ACL TOP - ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

☒ St. Joseph Medical Center, Tacoma, WA	☒ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
☒ St. Francis Hospital, Federal Way, WA	☒ St. Elizabeth Hospital Enumclaw, WA	☐ Harrison Medical Center, Silverdale, WA
☒ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

This procedure provides instructions for the analysis of Activated Partial Thromboplastin Time (APTT) using HemosIL SynthASil on ACL TOP Family analyzers.

PRINCIPLE

HemosIL SynthASil is a synthetic phospholipid reagent used for the *in vitro* determination of Activated Partial Thromboplastin Time (APTT) in human citrated plasma on IL Coagulation Systems.

BACKGROUND

Clinical Significance

SynthASil is sensitive to decreased concentrations of contact factors, factors in the intrinsic and common pathway, the anticoagulant effects of heparin and to the presence of inhibitors, particularly to the lupus-like anticoagulants.

Prolonged clotting times may be observed in the following situations: deficiency of Factor XII, XI, X, IX, VIII, V, II, or fibrinogen, liver diseases, Vitamin K deficiency, presence of heparin, lupus anticoagulant or other inhibitor.

Methodology

A plasma sample is incubated with HemosIL SynthASil and a negatively charged contact activator which initiates the activation of the intrinsic coagulation pathway. Calcium is added after incubating at 37ºC for a specific period of time to trigger the coagulation process. The time required for clot formation is measured and read at wavelength of 671 nm.

RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-W-CG-2300	Coagulation-Specimen Integrity
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
R-F-CG-1130	ACL TOP - Reagent Stability and Reconstitution
R-W-CG-2090	ACL TOP - Sample Analysis-Barcoded Specimens
R-W-CG-2091	ACL TOP - Sample Analysis-Non-Barcoded Specimens
R-W-CG-1131	ACL TOP - Reagent Management-Assay Reagents
R-W-CG-1132	ACL TOP - Reagent Management-Bulk Reagents
R-F-CG-2030	ACL TOP - Analytical Measurement Range (AMR) Chart

SPECIMEN

Specimen Requirement

Citrated blood (9:1 blood to anticoagulant) 3.2% sodium citrate. Follow CLSI NCCLS guidelines H3-A5 and H21-A5. No other anticoagulant is acceptable.

Specimen Storage and Stability

Specimen is stable for 8 hours at room temperature (20 +/- 5º C). If unable to complete testing in 8 hours, freeze at -20º C. Frozen plasma should be thawed only once at 37º C for 5 minutes.

Specimen Handling

Samples that are short-filled (refer to B-D Vacutainer fill chart), over-filled or clotted should be rejected. See also FHS Specimen Rejection/Cancellation Protocol and Coagulation Specimen Integrity.

Centrifugation of sample to achieve platelet poor plasma (<10,000 platelet count) is required.

REAGENTS

Contents of Kit

The SynthASil kit consists of:

- **APTT Reagent:** 5 x 10 mL vials of a buffered synthetic phospholipid reagent containing a colloidal silica activator, stabilizers, and a preservative.
- **Calcium Chloride:** 5 x 10 mL vials of an aqueous solution of calcium chloride (0.020 Mol/L) and a preservative.
- Package Insert

Reagent Preparation

SynthASil

1. The vial of APTT reagent must be equilibrated at 15-25ºC for at least 15 minutes.

2. Mix thoroughly before use.

Calcium Chloride

1. The reagent is ready for use.

Reagent Storage and Stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8ºC.

Open Stability	
APTT Reagent	30 days at 2-8ºC in the original vial
	10 days at 15ºC on the ACL TOP
Calcium Chloride	30 days at 2-30ºC

Additional Materials Needed

HemosIL Normal Control
HemosIL High Abnormal Control
NERL water
Factor Diluent

CALIBRATION

No Calibration is required.

QUALITY CONTROL

Normal and abnormal controls are recommended for a complete quality control program. HemosIL controls are designed for this program and must be performed every 8 hours. Controls should also be performed following loading of new reagents.

The HemosIL controls are:

- HemosIL Normal Control
- HemosIL High Abnormal Control

Quality Control Preparation

1. Dissolve the contents of each vial with 1 mL of NERL reagent grade water or equivalent.

2. Replace the stopper and swirl gently.

3. Keep the control at 15-25ºC for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.

Quality Control Storage and Stability

Unopened control is stable until the expiration date shown on the vial when stored at 2-8ºC.

Stability after reconstitution at 2-8ºC in the original vial is 24 hours.

Stability at 15-25ºC in the original vial on-board the ACL TOP is 24 hours.

PROCEDURE STEPS

Running QC and Patient Specimens

1. Load the APTT reagent and Calcium Chloride materials onto the ACL TOP.

2. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the ACL TOP in the D1 track.

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

4. Select APTT QC Normal and Abnormal, and choose the Run icon.

5. Place sample tubes in a sample rack with barcodes facing outwards.

6. Select an available sample track and load the sample rack when the barcode reader is in position.

7. Verify the samples have been identified and have a test ordered. If not, go to test box and add APTT test.

8. Choose the Run icon if the ACL TOP is not currently running.

CALCULATIONS

No calculations are required for APTT testing.

PERFORMANCE CHARACTERISTICS

Reporting Results

Patient results are reported in seconds to the nearest second (Example: 31.2 seconds, report as 31 seconds). If any flags or alarms are present, refer to Online Help. Standard time is 120 seconds and extended time is 400 seconds.

Reference Range

26 – 36 seconds

Critical Value

>60 seconds (Refer to FHS Critical Value Reporting Protocol)

Analytic Measurement Range (AMR)

Reportable Range: 16-270 seconds

If a result is <23, check for clots. If present reject sample. Assess for other tests that might be affected by the clotted sample. If not, report result and add the phrase code: **.PTTL** or **.BKRPTTL**
("REVIEWED: APTT result below normal range. Specimen checked for clot and fibrin. No clot or fibrin present.")

For low and high PTT results, report using alpha responses of <16 sec or >270 sec.

LIMITATIONS

APTT results may be affected by many commonly administered drugs. Further studies should be made to determine the source of unexpected abnormal results.

Interferences

PTT results are not affected by up to 500 mg/dL Hemoglobin, 26 mg/dL Bilirubin, or 1000 mg/dL Triglycerides.

Condition	Effect	Action/Additional Info
Lipemia	May affect	If lipemic may have to airfuge. See Coagulation-Specimen Integrity.
Hct ≥55%	False Prolongation	See Anticoagulant Adjustment for High Hematocrits.
Hemolysis	May affect	See Coagulation-Specimen Integrity.

REFERENCES

ACL TOP On-Line Help Manual Rev 2.0, Instrumentation Laboratory.

Clinical and Laboratory Standards Institute (formerly NCCLS). Collection, Transport, and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays – 4th Edition; Approved Guideline. NCCLS document H21-A4, Vol.23, No. 35, 2003.

Clinical and Laboratory Standards Institute (formerly NCCLS). One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline. NCCLS document H47-A4, 1996.

HemosIL SynthASil (PN 0020006800) package insert issued 10/2012, Instrumentation Laboratory.

Westgaard JO, and Barry PL. Cost-Effective Quality Control; Managing the Quality and Productivity of Analytical Process, AACC Press, 1986.