Status **Active** PolicyStat ID **12611085**

Sutter Health
Sutter Roseville Medical Center

Origination	11/9/2022	Owner	Alex Alba: Supervisor, Laboratory Analytic
Final Approved	11/9/2022		
Effective	11/9/2022	Policy Area	Lab - Coag
Last Revised	11/9/2022	Applicability	Sutter Roseville Medical Center
Next Review	11/8/2024		

Determination of Antithrombin III Activity

Principle

Antithrombin III (ATIII) exerts a powerful and immediate inhibitory action on thrombin when heparin is present. The assay procedure consists of two steps:

1. Plasma is incubated with a known excess of thrombin in the presence of heparin.
2. The residual thrombin is quantitated by its amidolytic action on the synthetic chromogenic substrate CBS 61.50 (pNA released at 405nm). Since the quantity of thrombin that is neutralized in the first reaction step is proportional to the ATIII level present in the plasma being tested, it follows that the residual thrombin in the second reaction step is inversely proportional to the ATIII level of the tested plasma.

Since the first report (1965) of a hereditary deficiency of ATIII and its consequences, ATIII has been considered an important parameter in spontaneous thromboembolic disorders.

The less frequent congenital deficiencies are qualitative (type II deficiency). In this case, it is recommended to look for the gene mutation. When the defect involves the AT-heparin binding, the incidence of thrombosis is low in the heterozygous patients. However, studies suggest that this risk is increased when this defect is combined with another anomaly of hemostasis, such as the presence of the factor V Leiden.

In additions to these congenital deficiencies, a number of acquired ATIII deficiencies have been described in DIC, nephritic syndrome, liver disease, and in L-asparaginase treatments.

Specimen

- Sample is collected in 3.2% trisodium citrate (blue top). The correct anticoagulant/sample volume ratio of 1:9 must be maintained.

- Centrifugation: Specimen is centrifuged to obtain platelet poor plasma (platelet concentration of <10,000/µl).
- Storage and Stability:
    - Specimens should be kept at room temperature and centrifuged within 4 hours of specimen collection. Plasma is stable at 15-25°C for 8 hours.
    - If testing is not completed within acceptable stability limits, plasma should be removed and frozen at -20°C for one month or at -70°C for longer-term storage. Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately.
- Unacceptable specimens: samples that are incompletely filled or overfilled, clotted, hemolyzed, unlabeled, hematocrits above 55% must be adjusted for the correct anticoagulant to blood ratio.

Supplies & Equipment

- STA Compact Max
- Centrifuge
- Pipettes & Tips
- Distilled Water

Reagents

- STA Stachrom AT III
    - Reagent 1: bovine thrombin lyophilized
    - Reagent 2: chromogenic substrate, lyophilized
    - Reagent 3: solvent containing heparin
    - The reagent in intact vials are stable until the expiration date indicated on the box label, when stored at 2-8°C.
    - Once reconstituted, the reagent is stable on the analyzer for 7 days or in its original capped vial for 21 days at 2-8°C.
        - In case of storage at 2-8°C, allow the reagent to stand at room temperature (18-25°C) for 30 minutes before use.

Reagent Preparation

Step	Action
1.	• Reagent 1: Take one vial of Reagent 3 and shake it well. Then pour its entire contents into a vial of Reagent 1. • Reagent 2: Reconstitute with 3 ml of distilled water.
2.	Allow the reconstituted materials to stand at room temperature (18-25°C) for 60 minutes.
3.	Swirl vials gently.

4. Place a new mini reducer and the perforated plastic cap (with the rubber stopper removed) on each vial.
5. Recommended to allow at least 10 minutes to equilibrate on-board before use.

Calibration

- Calibration verification must be performed:
    - Whenever the reagent lot is changed.
    - At least every 6 months.
    - Whenever QC fails to meet established criteria.
- Unicalibrator:
    - Reconstitute with 1.0 ml of distilled water.
    - Let stand at 18-25°C for 30 minutes.
    - Swirl vial gently; load on analyzer. Recommended to allow at least 10 minutes to equilibrate on-board before use.
    - After reconstitution, reagent stability is 4 hours on-board.
- Refer to the *Performing Calibrations on STA Compact MAX* procedure for programming, running, and evaluating the calibration.

Quality Control

- STA Coag Control N + ABN Plus. Refer to *Running and Evaluating Quality Control on the STA Compact Max* procedure.
- LIS QC mnemonic:
    - N control is KO1
    - ABN control is KO2
- QC schedule:
    - Every 8 hours of patient testing
    - Each time the reagent is replaced
    - Whenever patient results are in question
    - After calibration, major maintenance or service, or as required by manufacturer

Procedure

- Patients' plasmas are tested undiluted. The analyzer automatically prepares all dilutions of the calibrator, controls, and patient samples. Original sample dilution for ATIII is 1:20. Refer to the Linearity section for information regarding re-diluted samples that are above the Analytical Measurement Range (AMR).
- The results are reported out in % activity in whole numbers

Linearity

The analyzer is set up to automatically perform further dilution if the initial result is outside of the initial 1:20 dilution linearity range. If the auto re-dilution feature is necessary, the results are displayed on the screen in Blue numerals instead of the normal black numerals and the instrument will automatically correct the results for dilution.

The CRR (Clinical Reportable Range) is 9-200%.

- ATIII results under the CRR limit will be reported as "<9%"
- ATIII results over the CRR limit will be reported as ">200%"

Critical Values

- ≥6 months: <40%
- 3 months – 5 months: <30%
- <3 months: <20%

Reference Range

- ≥6 months: 80 – 120%
- 3 months – 5 months: 73 – 121%
- 1 month – 2 months: 48 – 108%
- <1 month: 39 – 87%

Appended Comments

- For all results, the ETC "ATFIN" will auto-append: Direct thrombin inhibitors may lead to an over-estimation of activity level.
- For results above the stated reference ranges, the ETC "SPKOIN" will auto-append: An elevated activity level is not clinically significant. Only deficiencies are associated with an increased thrombotic risk.
- For results below the stated reference ranges, the ETC "MEATFL" comment will auto-append: Antithrombin functional activity is decreased. This could be due to an inherited deficiency, but other causes of decreased production or increased loss should be excluded. Acquired deficiencies can be seen in DIC, sepsis, thrombosis, preeclampsia, liver disease, nephritic syndrome, malignancies, and L-asparaginase therapy. Evaluation of immunologic antithrombin will differentiate between Type I (decreased activity and immunologic) and Type II (decreased activity and normal immunologic) deficiencies.

Limitations and Procedural Notes

- This test procedure is not affected by therapeutic doses of heparin. It is therefore suitable for testing of plasmas collected from patients receiving heparin therapy.

- Thrombin inhibitors (e.g. hirudin, argatroban...) present in the sample to be tested may lead to an over-estimation of the AT level for this sample.
- The STA Stachrom AT III procedure is insensitive to the following substances:
    - hemoglobin (up to 0.7 g/dL)
    - bilirubin (up to 20.0 mg/dL)
    - triglycerides (up to 760 mg/dL)
- The STA Stachrom AT III procedure allows to detect the different types of AT deficiencies, particularly that of type II HBS (heparin binding site) which affects the binding of AT to heparin. In the described conditions, the specificity of the method is assured by:
    - the presence of heparin which makes the AT exert its inhibitory action fully and instantly, thereby rendering ineffectual any action that may be exerted by the progressive antithrombins (e.g. α2-macroglobulin);
    - the presence of aprotinin which ensures that the activity of plasmin is blocked if it is present;
    - the use of bovine thrombin and of a specific solvent which renders any interference by heparin cofactor II insignificant.
- The AT level in women up to the menopause period is a little lower than that in men. A diminution of this level is observed during pregnancy. In men, the AT level decreases with age. In children, the AT level is normally low until the age of 6 months; at this time it reaches the adult level.

References

- STA Stachrom AT III: Colorimetric Assay of Antithrombin III. Package insert. October 2018.
- STA Unicalibrator: Calibration Plasma for Functional Assays of Coagulation Parameters. Package insert. September 2017.
- STA Coag Control N + ABN Plus: Control Plasmas for Coagulation Tests. Package insert. March 2018.
- STA Compact Max Reference Manual.
- Clinical and Laboratory Standards Institute (CLSI). Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. H21-A5 vol. 28 No. 5.

All Revision Dates

11/9/2022

Approval Signatures

Step Description	Approver	Date

Medical Director	Lindsey Westerbeck: Director, Laboratory Services	11/9/2022
Laboratory Director	Lindsey Westerbeck: Director, Laboratory Services	11/1/2022