HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Haematology)		
Document:	Internal Policy and Procedure		
Title:	Anti –Thrombin III Test		
Applies To:	All Laboratory Staff		
Preparation Date:	January 07, 2025	**Index No:**	LB-IPP-070
Approval Date:	January 21, 2025	**Version :**	2
Effective Date:	February 21, 2025	**Replacement No.:**	LB-IPP-070 (1)
Review Date:	February 21, 2028	**No. of Pages:**	04

1. PURPOSE:

1.1 To describes the procedure for the determination of the functional activity of Antithrombin III in human plasma, using STACompact

2. DEFINITONS:

2.1 Anti-thrombin III is a naturally occurring plasma protein with anticoagulant activity. It inhibits thrombin and activated factor X. It forms an irreversible inactive complex with these enzymes.

3. POLICY:

3.1 In the presence of Heparin, ATIII has a powerful and immediate antithrombin action (inhibitory action).
3.2 Thrombin, which is present in excess, is inactivated in proportion to ATIII concentration.
3.3 The remaining thrombin releases as a dye from the chromogenic substrate and the color is measured at 405 nm, color is inversely proportion to ATIII concentration
3.4 The test procedure is not affected by therapeutic doses of heparin. It is therefore suitable for testing of plasmas collected from patients receiving heparin therapy.

4. PROCEDURE:

4.1 Specimen:
- 4.1.1 Collect the blood in a blue stopper vacutainer tube using a ratio of 9 parts of whole blood and 1 part of 3.2% buffered sodium citrate.
- 4.1.2 Centrifuge the specimen at 3500 rpm for 10 minutes
- 4.1.3 Remove the platelet poor plasma and transfer to a 12 x 75 plastic test tube. For the best results, test must be performed immediately
- 4.1.4 Freeze the plasma at -20°C or lower until ready to test (maximum 2 weeks)
- 4.1.5 Frozen plasma must be thawed directly at 37°C for 15 minutes before testing.
- 4.1.6 Specimens are stable for 4 hours at +20°C and 2 weeks at -20°C

4.2 Procedural steps:
- 4.2.1 After calibration and running controls in order to ensure accuracy and reducibility, run the patient's plasma. Patient plasma is tested undiluted. They are loaded in the instrument (see the reference manual of the analyzer). Dilutions with Owren- Koller buffer are automatically prepared by the instrument
- 4.2.2 Assay: refer to the "standardized operating procedure" of the instrument for full details on how to proceed from this point. The anti-thrombin III assay of the plasma to be tested is automatically carried out by the analyzer at 405 nm as soon as the samples have been loaded. If any of the patients results falls outside the working range of the assay, the instrument automatically resets the sample in question at an appropriate dilution provided that this option has been entered in memory in the test setup (see reference manual).

4.3 Quality Control.
- 4.3.1 System Control N.
- 4.3.2 System Control P.
- 4.3.3 Check values to ensure that results are within acceptable limits.
- 4.3.4 Patient results cannot be released if the run is rejected based on Westguard Rules.
- 4.3.5 Check reagents and controls for expiration date.
- 4.3.6 Refer to STA- Compact Antithrombin III leaflet insert to perform the quality control procedures

4.4 Reporting
- 4.4.1 The normal plasma (AT) level is usually in the range of 80%- 120%.
- 4.4.2 The (AT) level in women up to the menopause period is a little lower than in men. Administration of this level is observed during pregnancy
- 4.4.3 In men, the (AT) level decreases with age.
- 4.4.4 In children's, the (AT) level is normally low until the age of 6 months; at this time it reaches the adult level

4.5 Clinical Significance
- 4.5.1 Diagnosis of congenital ATIII deficiency
- 4.5.2 Diagnosis of acquired ATIII deficiency e.g:
  - 4.5.2.1 Diagnosis of consumption coagulopathy
  - 4.5.2.2 Severe liver disease, septicemia, nephrosis
  - 4.5.2.3 Screening for the risk of thrombosis

5. REAGENT AND EQUIPEMENT:

5.1 The reagent in intact vials is stable until the expiration date indicated on the box label and the vial, when stored at (+2° to +8 °C).

5.2 Reagent 1: take one vial of reagent 3 (R3) and shake it well. Then, pour its contents into a vial of reagent 1 of the same kit. Allow the re-constituted reagent to stand at room temperature (18°-25°C) for 60 min. after re-constitution, the reagent 1 is stable: in its original capped vial with STA- mini reducer and perforated cap in place; for 7 days on STACompact, but in its original capped vial, it's stable for 21when stored at (+2°C to +8°C).

5.3 Reagent 2: re-constitute each vial with 3ml of D. Water. Allow the re-constituted reagent to stand at room temperature (18°- 25°C) for 60 min. After re-constitution, the reagent 2 is stable for: 7 days on STA-Compact, but in its original capped vial, its stable for 21 days. But in its original capped vial, it's stable for 21 days when stored at (+2° to +8 °C).

5.4 Reagent 3: (R3) is ready for use. Shake vial well before use to re-constitute reagent 1.

5.5 STA- Owren-Koller

5.6 STA- Unicalibrator

5.7 STA- System controls N+ P

5.8 Analyzer of the STA line suitable to these reagents

5.9 STA- mini reducer.

5.10 Common clinical laboratory equipment and materials (centrifuge, D.W)

6 RESPONSIBILITIES:

6.1 This policy applies to all Hematology technologists involved in this special Hematology test

7 APPENDICES:

7.1 Reagent Preparation And Storage: (Antithrombin III)

8 REFERENCES:

8.1 STA- Compact Antithrombin III leaflet insert.

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Fatma Hassan Ahmed	Clinical Pathologist		January 07, 2025
Reviewed by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 08, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 09, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 12, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 21, 2025

Appendix 7.1

REAGENT PREPARATION AND STORAGE: (ANTITHROMBIN III)

Reagents	Preparation	Stability after reconstitution/ opening on board STA Compact®	Storage position on STA Compact®
Reagent 1 (Thrombin)	Transfer the contents of a vial of well-suspended Reagent 3 (Solvent) of the same kit. Allow the reconstituted reagent to stand at room temperature (18-25 °C) for 60 minutes. Gently homogenize. Then, place a new STA® - mini Reducer and the perforated cap.	7 days	Product drawer
Reagent 2 (Substrate)	AddC 0 3 ml (REF 00596) or 6 ml (REF 00672) of distilled water. Allow the reconstituted reagent to stand at room temperature (18-25 °C) for 60 minutes. Gently homogenize. Then, place a new STA® - mini Reducer and the perforated cap.	7 days	Product drawer
Reagent 3 (Thrombin Solvent)	3-ml vial (REF 00596) or 6-ml vial (REF 00672). Shake vial well before use.	—	—
STA® - Owren-Koller	15-ml vial. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes before use.	3 days	Sample drawer
STA® - Unicalibrator	Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes. Then, homogenize.	4 hours	Product drawer
STA® - System Control N **STA® - System Control P**	Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes. Then, homogenize.	8 hours	Product drawer
STA® - Coag Control N **STA® - Coag Control P**	Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes. Then, homogenize.	8 hours	Product drawer