STAGO PT / aPTT / Fibrinogen
General Procedure

Principle:
The Stago Coagulation analyzer is an automated system used for in vitro testing of the coagulation system. For PT/aPTT/Fibrinogen, the Stago analyzer measures the time of clot formation, using an electro-magnetic mechanical clot detection system.

The Prothrombin time (PT) is a basic coagulation screening test for the assessment of congenital and acquired deficiencies of the extrinsic pathway (factors II, V, VII, X). It is also used in monitoring warfarin therapy because of its sensitivity to variations in the concentration of vitamin-K dependent factors.

The activated partial thromboplastin time (APTT) is a basic screening test for the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II, and I). It is used to detect congenital and acquired deficiencies of these factors and to monitor heparin therapy.

Low functional fibrinogen levels may be seen in DIC, during massive bleeding, liver disease, in the rare congenitally deficient patient (afibrinogenemia), or patients with non-functional fibrinogen protein. Elevated fibrinogen levels can be observed in diabetes or inflammatory syndromes, and may be associated with an increased risk of cardiovascular disease and pre-thrombolytic states. The concentration of fibrinogen is inversely proportional to the clotting time when a known amount of thrombin (in excess) is added to dilute patient plasma. The measured time is read from a stored curve on the analyzer, and is the plasma fibrinogen level.

Safety:
- The required personal protective equipment for this procedure
  - Gloves
  - Impermeable lab coats, worn closed
  - Shield
  - Approved Protective eyewear
- Gloves and lab coats should be worn at all times during analysis of the samples.
- Samples that must be run in the secondary mode must be opened behind a safety shield.
- Gloves, lab coat, and a face shield or goggles/safety glasses must be worn when performing maintenance where there is a chance of splashing.
Specimen:

1. Specimen Collection:
   Specimens are collected in 3.2% sodium citrate tubes with a collection ratio of 9:1 blood to anti-coagulant. No other anticoagulant is acceptable. The sodium citrate tube should fill completely. The specimen should be gently mixed after collection.

2. Centrifugation:
   Specimens are centrifuged capped, in non-refrigerated centrifuges to achieve platelet-poor plasma. Refer to chart for centrifuge model, time and speed used at each site.

3. Unacceptable Specimens:
   - Specimens processed in a centrifuge that can not achieve platelet-poor plasma (<10,000 k/uL) are unacceptable.
   - Samples that are short draws, clotted, over-filled or hemolyzed may yield incorrect results and should be recollected.
   - Samples with a HCT >55% should be collected in tubes that have an adjusted amount of anticoagulant to insure accurate results. Use the “Criteria Nomogram for Acceptability of Citrated Plasma” (0544F) to visually detect a HCT >55%. Once it has been determined that citrate adjustment should occur, use the “Citrate Adjustment Chart” under Special Preparation for anticoagulant adjustments.

4. Special Preparation:

   **Citrate Adjustment Chart for HCT>55%:**
   
   *Small Plastic Citrate Tubes, 3 ml*
   
   *(Normal draw 2.7 ml blood/ 0.3 ml citrate)*

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Volume citrate to REMOVE from tube</th>
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</thead>
<tbody>
<tr>
<td>55.0 – 57.0</td>
<td>Remove 0.08 ml</td>
</tr>
<tr>
<td>57.1 – 59.0</td>
<td>Remove 0.09 ml</td>
</tr>
<tr>
<td>59.1 – 61.0</td>
<td>Remove 0.10 ml</td>
</tr>
<tr>
<td>61.1 – 63.0</td>
<td>Remove 0.11 ml</td>
</tr>
<tr>
<td>63.1 – 65.0</td>
<td>Remove 0.12 ml</td>
</tr>
<tr>
<td>65.1 – 67.0</td>
<td>Remove 0.13 ml</td>
</tr>
<tr>
<td>67.1 – 69.0</td>
<td>Remove 0.14 ml</td>
</tr>
<tr>
<td>69.1 – 71.0</td>
<td>Remove 0.15 ml</td>
</tr>
<tr>
<td>71.1 – 73.0</td>
<td>Remove 0.16 ml</td>
</tr>
<tr>
<td>73.1 – 75.0</td>
<td>Remove 0.17 ml</td>
</tr>
<tr>
<td>75.1 – 77.0</td>
<td>Remove 0.18 ml</td>
</tr>
<tr>
<td>77.1 – 79.0</td>
<td>Remove 0.19 ml</td>
</tr>
<tr>
<td>79.1 – 80.0</td>
<td>Remove 0.20 ml</td>
</tr>
</tbody>
</table>

   - Adjust the amount of anticoagulant in a sodium citrate tube according to the chart. Since the tube vacuum has been lost after removing the cap to do the adjustment,
the patient’s blood must be drawn using a syringe (draw 2.7 mL of blood), and then carefully delivered to the adjusted tube, capped, and mix thoroughly. Report results with the comment “specimen collected in anticoagulant adjusted tube”.

5. **Stability:**
Centrifugation and testing should be performed as soon as possible, to maintain the integrity of the specimen. While performing these tests outside the limits of their stability is unacceptable, specimens are capped and retained for at least 24 hours for purposes of specimen identity/integrity investigation.
If testing is not performed within 24 hours for PT and four hours for aPTT, plasma should double-spun and platelet-poor plasma should be frozen (-20°C up to two weeks, or -70°C for long-term storage). Frozen plasma should be rapidly thawed at 37°C, then gently mixed and tested immediately. **Mixing is critical before testing,** in order to resuspend proteins that may have been precipitated by freezing.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>8 hrs @ 15-25°C</td>
</tr>
<tr>
<td>aPTT</td>
<td>4 hrs @ 15-25°C</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>8 hrs @ 15-25°C</td>
</tr>
</tbody>
</table>

6. **Specimen Storage**
While performing these tests outside the limits of their stability is unacceptable, specimens are capped and retained for at least 24 hours for purposes of specimen identity/integrity investigation.

**Equipment and Materials:**
Centrifuge
Pipettes
Pipette tips
ABC Stago analyzer
Cuvette roll (contains 1000 cuvettes)
Magnetic stir bars
Reducer
Wash Solution
Clinical Laboratory Reagent Water or Commercially Bottled Purified Water
Reagents and QC Material:

<table>
<thead>
<tr>
<th>Product</th>
<th>Reconstitution</th>
<th>Stabilization time before use</th>
<th>Stability on STAGO (15-19°C)</th>
<th>Reconstituted reagent stability in refrigerator (2-8°C)</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastine Cl Plus*</td>
<td>Pour contents of Rgt. 2 vial into Rgt. 1 vial</td>
<td>Stand at RT 30 min. Swirl gently.</td>
<td>48 hours</td>
<td>8 days in original capped vial (stand at RT 30 min before use)</td>
<td>Use with stir bar &amp; reducer</td>
</tr>
<tr>
<td>PTT-Automate</td>
<td>5 ml Rgt. Grade Water</td>
<td>Stand at RT 30 min. Vortex on low for 5 sec before loading.</td>
<td>24 hours</td>
<td>7 days in original capped vial (stand at RT 30 min before use)</td>
<td>None</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>5 ml Rgt. Grade Water</td>
<td>Stand at RT 30 min. Swirl gently.</td>
<td>120 hours (5 days)</td>
<td>14 days in original capped vial (RT 30 min before use)</td>
<td>None</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>Load without white cap</td>
<td>None</td>
<td>72 hours (3 days)</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>Coag N &amp; ABN</td>
<td>Reconstitute each vial with 1 ml Rgt. Grade Water</td>
<td>Stand at RT 30 min. Swirl gently.</td>
<td>8 hours</td>
<td>8 hours</td>
<td>None</td>
</tr>
<tr>
<td>Owren-Koller</td>
<td>Load without white cap</td>
<td>None</td>
<td>72 hours (3 days)</td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
</table>

*Caution: Neoplastine Reagent 2 contains sodium azide as a preservative and should be discarded with care. When dumping waste in the sink, follow with copious amount water to clear the plumbing.

- Before loading any product, check surface for foam/bubbles and if present remove.
- RT = room temperature

Quality Control:
Coag Control N and Abn:
After the reconstitution period:
1. Load QC material onto analyzer:
   - Request product drawer to open through “Main Menu” under “Loading” “Reagents”.
   - Scan bar-coded controls, confirm volume, press <enter> on keyboard, and place in designated position on the analyzer.
2. To test QC:
   - Select “Main Menu”, “Calib/Control”, “Quality Control”, <enter>.
   - Place the cursor on the tests, and select each test by pressing “F1”.
   - When all the tests have been selected, press “F10” to run the QC.
Standardized

- When prompted, enter “QC or CQ” (depending on site) as the access code and press <enter>.
- Escape from QC screen to begin testing of controls.

Both levels of QC are tested every 8 hours of patient testing. QC also is tested if reagents are added, or if maintenance is performed during a shift.

Control ranges are monitored automatically by the analyzer. If any controls are outside the range stated on the manufacturer package insert, the analyzer will audibly and/or visually alarm the operator. All results for a 24-hour period will be held in the Stago QC file, and reduced to a “mean” value at midnight. This mean is plotted on the Levy-Jennings chart as a daily mean.

New lots of QC are sequestered annually for SLN and ranges are established during annual reagent lot switchover. System established ranges are then set up in the LIS and cannot exceed the ranges on the STAGO package insert. QC results entered into the LIS must meet the Westgard rules in the SLN QC policy.

To investigate an unacceptable control value:
- First check control for time of reconstitution.
- Repeat the QC run. If results are still out of range, reconstitute a fresh control sample and re-test.
- If results are still out of range, prepare new reagents and run QC.
- If results are still out of range, examine the analyzer set-up for any visible problems (i.e. bent needles, leaks).
- If no resolution, call technical service.
- Patient results cannot be reported until control value(s) are within acceptable limits.

Blind Duplicates:
A previously tested patient sample, with results in the normal range should be tested between analyzers at sites who alternate the use of two analyzers. This sample, along with QC samples, should be run on the newly set-up analyzer before running patient samples. The sample should be within the specimen age requirements for patients. If a result is out of range, troubleshoot and repeat the blind duplicate. If commercial QC is acceptable, choose another sample to use for a blind duplicate.

Calibration:
- No calibration is required for PT or aPTT.
- The pre-calibrated fibrinogen values are identical for all the vials of each lot.

Entering the data for the Fibrinogen calibration curve:
- When the operator scans a new lot of fibrinogen reagent, the STA / STA Compact will request the operator to scan the bar code printed on the bar code insert across the analyzer bar code reader. The curve is automatically read from this bar code label.
• The calibration curve will be validated for the lot in use once the two fibrinogen control levels have been tested. If the controls are out of range, the analyzer will not test patient samples or validate the curve.
• To examine the curve on the analyzer screen: Through “Main Menu”, under “Calib/Control”, select “Calibration”. Move the cursor to “FIB” and <enter>. Curve will display on the screen.
• To print the calibration curve: With the curve displayed on the screen, press ESC for options; select PRINT. A curve can not be printed while the analyzer is running.

Refer to STAGO Maintenance Procedure for instructions on Fibrinogen linearity verification.

Procedure:
Changing Sampling Modes
1. The Compact Stago can operate in two different patient loading modes, auto mode or manual mode. To change loading modes, select **Loading** from the main menu.
2. Select **Samples**. The mode will be displayed near the top of the screen. If you wish to change modes press ESC for options. Choose the desired mode.

Loading Patient Samples in Manual Mode
1. Choose **Loading** from the main menu.
2. Select **Samples** (the sample drawer will open)
3. Ensure that the sample mode is set to manual
4. Scan or type the accession and press enter on the keyboard
5. Load the tube into the sample drawer.
6. Select tests from the test menu.
7. Press **F10** to save.
8. Continue loading samples until all samples have been loaded, pressing **F10** after each sample.
9. After loading the last sample, press **ESC**. The drawer will close and processing will begin.

Loading Patient Samples in Auto Mode.
1. Choose **Loading** from the main menu.
2. Select **Samples** (the sample drawer will open)
3. Ensure that the sample mode is set to auto.
4. Scan or type the accession and press enter on the keyboard
5. Load the tube into the sample drawer.
6. After loading the last sample, press **ESC**. The drawer will close and processing will begin.
Microsample pour-offs
- Select F8 to indicate volume once drawer has opened. Be sure to turn off “micro volume” before running normal volume samples again.

Instrument Processing
- As soon as the sample drawer closes, the “Test Status” screen will appear. If there are insufficient reagents to run the test, the “Blocked Sample Pipetting” will appear in red with the amount of deficiency. This deficiency will BLOCK the sample pipettor. When this occurs, add the necessary reagents and run QC.
- Dilutions are automatically prepared by the analyzer, according to the established parameters that are entered in each TEST SETUP at time of installation. If the patients’ results fall outside the assay range, the analyzer will automatically re-test that sample at an appropriate dilution, if the test has been previously defined for this response.

Results:
- All patient results display on the TEST PANEL screen of analyzer’s monitor.
- Results will automatically transmit to the LIS.
- Results may automatically print or be batch printed at the end of each day.
- For results in question that need operator intervention, cursor to the identification number in the “Test Panel” screen; <enter>. This will display the File Processing screen. Follow the options in the left hand corner of the screen, (i.e. re-run test).

Reporting Results:
- See Stago Information Sheet, #0716C and Stago Compact Error Codes, #0544C, for result reporting and error code information.

Reference Range:
See Stago Information Sheet, #0716C for current reference ranges.

<table>
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<th>Therapeutic Ranges</th>
<th>Comments</th>
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<tr>
<td>INR</td>
<td></td>
</tr>
<tr>
<td>&lt;1.5</td>
<td></td>
</tr>
<tr>
<td>1.5-2.0</td>
<td>Low-intensity Therapeutic Range</td>
</tr>
<tr>
<td>2.0-3.0</td>
<td>Mod-intensity Therapeutic Range</td>
</tr>
<tr>
<td>2.5-3.5</td>
<td>High-intensity Therapeutic Range</td>
</tr>
<tr>
<td>3.0-4.0</td>
<td>High-intensity Therapeutic Range</td>
</tr>
<tr>
<td>5</td>
<td>Common Critical/Alarm Value</td>
</tr>
<tr>
<td>10</td>
<td>Common Upper Limit reported</td>
</tr>
</tbody>
</table>
Critical Values:
Critical values must be verified, called immediately, and documented in the LIS. See Stago Information Sheet #0716C for current critical values.

Procedural Notes:
1. New lot numbers of Neoplastine, PTT-A, and Fibrinogen reagent are changed annually and are done as a System. No changes are made by individual sites. Refer to “Annual Lot Conversion” procedure for instructions.
2. Patients receiving thrombolytic therapy will have a rapid drop in the plasma Fibrinogen level. These samples must be collected with an anticoagulant containing a plasmin inhibitor to determine accurate Fibrinogen result.

DIC Panel Reporting:
The panel for testing suspected cases of Disseminated Intravascular Coagulation (DIC) includes the following tests: PT, APTT, Fibrinogen, D-dimer, Platelet count, and smear review (Tech Review) for the presence of shistocytes. The panel tests appear collectively on the patient report when ordered as a panel. This panel is available for testing only in the hospital setting.
NOTE: when reporting the “Smear Review” portion of the panel, report either “schistocytes noted on the smear” or “no schistocytes on the smear” using site specific entry codes.

Limitations of Procedure:
1. STA Neoplastine CI and CI+, both contain a specific inhibitor of heparin. Therefore, only levels of heparin outside of the therapeutic range will affect the PT results
2. Many commonly administered drugs may affect the results obtained in APTT testing. (Example: heparin and coumadin).
3. High levels of paraproteins may interfere with the fibrinogen assay.
4. The clinical use of topical bovine thrombin can cause the generation of antibodies which can lead to artificial prolongation of fibrinogen results.
5. The STA-Fibrinogen reagent contains a specific inhibitor of heparin. However, heparin levels above 2 IU/mL may interfere with this assay.
8. The STA Fibrinogen procedure is insensitive to hirudin levels up to 3 ug/mL.
9. Fibrinogen levels will be falsely low in the presence of thrombolytic agents (tPA, streptokinase). Plasma from patients receiving these agents must be treated with an inhibitor (ie, aprotonin) before testing for fibrinogen in order to obtain accurate values.
References:
## Authorizing Documentation

### Document Preparation

<table>
<thead>
<tr>
<th>Prepared by / Date</th>
<th>Supersedes Procedure #</th>
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<tbody>
<tr>
<td>Donna Hogan, MT, (ASCP) SH &amp; Susan Soles, MT(ASCP) / September 17, 2008</td>
<td>0701</td>
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### Review by Corporate Medical Director

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<th>Corporate Medical Director Signature</th>
<th>Adoption Date / Review Date</th>
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<tr>
<td>Janice J. Hessling, MD PhD</td>
<td>09/30/08</td>
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<tr>
<td>[Signature]</td>
<td>09/30/09</td>
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### Review by Performing Lab’s Site Director or Technical Supervisor

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### Review by Performing Lab’s Medical Director or Designee

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### Subsequent Review / Revisions by Standardization Group

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<th>Revision Date</th>
<th>Retired Date</th>
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<tr>
<td>Susan Soles, MT(ASCP)</td>
<td>07/30/09</td>
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### Document History:

- 09/08 New procedure created by combining 0544 Operating, 0542 PT, 0543 aPTT, and 0537 Fibrinogen procedures. Updated specimen and reagent stabilities per revised STAGO package inserts.
- 07/09 Added Safety Section. Minor formatting changes. Removed the Reference Range and Critical Values numbers and referred the reader to Stago Information Sheet #0716C.