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Antithrombin-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this procedure is to give the steps on how to perform Antithrombin test on IL ACL TOP.

II. PRINCIPLE:

A. Antithrombin (AT), or Heparin Cofactor I, is the major inhibitor of blood coagulation and is essential for effective heparin therapy. By inhibiting the coagulation proteases, especially thrombin, FXa and FIXa, AT prevents uncontrolled coagulation and thrombosis. (AT) deficiency is associated with a high risk of thromboembolic disorders. The (AT) assay can be used to exclude or diagnose hereditary deficiency in patients with a tendency toward thromboembolism, in pre-operative stages, before prescription of oral contraceptives, DIC, nephritic syndromes, liver diseases and in therapy with heparin or antithrombin concentrates.

B. (AT) levels in patient plasma are measured automatically on IL Coagulation Systems in two stages:

   1. Incubation of the plasma with Factor Xa reagent in the presence of an excess of heparin.
   2. Quantification of the residual Factor Xa activity with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the Antithrombin level in the test sample.

III. ACRONYMS:

A. Deionized (DI)
B. Instrumentation Laboratory (IL)
C. Laboratory Information System (LIS)
D. Low Molecular Weight (LMW)
E. Unfractionated (UF)
F. Quality Control (QC)

IV. SPECIMEN COLLECTION AND HANDLING:

Refer to Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only) procedure

V. SUPPLIES:

A. **Equipment:**

1. IL Coagulation Analyzer
2. Cuvette
3. IL Reagent racks and sample racks
4. Cleaning and Rinse solutions
5. Serological and automatic pipettes

B. **Reagents**:

1. The HemosIL Liquid Antithrombin Kit contains:
    a. **Chromogenic Substrate**: 4 vials of the chromogenic substrate S-2765, N-α-Z-D-Arg-Gly-Arg-pNA.2HCl (6 mg/vial), surfactant and buffer. Vial contents ready for use; no reconstitution necessary. Opened reagent is stable for 5 weeks at 2-8˚C in the original vial or 48 hours at 15˚C on the ACL TOP® Family instrument. Do not freeze.
    b. **Factor Xa Reagent:** 4 vials of a solution containing bovine Factor Xa (40 nkat/vial), heparin, buffer, bovine serum albumin and preservatives. Vial contents ready for use; no reconstitution necessary. Opened reagent is stable for 5 weeks at 2-8˚C in the original vial or 48 hours at 15˚C on the ACL TOP® Family instrument. Do not freeze.
    c. **Cleaning Agent (Clean B Diluted)**: Dilute 1 part of Cleaning Agent + 7 parts of deionized (DI) water or equivalent.
    d. **HemosIL Factor Diluent**: Ready to use. Stable on instrument for 24 hours.

C. **Controls**:

1. **HemosIL Normal Control, ASSAYED**: Lyophilized human plasma containing buffer, stabilizers and preservatives. Dissolve the contents of each vial with 1mL of DI water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25˚C for 30 minutes and invert to mix before use. Do not shake to avoid foam formation. Reconstituted material is stable for 24 hours stored at 2-8˚C or 15-25˚C.
2. **HemosIL Low Abnormal, ASSAYED**: Lyophilized human plasma containing buffer, stabilizers and preservatives. Dissolve the contents of each vial with 1mL of DI water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25°C for 30 minutes and invert to mix before use. Do not shake to avoid foam formation. Reconstituted material is stable for 24 hours stored at 2-8°C or 15-25°C.

D. **Standard**:

1. **Calibration Plasma:** Dissolve the contents of each vial with 1mL of DI water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25˚C for 30 minutes and invert to mix before use. Do not shake to avoid foam formation. Reconstituted material is stable for 24 hours stored at 2-8˚C.

VI. QUALITY CONTROL:

A. Quality control consists of HemosIL Normal Control, ASSAYED and HemosIL Low Abnormal, ASSAYED.

B. Frequency of Control Use:

1. Controls should be run at least once every 8 hours, with reagent replacement, and with a new calibration curve.

C. The AT assay on the ACL TOP® Family instrument is also monitored through the use of CAP proficiency surveys or equivalent.

VII. PROCEDURE:

A. Gently invert vials of reagents and QC. Check for bubbles before loading reagent and controls on IL ACL TOP.

B. Load chromogenic substrate in R3-R6 lane

C. Load factor Xa reagent in D3, R1-R4 lane

D. Load Clean B diluted in D3, R1-R4.

E. Run both levels of QC for AT test.

F. Verify that all levels of QC are within acceptable range before running patients.
Note: If you run a capped sample in the Yellow sample rack, you will crash the Probe. If you run an uncapped sample in the Blue sample rack, the alignment of the probe and its coordinates will be affected.

G. Document any troubleshooting performed if QC fails.

H. Refer to IL Operations procedure for any instrumentation details.

VIII. EXPECTED VALUES:

A. Any unreasonable result is to be repeated.

B. Resulting is performed in LIS.

C. Normal Ranges:

1. Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.

D. Reportable Ranges:

1. Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.

E. Turn Around Time:

1. AT assays will be performed at least once each week. Completion of results should be available within 7-10 days of specimen collection date.

IX. LIMITATION:

A. Antithrombin results on the ACL TOP® Family are not affected by:

1. Heparin (UF or LMW) up to 4 U/mL
2. Alpha1-antitrypsin up to 4 mg/mL
3. Alpha2-macroglobulin up to 7 mg/mL
4. Heparin Cofactor II up to 4 U/mL
5. Hemoglobin up to 500 mg/dL

6. Bilirubin up to 40 mg/dL
7. Triglycerides up to 2300 mg/dL.

X. REFERENCES:

A. HemosIL liquid Antithrombin package insert 06/2017.
B. ACL TOP Online Operator's Manual, Version 2.2, 06/2017.
C. HemosIL Calibration plasma package insert 06/2017.
D. HemosIL Normal Control ASSAYED package insert 04/2019.
E. HemosIL Low Abnormal Control ASSAYED package insert 04/2019.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	6/24/2021
Coagulation Medical Director Designee	Marc Smith: System Med Dir, Coagulation	6/9/2021
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	6/4/2021
Policy and Forms Steering Committee Approval (if needed)	Tamara Sabih: Medical Technologist Lead	6/3/2021
System Manager	Rebecca Bacarella: Mgr Laboratory	6/3/2021
	Tamara Sabih: Medical Technologist Lead	6/2/2021

Applicability

Royal Oak