Lupus Insensitive aPTT on the ACL TOP

I. PRINCIPLE

The activated partial thromboplastin time is a global screening procedure used primarily to evaluate coagulation abnormalities in the intrinsic pathway. The presence of non-specific inhibitors, such as the lupus anticoagulant may prolong the aPTT but this effect is variable and is generally recognized as being related to the nature of the aPTT reagent employed. Actin FS, the reagent used in this assay, is known to have a low sensitivity to the lupus anticoagulant and when compared to an aPTT using a sensitive reagent, can help to confirm or exclude the presence of a lupus anticoagulant.

In this assay, factors of the intrinsic coagulation system are activated by incubating the plasma with the optimal amount of phospholipids and a surface activator. The addition of calcium ions triggers the coagulation process, and the clotting time is then measured.

II. SPECIMEN

A. Sample Collection

Obtain venous blood while avoiding stasis and contamination of the specimen with tissue fluids. It is not recommended that blood for coagulation testing be drawn through arterial lines because heparin can contaminate the specimen.

Immediately mix the blood being certain that exactly 9 volumes of blood is mixed with 1 volume of anticoagulant. The anticoagulant of choice is sodium citrate 3.2%. The ratio of blood to anticoagulant is critical to obtaining precise results. Increased or decreased volumes of anticoagulant will result in falsely high or low results. All tubes must be checked for proper filling; acceptable ranges are as follows:

1. $2.7 \text{mL} \pm 0.25 \text{mL}$
2. $4.5 \text{mL} \pm 0.5 \text{mL}$

Note: The taller 100 x 13 mm tube is not designed to run on the ACL TOP. Plasma after centrifugation of these tubes must be placed in sample cups.

For patients with hematocrits above 55% (usually men, polycythemia patients or neonates) a special plastic tube containing 40% sodium citrate must be prepared and sent to the floor where the sample will be drawn. This is done to prevent falsely prolonged results caused by the
disproportionately high ratio of anticoagulant to plasma in patients with elevated hematocrits. Refer to the HEM 4.12.x, “Anticoagulation of specimens from patients with high hematocrits”.

B. Sample Preparation

Obtain a properly filled sodium citrate vacutainer tube.

For samples that are run fresh, it is not necessary to check the specimen for the presence of clots or fibrin strands as the clot curves for abnormal results will be reviewed and suspect samples checked for clots before results are released. In addition, samples with short clotting times of less than 24.0 SEC must be checked for clots or fibrin strands prior to results being released. Samples may be centrifuged at 3000 RPM s for 20 minutes to ensure platelet poor plasma (10K/ul or less) or they can be centrifuged in the stat spin (express) for a minimum of 2 minutes at 10,000 RPM. For specimens that will be frozen, check for clots and centrifuge the specimen at approximately 3,000 rpm for 20 minutes at 4°C to obtain platelet poor plasma (PPP). Transfer PPP to polypropylene tubes and perform a platelet count to verify that it is less than 10,000/µL. If not, the plasma must be re-spun until the desired count is obtained. Immediately place the PPP on ice. Aliquot into one mL cryotubes, place in ice block, and freeze at -70°C within four hours of centrifugation. Specimens may remain at -70°C for up to six months. When ready to use, the specimen is thawed in a 37°C waterbath for 5 minutes. Mixing of the sample is critical before testing, as precipitation of certain proteins may occur with freezing.

The allowable time between specimen collection and the centrifugation and removal of the plasma is 4 hours.

Technologists must wear gloves throughout the procedure.

III. REAGENTS

Dade Actin FS Activated PTT Reagent (pn B4218-20), purified soy phosphatides in ellagic acid with added buffer, stabilizers and preservative.¹

HemosIL Calcium Chloride, 0.020 mol/L (pn 20006910) in liquid form.²

The following are not supplied with the reagent and must be purchased separately.²

<table>
<thead>
<tr>
<th>Control Number</th>
<th>Code</th>
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<tr>
<td>HemosIL Normal Control 1 Unassayed</td>
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<tr>
<td>HemosIL Low Abnormal 2 Control Unassayed</td>
<td>20003220</td>
</tr>
<tr>
<td>HemosIL High Abnormal 3 Control Unassayed</td>
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A. Reagent Preparation and Storage

**Actin FS:** Store unopened at 2 - 8°C and use by the labeled expiration date. Once opened, the reagent is stable for 7 days at 2 - 15°C. **Do not freeze.** Before use, mix by inverting, and transfer reagent to IL 4 mL reagent bottle (pn 18924104). (Intermediate reagent. **Place in reagent rack R1-R3**)
Calcium Chloride: Opened reagent is stable for 30 days at 2 – 30°C. (Start reagent. Place in reagent rack R4-R6).

IV. PROCEDURE


2. Define Results Units and Rerun Rules in the Actin FS Test Definition if necessary (Setup, Tests, Result Units and Rerun Rules from the On-Line Help Manual).

3. Create/Edit QC files, if necessary (Setup, QC, QC List and QC Definition from the On-Line Help Manual).


5. Add the appropriate Actin FS reagent and Calcium Chloride to the Materials Programming Window if necessary (Setup, Display Configuration, Materials Programming Window from the On-Line Help Manual).


7. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the TOP in the D1 or D2 tracks. (If running the QC from the sample rack refer to Quality Control, Performing a QC Test in the On-Line Help Manual).

8. Choose QC from the Main Menu and select Test Status List. Double-click any QC name to show Test Materials Definition tree.

9. Select Actin FS QC Normal, Low, and High and choose the Run icon.

10. Place capped sample tubes in a cap-piercing sample rack with barcodes facing outwards. Select an available sample track and load the sample rack when the barcode reader is in position.

11. Place patient samples in sample cups and load onto a non-cap piercing sample rack. Go to the sample area and double-click on the off-line rack to access the Rack Details screen. The Sample Type field should be left on “Patient”. Click on the Sample ID field corresponding to the position of each patient sample in the rack and enter the accession number in numeric format.

12. Verify the samples have been identified and have a test ordered. If not, go to test box and add Actin FS test.
13. Choose the Run icon if the TOP is not currently running.²

For more instructions on loading samples without barcodes or LIS, please refer to Samples Analysis, Managing Patient Samples, Programming Bar Coded Samples and Programming Non-Bar Coded Samples in the OnLine Help manual.³

V. QUALITY CONTROL

Three levels of control material must be run on each shift during which this test is ordered. Values will be transmitted to the Sunquest LIS which automatically flags data that are outside of the acceptable limits (+/- 2 standard deviations). The section supervisor or designee will review Levy-Jennings Charts on the analyzer, which are used to detect trends.

If a control does not fall within the expected range, it should be rerun and/or made up again before proceeding with patient testing. If one or more control values are not within acceptable limits after rerunning or after reconstituting new vials of control and/or switching to new bottles of reagent, or if an obvious trend is visible on the Levy-Jennings Chart, do not proceed with testing. Switch to a back-up instrument to rule out instrumentation problems, and immediately notify the section supervisor on your shift.

Precision is to be performed twice a year on each instrument. Patient specimens with normal and abnormal high LPTT results are to be pooled separately, and separated into 10 sample cups for each level. Run 10 LPTTs on each level on each instrument. Controls can be substituted for patient samples. Calculate the CV% of all three levels for each instrument.

Expected Precision limits:
Within run - LPTT: normal level (<4.0%); abnormal levels (<4.0%)
Between run – LPTT: normal level (<4.0%); abnormal levels (<4.0%)

VI. RESULTS

The adult normal ranges for all coagulation tests are re-calculated whenever new lots of reagents are put into use. Refer to the Special Hematology "Table of Reference Values" chart for the current normal ranges.

For infants less than 6 months old, contact the pediatric hematologist on call for the appropriate aPTT reference ranges.

Interpretation of Results

Non-specific inhibitors such as the lupus anticoagulant may or may not prolong the aPTT due to the variability in the nature of aPTT reagents. In this laboratory SynthasIL, a reagent which is sensitive to the presence of the lupus anticoagulant, is used to perform routine aPTTs. When an aPTT is prolonged, the test can be repeated using Actin FS, a reagent with a low sensitivity to the lupus anticoagulant. A shortening of the aPTT into the normal range with Actin FS suggests the presence of a lupus anticoagulant. A prolonged result with Actin FS suggests the presence of factor deficiencies, anticoagulants, or non-LA inhibitors.
Reporting of Results

The Lupus Insensitive aPTT is lab orderable only. This test will typically be run at the request of the laboratory director or a hematologist.

The TOP is programmed to automatically perform the extended-mode test for Lupus Insensitive aPTTs $\geq 100$ seconds or LPTTs that fail. All other specimens yielding critical or questionable results must be manually reprogrammed and rerun.

The test range for the Lupus Insensitive aPTT on the ACL TOP is as follows:  
Delay Time: 16 Seconds (amount of time that passes before clotting time starts)  
Standard test time: 120 seconds  
Extended test time: 400 seconds  
Test range: 10 – 400 seconds

ALWAYS REVIEW THE ERROR MESSAGES AND CURVES FOR FAILED TESTS

Results from failed tests may be able to be estimated with some accuracy from the clot curve. In order to release an estimated clotting time, the clot curve needs to have the following elements: Baseline, acceleration and endpoint. If these elements are present and the instrument has not yielded a result, the estimate can be obtained by holding the mouse pointer over the peak of the second derivative (light blue curve) and recording the displayed number.

All validated results, whether they have been autovalidated or validated by the user, are uploaded to the LIS. The results are then released in OEM. The device is either TOP1 or TOP2. When manually entering a result using MEM, the worksheet code and the test code are both “LPTT”.

A comment with the code “ACT1” is appended to each result and reads as follows: “A normal result for Lupus Insensitive PTT supports the presence of lupus anticoagulant (LA) if a screening PTT is prolonged. A prolonged result for Lupus Insensitive PTT suggests the presence of factor deficiencies, anticoagulants, or non-LA inhibitors.”

VII. LIMITATIONS

APTT results may be affected by many commonly administered drugs. Further studies should be made to determine the source of unexpected abnormal results, such as hemolysis, lipemia or icteric plasmas.

X. REFERENCES


Appendix A: