

ACL TOP Family Analyzer: 300 series

Specimen:

3.2 % Citrated platelet poor plasma. Stability is 24 hours at 24-27 degrees. Specimens are maintained at 15 degrees while stored on the instrument. Upon test completion, specimens should be removed from the instrument and stored at room temperature.

Reagents:

HemosIL RecombiPlasTin 2G Reagent (Instrumentation Laboratory)

- Allow RecombiPlasTin 2G reagent and diluent to equilibrate at 15-25 degrees C for at least **15 minutes prior to reconstitution.**

- **Using automatic pipet, dispense 20.0 ml** RecombiPlasTin Diluent into RecombiPlasTin 2G lyophilized reagent.
- **Do not dispense entire diluent vial into lyophilized reagent. Reconstitution will be inaccurate.**

- Mix well (by inversion 3-4 times) to ensure complete hydration. Allow to stand for 30 minutes at room temperature. At the end of reconstitution time, gently invert 3-4 times to ensure homogeneity.
- Stability after reconstitution is 10 days at 15 degrees C when stored in reagent rack of the analyzer, or 10 days stored at 2-8 degrees in original vial.

HemosIL Normal Control, Assayed (Instrumentation Laboratory)

Reconstitute with 1.0 ml deionized water using automatic pipet. Allow to stand for 30 minutes at room temperature to assure complete hydration. Swirl gently to mix before use. Stability is 24 hours when stored in diluent rack in instrument track D1.

HemosIL High Abnormal Control, Assayed (Instrumentation Laboratory)

Reconstitute with 1.0 ml deionized water using automatic pipet. Allow to stand for 30 minutes at room temperature to assure complete hydration. Swirl gently to mix before use. Stability is 24 when stored in diluent rack in instrument reagent track D1.

Diluted Clean B Solution: 1: 8 dilution HemosIL Critical Care Cleaning Agent (Sodium Hypocholorite), (Instrumentation Laboratory)

Prepare Daily: 2.0 ml Critical Care Cleaning Agent (Clean B) + 14.0ml distilled water in appropriate glass vial.
Stability is 24 hours at 15 degrees C while on the instrument.
Must remain on analyzer in position 6 in order for all assays to run.

Reagents/Controls	ACL TOP 300 Location

HemosILControls Nl, High	D1
Recombiplastin PT Reagent	R1 only
Dilute Clean B Solution (1:8 Dilution)	One vial in position 6 or R1

Test Procedure: PT-RP

1. Load reagents into appropriate locations onto ACL analyzers (see chart above). Testing will only occur if reagents/controls and cleaning solutions are on board in appropriate instrument locations and quantities.

2. When testing begins, the instrument sample probe pipets 0.05 ml of control (or specimen) into a cuvette and warms it to 37 +/- 1 degrees C and then 0.1 ml of warmed RecombiPlasTin 2G is pipetted by the reagent probe into the cuvette and timing for clot detection simultaneously begins.

2. PT-RP is performed in single determination testing. Results are reported in seconds, to the nearest tenth of a second.

3. PT-RP clot detection occurs up to 100 seconds. Results (sec) are displayed on test screen and downloaded by the LIS. Results are auto-verified by the LIS, or manually verified by the technologist, as defined by the limits established by this laboratory.

4. Repeat testing is automatically performed by the instrument when INR $\geq$ 5.0.

4. PT-RP (E): PT-RP Extended:
PT testing with extended endpoint detection up to 320.0 sec is automatically performed by the instrument when

- INR $\geq$ 5.0,
- and/or PT $\geq$100 sec.

Instrument displays PT-RP (E) when extended test is in progress.

7. Verification of PT no endpoint clotting time is performed as described in this procedure using evaluation of "Extended PT" and "Short PT" clotting times and review of clot signature.

Quality Control:

1. HemosIL Normal and High Abnormal Controls are assayed prior to any patient specimens and will be assayed automatically by the instrument every 8 hours, as needed.

2. Controls must fall within the ranges specified by the laboratory. The normal control AND abnormal control must fall within established limits in order to report patient results.

3. Controls that fail to fall within the specified ranges will be automatically repeated by the instrument when specimens are assayed.

4. Controls are assayed automatically whenever new reagent is placed on the instrument.
   - New vials controls and/or reagent should NOT be placed in same locations(s) as vials currently in use. Different location is a signal to analyzer that the vials are freshly made, reagents and QC will be run automatically.

   - Prepare new Recombiplastin or controls when "low reagent" notification alarm occurs.

5. Controls are assayed by the technologist performing the testing and are tracked by the instrument database via technologist log on procedure.

To order Quality Control: PT-RP

1. From Menu Bar
   - Select: QC > Results List
   - Double click (left side of screen) on any test in the test selection column
   - Select Material/Tests Definition Tree
   - Select the check box next to controls for test: **PT-RP**
   - Select "Program QC" (running man icon) to start analysis

   - To remove all previously selected test requests:
     - From the Menu Bar: Select Action
     - Hover on "QC", scroll down to locate "Clear QC selections"
     - Click on "Clear QC Selections"

2. All levels of Control for the selected test will be automatically assayed using single determination testing.

3. QC results are displayed on instrument test screen and may also be printed by the instrument.

4. QC Failure alarm will sound and icon will appear in status area of the instrument screen in the event of any failed control.

    - To view/repeat QC: Click on the alarm icon at bottom of screen;
    - Click on message to view message & determine the test and level of failed control
    - Select "QC Results List"
    - Clear previous selections
    - Select the individual level of control to be repeated
    - Select Program QC (running man) icon to start test

5. Enter Corrective Action Comment for Failed QC results
    - From the QC results list, select the QC result (check mark will appear)
    - Enter the action take to resolve the QC failure and your intials (repeat qc, made new control, made new reagent etc)

6. Patient specimens may be assayed (single determination), when control values fall within established limits.

To assay Patient Specimens:

1. Load patient specimens into appropriate sample racks (see below). Be sure entire barcode (top to bottom) is facing outward.

2. Tubes with blue hema-guard vacutainer caps on MUST be run in BLUE tabbed, Closed Tube System rack. The instrument has cap-piercing ability for this type of tube.

3. FAILURE TO USE BLUE RACK FOR CAPPED TUBES WILL RESULT IN PROBE DAMAGE, ASPIRATION ERRORS AND INSTRUMENT FAILURE.

4. Un-capped vacutainer specimen tubes and plasma in pour tubes may be run in appropriate BLACK open tube (OTS) rack as follows:

Tube	**Rack**	**Cap**
Capped Blue top vacutainer 4.5, 3.2 ml, 2.7 ml	Blue Closed Tube System (CTS) Sample Rack	CAP ON
Uncapped Vacutainer tube **or sample cup**	Yellow rack: Open Tube System (OTS) Sample Rack	**CAP OFF**
12x75 mm polypropylene tube	Yellow rack: Open Tube System (OTS) **Sample Rack with Adapter**	**CAP OFF**

5. Load racks into Specimen rows (S tracks):
    - Barcodes on specimen tubes will be scanned and the appropriate test will be performed by the instrument.

- Specimens that are not identified will be displayed on screen with "?" symbol. Remove rack, reposition or reprint specimen/label and insert into instrument.

6. Testing is performed in single determination;
   - Clot endpoint is determined when all four parts of clot signature are present
   - Delta Absorbance (mAbs) (change in Optical Density) for clot formation is PT-RP $\geq$15
   - (View the y-axis of clot signature screen for actual values)
   - Vertical line indicating calculation of endpoint is present
   - No error flags are present: ie SE, HE, RE, CE, RW

7. **Automatic PT-RP repeat testing** with extended endpoint detection PT-RP (E) up to 320 seconds is performed by the instrument in the event of:

   - INR $\geq$5 and/or

   - PT-RP with no result within 100sec clot detection time

8. **PT-RP "FAILED"** result message indicates a clot was not detected during 100 second detection time;
   Results may be due to:
   - PT result that has an endpoint >100 sec and <320 sec OR
   - PT result is >320 and/or has no endpoint OR
   - PT result that is unusually short (< 5sec) and occurred during the instrument blank time

9. All "FAILED" results must be verified by the following procedure to determine if PT-RP is a no endpoint clot or an unusually short clot.

Verification of "Failed" PT Result

1. **To confirm the No Endpoint PT, and differentiate it from unusually short clotting time, "Short PT" test is automatically performed by the ACL TOP.**
   - During the "Short PT" test, the instrument uses 10 measurement/sec in the first few seconds of testing to search for the development of an unusually short PT clot (ie 5 sec).

"FAILED" s (seconds) result may be accompanied by
" CE", Coagulation Error Flag
"RE" Result error Flag

2. To differentiate a No Endpoint PT result from an unusually short clot PT, be sure there are no other instrument error flags and then view the clot signature for this specimen.

- Review the PT Clot Signature for the extended PT- RP(E) and the "short PT" tests: Click on sample Extended result
- Select the Test Details icon (magnifying glass with test tube)
- Select the Sample details icon (Magnifying glass with multiple test tubes)

a. Interpret/Evaluate the Clot Signature

- Look for presence or absence of the 4 stages of clotting reaction (blue line): Baseline, acceleration, deceleration and end

- Look for presence or absence of red vertical line detecting the clotting endpoint for clot signature
  A valid clot signature must be present to release results.

b. **A No endpoint PT will have the following characteristics:**

- "Failed" PT result with 320.0 sec testing
- CE (Coagulation Error) flag
- Lack of 1 or more stages of a complete clot signature
- No red vertical line to determine endpoint for the clot signature.

3. Report PT-RP as INR >12.0 if no clotting endpoint is detected

4. For all short PT, critical PT and no endpoint PT clotting times, check to be sure there is no clot in the plasma or red cell mass of the specimen by inserting 2 applicator sticks down to the bottom of the specimen and withdrawing them slowly. Perform this step using a plexiglass shield or goggles to protect from splatter or spray.

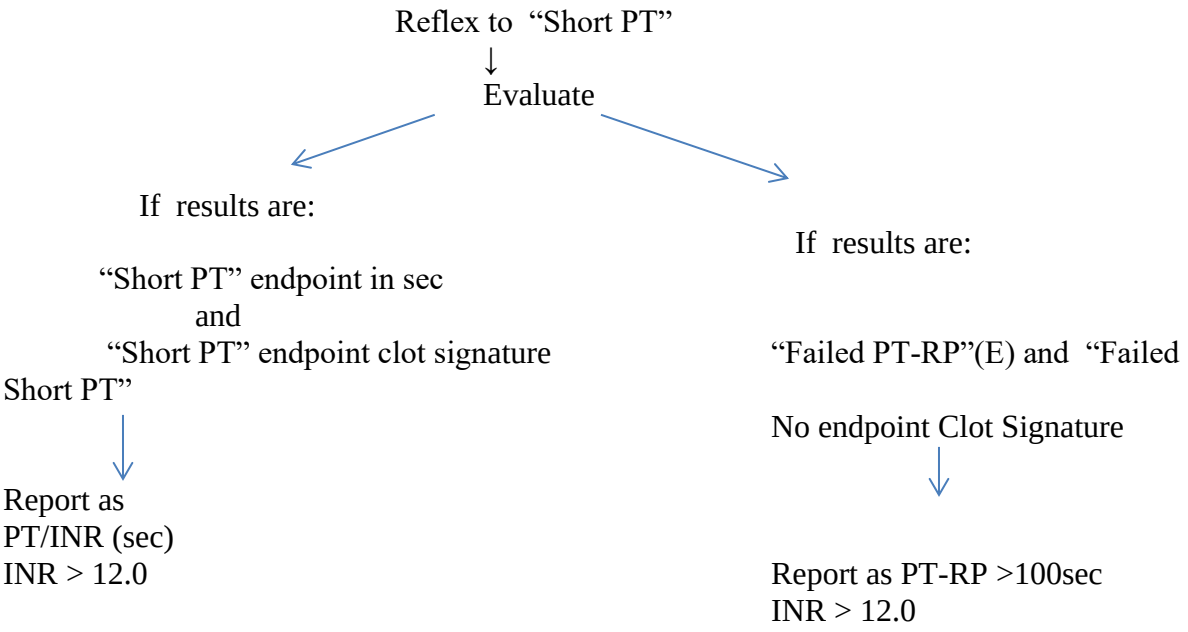
5. Examples of valid endpoint and No endpoint clot signatures can be found at the end of this procedure.

6. Report PT-RP in sec if unusually short PT endpoint is detected

PT -RP
↓
"Failed" result
↓
Automatic Reflex PT-RP (Extended test)
↓
If test is "Failed" result

Automatic Reflex to "Short PT"
↓
Evaluate PT-RP Extended Clot signature

↓



Data/Error Flags:

1. Under normal conditions, when samples are displayed on screen with no data flags, patient results may be reported.

2. Error Flags are displayed in capital letters on sample list/results screen. If a test and/or sample has multiple flags/error codes, the error with the highest priority is displayed with both capital letters underlined to indicate there are more flags.

3. All applicable flags and codes can be viewed, along with detailed explanation, by clicking on error code and then viewing explanation in "Details".

Common Error Flags:

- Coagulation Error (CE)
- Coagulation Warning (CW)*
- Sampling Error (SE)
- Mechanical error (ME)
- Hardware error (HE)

c. Each flag, material alarm and instrument alarm has its own numeric code number. View details to read the exact description type of error, and suggested action to resolve.

***CW Error: Do not report results in presence of a CW (Coagulation Warning). This message indicates the presence of an unusual clot signature. ACL TOP results may be reported if the test is repeated on the StArt instrument and results are**

+/- 10% of TOP results. Send specimen to main hospital lab for PT Procedure on STArt instrument Method.

*** The STArt mechanical clot detection process confirms that the TOP clot signature is valid.**

5. Interferences:

Gross hemolysis, bilirubin or lipemia rarely interfere with clot detection by the ACL TOP. In a rare instance, a clotting endpoint may not be detected if the instrument fails to detect a significant delta value (change in optical density Delta Optical Density for clot formation PT-RP ≥15) or a clot signature that meets the instrument parameters for determining a clot endpoint.

Send specimen to main hospital lab for testing to be repeated on the STArt instrument to obtain a result when interference prevents clot detection by the ACL TOP analyzer. See PT procedure STArt instrument manual.

6. Lipemia: In rare instances, excessive lipemia may interfere with testing. Specimens from patients receiving TPN (nutritional supplements) may be re-drawn when TPN infusion does not interfere with specimen collect time.

Specimens that cannot be re-drawn may have some lipids removed by high speed-ultra-centrifugation as follows.

- Ultra centrifugation can be performed in the chemistry area: - send specimen to main hospital lab for further testing: Chemistry tech will prepare 0.5 ml ultracentrifuged plasma-place into cup, use a yellow tab cup rack and load onto ACL TOP and repeat testing,
- The supernatant (top) layer is discarded and the plasma should be removed to pour off tube.
- If interference persists, send specimen to main hospital lab to perform testing on STArt (mechanical detection) insturment. If unable to obtain a result, report result as ".SEE NOTE" with Unable To Report (UTR) footnote as appropriate explanation in the LIS. Obtain repeat specimen for testing.

Results:

1. The PT is reported in time in seconds, to the nearest tenth of a second.

2. The PT (sec) is used calculates the INR (International Normalized Ratio) value that is used to monitor patients on stable anticoagulant therapy. The instrument

calculates the INR, and sends results to LIS. The INR result is report to the nearest tenth of a second. See Procedure for explanation and calculation of INR in this manual.

3. Reportable Results:

Auto-verification of PT results occurs ONLY when (no autoverification at Bristol Site)

- Patient results are normal or abnormal, but do not fall within the limits established for critical values
- Quality control for the specified assays falls within limits established by the laboratory
- Results are **not** accompanied by clot detection error codes, such as critical value/panic message, short clot, no endpoint clot, or absurd result
- Results are **not** accompanied by analyzer sample error message, sample processing error or analyzer maintenance or failure message.

    - PT results that are auto-verified by the LIS are accompanied by the tech name "I/AUT".

Reportable Results: Prothrombin Time (PT/INR)	
PT (seconds)	INR
Normal Reference Interval: 10.0-13.0 sec	Normal Reference Interval 0.8-1.2
Lowest Reportable PT < 5.0 sec	Lowest Reportable INR < 0.5
Highest Reportable PT > 100.0 sec	Highest Reportable INR >12.0
Critical Result: N/A	Critical Result > 5.0

4. **Critical Result: INR > 5.0**
   This is a critical result, notify the nurse or doctor taking care of the patient and report the results in the LIS with the appropriate critical result footnote.

    - The PT result (in seconds) is not reported as a critical result parameter.

5. **No Endpoint Clot: Report the PT as >100.0 seconds, INR >12.0**
   This is a critical INR result, notify the unit and report the results in the LIS with the appropriate critical result footnote.

6**. Unusually Short PT: Report PT as < 5.0 seconds if a short clot** has been detected during the instrument blank time. Ensure that the specimen is not clotted or QNS.

These parameters indicate that a "short clot" < 5.0 seconds occurred during the instrument blank time after Recombiplastin reagent was added to test sample.

7. **UTR: Report PT results as UTR (Unable To Report)**

If any of the following criteria apply to the specimen do not report results; request a new specimen for repeat testing:

- Gross hemolysis, icterus or lipemia that causes unreproducible results and/or interference with clot detection on the ACL TOP analyzer and the STArt instrument..
- Unable to obtain reproducible results (+/- 10%) with duplicate tests and on STArt instrument
- Interference observed in clot signature and/or unable to obtain reproducible result on STArt instrument.

References:

ACL TOP Operator's Manual, Instrumentation Laboratory (2010).

ACP TOP Instructional Webinar: Clot Signature Analysis (2011).

Package Inserts, RecombiPlasTin 2G (6/2017), HemosIL Normal (4/2018), Abnormal High Controls, (6/2017) .

Verification & Performance Procedure, INR Calculation (ACL TOP), pn V&P00007, Beckman Coulter, Inc., 2004.

Clinical and Laboratory Standards Institute (CLSI). Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis asssys– 5th Edition; Approved Guideline. NCCLS document H21-A5, 2008