FIBRINOGEN ON THE STA-R EVOLUTION

Purpose

This document describes the procedure for the performance of the Fibrionogen assay on the Sta-R Evolution analyzer.

Principle

The STA® - Fibrinogen 5 ml (Cat # 00674), is intended for the quantitative determination of fibrinogen in plasma by the clotting method of Clauss. In the presence of an excess of thrombin, the clotting time of diluted plasma is inversely proportional to the level of plasma fibrinogen. The clot is detected by the STA–R®/STA-R Evolution®, a fully automated coagulation instrument, which uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette with the thrombin and diluted plasma is monitored by the STA–R®/STA-R Evolution®. When the oscillation of the steel ball is slowed by clot formation, the sensor determines the time in seconds. The time is read from a stored curve on the STA–R®/STA-R Evolution®. An increase of the fibrinogen level is observed in cases of diabetes, inflammatory syndromes and obesity. A decrease of the fibrinogen level is observed in DIC, fibrinolysis, thrombolytic therapy and inherited disorders. Fibrinogen seems to be involved in the pathogenicity of thrombotic cardiovascular events.

Sample Preparation and Requirements

1. Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Follow CLSI guidelines H3 – A6 and H21-A5, or latest revision.
2. Centrifugation: 5 minutes at 5100 g
3. Plasma storage: 8 hours at 20 ± 5 °C
4. Unacceptable Specimens: Samples that are short draws, clotted or hemolyzed may yield incorrect results.

Equipment, Materials, Reagents and Supplies

- Centrifuge
- Reagent Grade Water
- Pipettes
- Cuvette roll 1000 cuvettes (Cat. No. 38669)
- Pipette tips
- STA–R®/STA-R Evolution®
- STA®- Owren-Koller Buffer (Cat. No. 00360)
- STA®- System Control N + P (Cat. No. 00678)
- STA® - Coag Control N + ABN PLUS (Cat. No. 00677)
- STA® - Fibrinogen (Cat. No. 0674)

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Reagents Prep

1. Reagent 1: STA® - Owren-Koller Buffer: Ready to use. (Cat. No. 00360) Ready to use buffer. Used by the STA–R®/STA-R Evolution® to perform dilutions of controls and patients' plasmas. Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R0**. Stability is 72 hours.
2. Reagent 2: STA® - Fibrinogen (Cat. No. 0674): Freeze-dried titrated human calcium thrombin (approx. 80 NIH unit/ml) containing a specific heparin inhibitor. Reconstitute each vial with 5.0 ml of reagent grade water. Let stand 30 minutes at room temperature. Swirl gently. Replace the perforated plastic cap on the vial. Reconstituted stability:
   5 days on board with the perforated cap in place
   14 days at 2 – 8° C in its original capped vial. Allow the refrigerated reagent to stand at room temperature for 30 minutes before use.
   These durations should not exceed the above mentioned figures which have been determined under controlled conditions.

   Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R2**.
3. STA® - Coag Control N + ABN **(Cat. No. 00677)**: citrated control plasmas normal and abnormal levels, both freeze-dried. Reconstitute each vial with 2.0 ml reagent grade water. Let stand 30 minutes at room temperature. Swirl gently. Reconstituted stability on the STA–R®/STA-R Evolution® is 24 hours.
   Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R0**.
4. STA® – DESORB U (Cat. No. 0975): is a decontaminating solution (contains KOH < 1%) for use with the STA® line of instruments. Install a new STA® - maxi reducer (REF 00801) and the perforated cap on a freshly opened bottle before loading in the reagent drawer. The analyzer will state "BLOCK SAMPLE PIPETTING" when there is not sufficient STA® – DESORB U to run requested testing. Stability is 5 days on STA–R® Evolution. Request the product drawer to open by clicking the Products menu  icon, select load products, bar code the reagent and place the reagent into the product drawer, **R0**, **R1**, and **R2**.
5. STA® - Cleaner Solution (Cat. No. 0973): is a washing aqueous solution

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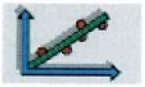
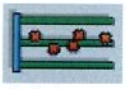
used on the STA® line of instruments. Sufficient STA® - Cleaner Solution must be loaded to operate the analyzer.

Calibration

1. The kit reagents are pre-calibrated: this calibration is identical for all reagents of each lot.
2. Entering the data for the calibration curve: When the operator scans a new lot of fibrinogen reagent, the STA–R®/STA-R Evolution® will request the operator to scan the barcode printed on the barcode insert across the barcode reader.
3. The calibration curve will be validated for the lot being used when the two fibrinogen control levels have been run. If the validation controls are outside the assayed range, the STA–R®/STA-R Evolution® will not run fibrinogen until the controls are validated
4. To view the calibration curve:
   - Click on Calibration menu  icon
   - Click **FIB TEST** (on the test abbreviation)
   - Calibration screen includes current calibration curves and product information
   - Position for two different lots of reagent are available
   - Click on the calibration icon  or calibration icon 
   - Confirm by clicking on **Calibrate**
   - Select the lot # to be calibrated  (the one highlighted in green)
5. Click the Print icon

Quality Control

1. STA® - Coag Control N + ABN PLUS (Cat. No. 00677), After t reconstitution period, request the product drawer to open by clicki the Products menu  icon, select load products, bar cc the controls and place the controls in the product drawer, **R0**.
2. QC can be run automatically at pre-set intervals (in Test Set-up) by ordering manually from the Quality Control Menu. To order (manually:
   - Click the Quality Control menu  icon
   - Select the test abbreviation for which a quality control has to be run (Single Click only)

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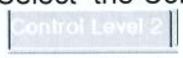
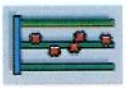
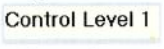
- Select the Control Level tab # 

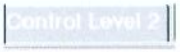
or 

(#corresponds to the number of the desired level – Single Click only)
- Single Click on  icon.
- Confirm by clicking on 

.
- All control ranges are monitored automatically by the STA–R®/STA-R Evolution®. If any controls are outside of the bar coded ranges or the site specific QC range, the STA–R®/STA-R Evolution® will audibly and visually alert the operator by the Quality Control menu  icon blinking at the System Panel Area.

3. Control results are automatically filed in the STA–R®/STA Evolution® QC file. All results for a 24-hour period will be convert to a "mean" value at midnight. This mean is used in the statisti data and is plotted on the Levy-Jennings chart as a daily mea Each point can be viewed on the Levy-Jennings Daily control ch by clicking the left arrow. To print all the QC data points for any t used to calculate the mean for a specific day, perform the followi procedure.

- Click the Quality Control menu  icon.
- Select the test abbreviation for which a quality control has to be run (Single Click only)
- Select the 

or 

tab (#corresponds to the number of the desired level – Single Click only)
- Select the date on the Levy-Jennings chart 

- Click the Print icon.
- Then select the appropriate button 

,
- Confirm by clicking **Validate.**

Procedure

1. Refer to the START-UP procedure for the STA–R®/STA-R Evolution® before running patient specimens on STA–R®/STA-R Evolution® at the start of each shift.

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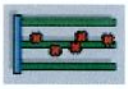
2. Request quality control.
   - Click the Quality Control menu  icon
   - Select the test abbreviation for which a quality control has to be run (Single Click only)
   - Select the Control Level 1 or Control Level 2 tab (#corresponds to the number of the desired level – Single Click only)
   - Single Click on  icon
   - Confirm by clicking on Ok
3. Load patients' samples:

Barcode labels with LIS downloading of tests

- Click the Patient Menu  icon
- Click on the **(Un)loading** tab
- Click the box next to **Downloading** to add a check
- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded, their barcode labels read and the job list will be fed to the host computer for each tube. The tests will be automatically carried out by the STA–R®/STA-R Evolution®

Barcode labels with automatic profile

- Click the Patient menu  icon
- If necessary, specify the automatic profile
- Click on the **(Un)loading** tab
- Check the Add Automatic Profile … **box**
- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded, their barcode labels read and the test profile will be added to each patient's identification. The tests will be automatically carried out by the STA–R®/STA-R Evolution®

Barcode label and manual test selection

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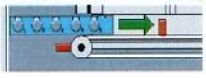
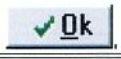
- Place the previously centrifuged tubes in the rack(s)
- Place the rack(s) in the rack tray
- Place the rack tray in the instrument: the tubes will be automatically loaded and their barcode labels read
- Add the test(s): either via the Test panel or via the Patient File Acquisition screen. (refer to manual for adding a test)

Barcode and Stat

- Click on STAT menu  icon.
- Place previously centrifuged **Stat** tube(s) in the rack
- Make sure loader is ready to accept Stat rack  (screen with green arrows)
- Slide rack into loader area
- STA–R®/STA-R Evolution® will automatically read barcode label: test information will be determined as previously mentioned.

4. Reagent Forecast
   - When patient samples are loaded, if a product is missing or insufficient to run tests  icon will blink in the SYSTEM PANEL ALARM BOX
   - Double click on  icon, the  tab will display
   - The missing reagent will be listed with **RED triangle**
   - Load the necessary product and the tests will resume
5. All patient results are displayed on the TEST PANEL screen and automatically printed out and transmitted if selected.
6. Operator intervention of sample(s)

Re-Run Result

- Double click result in question
- Click **Rerun**
- Confirm by clicking on 

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Add (insert) Test to Sample

- Scroll on the file identity (accession number…)
- Double click test box to be added for accession number
- Confirm by clicking on 

Change Priority of Loaded Sample

- Double click on the file identity (accession number…)
- Click STAT  icon

7. All dilutions of controls and patients' samples are automatically prepared by the STA–R®/STA-R Evolution® according to the parameters entered in the Test Set-up.
   If the patients' results fall outside the assay range, the STA–R®/STA-R Evolution® automatically re-tests the sample in question at an appropriate dilution, provided that the option has been entered in the Test Set-up under re-dilute conditions.

Calculations

The STA–R®/STA-R Evolution® automatically converts the results in seconds from a standard curve (log-log) to the main unit selected in the test setup (i.e. mg/dL, g/L). The assay uses a dilution of 1:20 sample plasma to buffer. The STA® System automatically dilutes this sample to a 1:8 dilution on samples with a concentration <150 mg/dL (<1.5 g/L) or a 1:40 dilution if the value is >900 mg/dL (>9.0 g/L). If the auto redilute feature is necessary the results are displayed on the Screen in Blue numerals, instead of the normal Black numerals.

Reference Range

218 – 441 mg/dL

Test Analytical Reportable Range (AMR)/Clinical Reportable Range (CRR)

1. The package insert states that the Analytical Measurement Range (AMR) of the reagent on the STA® System instrument is 150-900 mg/dL (1.50 - 9.00 g/L). This is at the normal dilution (1:20) the instrument uses to assay samples.
2. The Clinical Reportable Range on the STA® System instrument is expanded to 60-1800 mg/dL (0.60 - 1.80 g/L) (see the bar-coded Calibration Curve) with the addition of alternate dilutions defined in the Test Setups, used for the auto redilution: 1:8 if < 150 mg/dl (< 1.50 g/L) and 1:40 if > 900 mg/dL (> 9.00 g/L).

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Notes

1. When the STA–R®/STA-R Evolution® redilutes a patient sample at a more appropriate dilution (as pre-determined in Test Set-up) the results in the TEST PANEL screen will appear in blue have already been corrected by the analyzer for the dilutional difference.
2. Patients receiving thrombolytic therapy will have a rapid drop in the plasma fibrinogen level and these samples **MUST** be collected with an anticoagulant containing a plasmin inhibitor such as Aprotinin, Cat # 0820, to determine an accurate fibrinogen result.

Special Notes Related to Fibrinogen

The results >Mmax and <Mmin are prompted on raw data (sec) and FIB is reported in concentration (mg/dL or g/L). The Fibrinogen curve is an inverse curve, thus:

1. On the STA–R®/STA-R Evolution® a result of **>Vmax** for fibrinogen means the fibrinogen value is **extremely low**.
2. On the STA–R®/STA-R Evolution® a result of **<Vmin** for fibrinogen means the fibrinogen value is **extremely high**.
3. See Note # 1 in Notes section directly above. It is possible to have a **>Vmax** or **<Vmin** result after the instrument does the auto redilute.

Limitations

1. In patients receiving drugs that affect the fibrinolytic system, the plasma levels of fibrinogen degradation products (FDP) may be extremely high. FDPs may inhibit both thrombin action of fibrinogen and fibrin polymerization. At normal fibrinogen concentrations, FDPs have a minimal effect on the fibrinogen assay. At fibrinogen concentrations below 150 mg/dL, FDPs greater than 130 µg/mL increasingly inhibit the thrombin clotting rate assay. High levels of paraproteins may interfere with the polymerization of fibrin monomers.
2. The clinical use of topical bovine thrombin has led to the generation of antibodies in some patients. These antibodies may lead to artifactual prolongation of the thrombin clotting rate assay of fibrinogen.
3. Heparin may interfere with this assay. However, the STA® - Fibrinogen reagent contains a specific inhibitor of heparin. Any prolongation of the assay is therefore, related to a real coagulation factor deficiency of fibrinogen.
4. The STA® - Fibrinogen procedure is insensitive to the following substances: fibrin degradation products (up to 130 µg/mL), hirudin (up to 3 µg/mL), and heparin (up to 2 IU/mL).

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References

- STA® - Fibrinogen, Quantitative Determination of Fibrinogen by STA® Analyzers. Package insert for use in Fibrinogen determinations. Ver. English 2 - Revised July 2012.
- STA® - Coag Control N+ABN PLUS (Cat. No. 00677): citrated control plasmas normal and abnormal levels;Control Plasmas for Assays of Coagulation Parameters on STA® Analyzers. Package insert, December 2017.
- STA® - Owren-Koller Buffer (Cat. No. 00360) Buffer Solution for Coagulation Testing. Package insert 23070 06 – April 2012.
- STA - Desorb U (Cat. No. 0975) Decontamination solution for STA® analyzer systems. Package insert #26265 – revised June 2011.
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- Clinical Laboratory Standards Institute: Interference Testing in Clinical Chemistry: Approved Guideline. Second Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2005. Document EP 7-A2.

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HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Area Lab Manager/ Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
NEW		M. Acosta 07-24-14	J. Wolf 07-24-14	S. Wirio MD 07-29-14	08-12-14
Major	• New STA® - Coag Control N+ABN PLUS (Cat. No. 00677)	11-15-18	11/21/18	11/21/18	11/21/18