

 Proc. #4840-CO-0625

**TITLE: AT3 (Innovance Anti-thrombin ) on the CS-2500**

**PRINCIPLE:**

INNOVANCE® Antithrombin is a chromogenic assay for the automated quantitation of functionally active anti-thrombin in human citrated plasma and can be used as an aid in the diagnosis of anti-thrombin deficiency.

 The INNOVANCE® Antithrombin assay utilizes a chromogenic measuring principle. An excess of factor Xa is added to citrated plasma. In the presence of heparin, a portion of the enzyme is complexed and inactivated by the antithrombin present in the sample. Excess, uninhibited factor Xa then cleaves a specific chromogenic substrate, causing the release of a dye. The rate of the substrate cleavage is determined by the increase in the absorbance value at 405 nm.



The release of dye is inversely proportional to the inhibiting activity of antithrombin in the plasma sample, i.e. the smaller the concentration of functionally active antithrombin, the higher the absorbance signal per time unit.

**SPECIMEN:**

 **Type:**

 Blue top(3.2%) sodium citrate Vacutainer tube. Both the 2 mL or 3 mL tubes are acceptable as long they are filled properly (nine parts of freshly collected blood with one part of 0.11 mol/L (3.2%) sodium citrate). For proper collection, see policy 4840-CO-0120 Proper Collection & Preparation of Patient Samples.

**SPECIMEN HANDLING:**

The whole blood specimen is checked for clot formation by gentle inversion and rimmed with sticks prior to centrifugation. Centrifuge the blood specimen for a minimum of 3 minutes at 4500 RPM as soon as possible after collection to obtain platelet poor plasma. Patient plasma should be tested within 4 hours if stored at room temperature and within 6 months if stored at -70°C (Do not freeze multiple times). If testing is not complete within 4 hours, the plasma must be frozen. Frozen plasma samples must be rapidly thawed at 37°C and tested immediately after thawing.

**EQUIPMENT AND MATERIALS:**

Equipment:

Sysmex® CS-2500

SLD Mini cups

4.0 mL Sample Cups

Cuvettes (SUC-400A)

Materials:

Standard Human Plasma (for calibration)

Innovance Antithrombin Test Kit

Control N

Control P

CA Clean™ I

CA Clean™ II

Preservative-free distilled or deionized water

**Preparation of Reagents:**

 The Innovance Antithrombin test kit components are liquid. To ensure homogeneity, gently swirl the reagents shortly before use, do not shake and avoid foam formation.

**Stability of Innovance Antithrombin Test Kit:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Condition** | **Innovance Antithrombin reagent** | **Innovance Antithrombin Substrate** | **Innovance Antithrombin Buffer** |
| **On board in SLD mini cup** | **24 hours (closed vial)** | **24 hours** | **24 hours** |
| **+2 to +8°C** | **4 weeks (closed vial)** | **4 weeks** | **4 weeks** |
| **15-25°C** | **8 hours** | **8 hours** | **8 hours** |

**CA Clean I** is liquid and ready to use. Stable on-board analyzer for 120 hours.

Opened bottle stable for 30 days at 2-8 °C

Stable unopened at 2- 8°C until expiration date on bottle.

**CA Clean II** is liquid and ready to use. Stable on-board analyzer for 120 hours.

Opened container or bottle is stable for 60 days.

Stable unopened at 5-35 °C until expiration date.

**Standard Human Plasma (for Calibration)** Lyophilized preparation of pooled human, normal citrated plasma and HEPES buffer solution (12 g/L). **Used for the calibration of coagulation and fibrinolysis tests.**

* + Reconstitute lyophilized Standard Human Plasma with 1.0 mL distilled or deionized water.
	+ Shake carefully to dissolve (without foam formation).
	+ Let stand at +15 to +25 °C for at least 15 minutes.
	+ Before use, again shake carefully.

 **Stability after reconstitution:** This calibrator is intended for immediate use; no on-board stability data has been established.

 **5. Control Plasma N, Control Plasma P**
 Lyophilized preparation of pooled normal plasma stabilized with HEPES buffer solution (12 g/L). Used for Quality Control (Normal and Pathological).

* Reconstitute Control Plasma N and Control Plasma P with 1.0 mL distilled water or deionized water.
* Shake carefully to dissolve (without foam formation).
* Let stand at +15°C to +25°C at least 15 minutes.
* Before use, again shake carefully.
* Do not use distilled water containing preservatives.

 **Stability of QC after reconstitution:**

|  |  |  |
| --- | --- | --- |
| **Condition** | **Control N** | **Control P** |
| On-board in SLD mini cup | 24 hours | 24 hours |
| -20°C | 4 weeks | 4 weeks |
| \*Frozen and thawed once | YES | YES |

Control N and Control P may be frozen in the original contained and thawed once after reconstitution. The previous storage time at 15-25 °C must not have exceeded 4 hours. The plasma must be well sealed and frozen as quickly as possible. Thawing must be completed at 37 °C and within a maximum of 10 minutes. Control N and Control P should not stand for more than 4 hours at 15-25 °C after thawing. Control N and Control P should not be used if they contain visible clots.

**QUALITY CONTROL**

1. Controls should be tested at the initiation of testing, upon reagent changes, and at least once each 8 hour shift.
2. Controls should be run in the same manner as the test samples.

3. Controls should be within the tolerance limits established for the specific lot number of control. If control values are outside of determined range:

* Check controls, reagents and instrument performance.
* Document actions taken to identify and correct the problem before reporting any patient data.
* Corrective action when tolerance limits are exceeded include:

 1. Rerun out-of-range control material.

 2. Verify reagent performance.

 3. Check instrument performance.

4. Document actions taken to identify and correct the problem before reporting any patient data.

* The calibration curve should be rerun if the control is repeatedly outside the assigned range.
* New control ranges should be established for unassayed controls with changes in each lot of reagent or control material.

PROCEDURES:

 Loading Reagents onto the Reagent table

 1. Press **Reagent** icon on the toolbar

 2. Highlight a reagent position for removal

3. Press **Change/Add.**

4. Lift the reagent section lid.

5. Verify reagent table cover LED is solid green.

6. Slide the lock lever and remove cover.

7. Lift out rack and remove empty or expired reagents.

8. Add new vial to rack with barcode showing

9. Load rack into the reagent table.

10. Replace cover and slide the lock lever, close the reagent section lid

11. Press **OK** on the screen to read barcode.

12. Wait for the barcode to finish reading.

13. On the reagent screen, **touch reagent position just loaded**, and press **Change** to update date and time.

Loading QC or Calibrator:

Use C-rack and SLD Mini cup

1. Reconstitute vial observing package insert instructions.
2. Aspirate entire contents of vial into a new SLD mini cup, avoid bubbles.
3. Set SLD mini cup into corresponding vial.
4. Carefully check for bubbles and remove if necessary with a small pipette.
5. Remove C-rack and insert vials with SLD mini cups into the rack.
6. Place C-Rack back into the reagent table
7. Lock Lever and press OK to read barcode.
8. On reagent screen, **highlight vial just loaded** and press **Change** to update date and time.

***Note:***  *SLD mini cups are for the C-rack only*

Loading Consumables and Discarding Waste Material:
(do the following as necessary)

1. Replenish Reaction Tubes- Reaction tubes should be replenished as needed. Do not fill cuvettes above the red line. Make sure you use only the Cuvette SUC-400A reaction tube.

2. Replenish DI water

Rinse the DI water tank with 70% isopropyl alcohol followed by DI water before replenishing with

 DI water.

3. Dispose of Used Reaction Tubes from the Trash Box. Reset the trash counter.

Loading consumables should be done along with the daily maintenance. See the SOP for complete directions for performing maintenance.

**CALIBRATION**

A new reference curve should be established with each change of reagent lot, or with any deviation from control or proficiency testing limits. In order to generate a standard curve, a calibrator must be loaded onto the CS-2500 analyzer. Standard Human Plasma is used to calibrate AT3 Assay. Both levels of QC must be run following a calibration to validate the new curve.

 To calibrate a new lot of reagent:

1. Enter the reagent and calibrator lot information in the **Reagent Lot Master** tab.

2. Load reagents, calibrator and buffer. Calibrator plasma (Standard Human Plasma) should be placed into a SLD mini cup and put inside the vial and placed in the C-Rack

3. Select **Order**

4. Select **Switch Order**.

5. Select **Holder Calib Curve Order**.

6. Select the desired assay to calibrate

7. Select **Change** and select the correct lot number. Select **OK.**

8. Select the correct calibrator lot number from the list.

9. For manual entry of assay values: place the cursor in the **Assay sheet Value**. Select **OK.**

10. Select **Start**.

11. To view the calibration status and progress, press **Joblist**.

QUALITY CONTROL PROCESSING

**Processing QC from the reagent table: QC files**

1. Load QC onto a C-Rack.

 2. Select **Order.**

 3. Select **Switch Order.**

 4. Select **Holder QC Order**.

 5. Press **Order Entry**.

 6. Select **QC01-QC20** radio button.

 7. Select **QC file** from the list on the right.

 8. Select appropriate assays. Press the down arrow to order the next control.

 9. Press **OK** once controls have been ordered.

 10. Press **Start**.

SAMPLE PROCESSING

After a valid standard curve has been established followed by acceptable Quality Controls, sample testing can begin.

**Samples can be loaded on the analyzer using sample barcode and the host connection. The CS-2500 has an automatic cap piercer- caps do not have to be removed from the sample tubes.**

 Sample ID number (barcoded sample) read by barcode reader /
 Automatic inquiry of tests (host connection operational)

1. When the host computer is connected using bi-directional communication, host inquiry takes place when the sample ID is read and the analysis parameters are automatically registered
2. Load the barcoded sample tube on the sampler.
3. Check host connection (HC) status, HC status icon must be green or orange.
4. Press **Start** to begin processing.

5. After barcode reading, confirm sample order status and progress on the **Joblist** screen

**Manual order processing (When no bar-code is available or the LIS is down)**

1. Place rack with sample tubes on sampler.

2. Press **Order**.

3. Enter rack number.

4. Select tube position to input an order.

5. Press **Order Entry**.

6. Press **Ordinary Sample.**

7. Place cursor in sample no. and input sample ID if the sample does not have a barcode label.

8. Select assays to be analyzer.

9. Press the down arrow to order the next sample.

10. Press **OK.**

11. Press **Start.**

12. Confirm sample order status on the **Joblist**.

**Processing samples in Micro-Mode**

1. Follow steps listed above for manual order processing.

2. Press **Mc** column on **Order screen**.

3. Load **uncapped** tube on to the system.

4. Press **Start**.

**Change to a longer measurement time.**

 1. Follow steps listed above for manual order processing.

 2. Press **Detailed Settings** button.

3. Click box below **Measurement Time**.

4. Select measurement time

5. Select **OK**

6. Select **Start**.

REPORTING RESULTS

The AT concentration in % is calculated automatically by the analyzer based on the reference curve.

Record and report patient and quality control values according to laboratory procedure.

Hemolyzed, lipemic, or icteric samples must be noted with the result.

The reference value of the curves may change; we report >140 when the result is above 140.0%.

Reference Interval:

Reference interval values determined for INNOVANCE Antithrombin are 83-111 %.

**\*NOTE\*:** Results are read off and based on the current curve. Control P is at the lower end of the curve (approximately 25-45%). Control N is at the normal range level (approximately 88-108%).

Patient results that have the (-) sign in front of the result indicates the result is below the reference range. Any result less than 10.0 is reported as <10.0 and crosses the interface as such.

**PROCEDURE NOTES**

Overall performance of INNOVANCE Antithrombin testing is dependent on reagent and instrument performance. Acceptable variability (imprecision) should be such, that the total coefficient of variation (CV) of the analytic system is less than 10% on the same lot of control plasma.

**Reference Ranges:**

Reference Range determined for AT3: 83% - 111.0%**.**

**Analytical Measuring Range:** 9% - 128%

**Instrument Operations**

System Shut-down

 1. Verify CA Clean 1 is set on reagent table A.

 2. Press **Shutdown**. Select option: **Turn the main unit OFF**.

 3. Press **OK.**

 4. Press **OK** after shutdown process is completed.

 5. Press the **X** in the upper right of the screen.

 6. Press **OK**.

 7. Select **Windows Start** icon.

 8. Select **Shutdown**.

 9. Turn the analyzer power **OFF**.

System Start-up:

 1. Power **ON** the IPU computer.

 2. Windows Logon: Press **CSAdministrator icon**- enter password: CS Admin+2304

 3. CS software logon: Press IPU Logon: admin- enter password: admin

 4. Turn the analyzer power **ON**.

Emergency Stop:

 To be used if the instrument encounters a sudden malfunction or other problem.

 1. Press the Mechanical Stop switch on the Main Unit.

 2. The operation of the main unit stops and an alarm sounds.

 3. The samples already in process become will need to be repeated.

**LIMITATIONS OF THE PROCEDURE**

Some very rare antithrombin gene variants with reduced functional activity may yield results within the reference range.

User defined modifications are not supported by Siemens since they may affect performance of the system and assay results.

Results of this test should always be interpreted in conjunction with the patient’s medical history, clinical presentation and other findings.

**INTERFERENCES:**

 No interferences up to:

|  |  |
| --- | --- |
| Triglycerides | 425 mg/dL |
| Hemoglobin  | 1000 mg/dL |
| Bilirubin unconjugated | 60 mg/dL |
| Bilirubin conjugated | 40 mg/dL |

REFERENCES

 1. Application sheet for Control P and Control N CA-500/CA-600.

 2. Sysmex® CS-2500 System Operator’s Manual.

 3. Application sheet for Innovance Antithrombin for CA-2500, Siemens Healthcare.

 4. Package Insert for Standard Human Plasma, Siemens Healthcare