

 Proc. #4840-CO-0605

**TITLE: Activated Partial Thromboplastin Time – Sysmex CS-2500**

**PRINCIPLE**

The activated partial thromboplastin time (PTT) is a screening procedure for deficiencies in coagulation factors of the intrinsic coagulation pathway, factors VIII, IX, XI, XII, Fletcher factor and Fitzgerald factor. Severe deficiencies of fibrinogen and factors II, V and X can also be detected by the PTT. It is a useful and effective method for screening patients with a bleeding tendency, for evaluating the effect of therapy in pro-coagulant disorders and as the basis for several specific coagulation factor assay procedures. The PTT is widely advocated as a test for monitoring and regulating heparin therapy. The presence of non-specific inhibitors, such as the lupus anticoagulant, may prolong the PTT; this effect is variable and is generally recognized as being related to the nature and level of the inhibitor present. The PTT consists of recalcifying plasma in the presence of a standardized amount of platelet-like phosphatides and an activator of the contact factors of the intrinsic coagulation pathway measured at 37 degrees. The PTT is the time in seconds, required for a fibrin clot to form.

**SPECIMEN TYPE**

 Blue top(3.2%) sodium citrate vacutainer tube. Both the 2mL or 3mL 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.

 **Handling Conditions:**

The specimen should be transported at room temperature. Prior to centrifugation, the whole blood specimen is checked for clot formation by gentle inversion and an applicator stick. Centrifuge the capped specimen for a minimum of 3 minutes at 4500 RPMs or at a speed and time that will produce platelet poor plasma. Although it is recommended to test samples as soon as possible the specimen is stable for 4 hours when stored at 15-25°C. Do not store on ice or refrigerated. If testing is not complete within 4 hours, specimens can be frozen at -20°C or below for short term storage for up to 2 weeks or -70°C or below for long term storage. Frozen plasma samples must be rapidly thawed at 37°C while gently mixing and tested immediately.Do not store at 37°Cfor more than 5 minutes. **Mixing is critical before testing.**

**EQUIPMENT AND MATERAILS:**

**Equipment:**

Sysmex® CA-2500 Instrument

4.0 mL Sample Cups

Reaction Tubes (only use Cuvette SUC-400A)

**Materials:**

 Dade® Actin® FSL Activated PTT Reagent

Calcium Chloride, 0.025M

Dade® Ci-Trol® Coagulation Controls, Level 1, Level 3

CA Clean I

 Preservative -free distilled or deionized water

 **Preparation:**

 **1**. **Dade® Actin® FSL Activated aPTT Reagent** -purified soy and rabbit brain phosphatides in 1 x 10-4 M ellagic acid with added buffer, stabilizers and preservative.

Dade® Actin FSL Reagent must be mixed gently by inversion (5 to 8 times) before use. The reagent is liquid and ready for use.

Store at 2-8°C. **Do not freeze.**

 **Stability**:

 On board 72 hours (cooled position)

 24 hours (non-cooled position)

 2 to 8°C used by the labeled expiration date when unopened

 2 to 8°C use within 7 days after opening

 **2. Calcium Chloride (0.025M)**

The reagent is liquid and ready to use.

 Store at 2-25°C when not in use.

**Stability:**

 On board 72 hours

 + 2 to 25°C Stable until the date indicated on the vial label date

**This product is for in vitro diagnostic use.**

**3. Dade® Ci-Trol® Coagulation Control, Level 1** (Normal) -Lyophilized preparation of human plasma, stabilizers and buffers. Used for Quality Control.

**4. Dade® Ci-Trol® Coagulation Control Levels 3** (Abnormal)-Lyophilized preparations of

Human plasma, stabilizers and buffers. Used for Quality Control.

**Stability after reconstitution:**

|  |  |
| --- | --- |
| **Condition** | **Ci-Trol 1, 3** |
| **On-board** | **24 hours (SLD) mini cup** |
| **+2 to +8°C** | **16 hours (closed vial)** |
| **+15 to +25°C** | **8 hours (closed vial)** |
| **-20°C** | **--------------** |
| **\*Frozen and thawed once** | **NO** |

* + *Note: Do not expose thawed control to 15 to 25°C for longer than 2 hours.*

**5. CA Clean I is liquid and ready for use.**Store at 2-8 °C. Do Not Freeze.

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

 Opened bottles are stable for 30 days at 2-8 °C.

**6. CA Clean II is liquid and ready for use.**

 Store at 5-35°C

 Opened stability is 2 months.

 **These products are for in vitro diagnostic use.**

**Performance Parameters:**

Indication of deterioration: Controls will show deviations in results from the established laboratory range.

**QUALITY CONTROL**

Dade® Ci-Trol® Control Level 1

Dade® Ci-Trol® Control Level 3

(1) Controls should be tested at the initiation of testing each day, upon reagent changes, and at least once each 8 hour shift of instrument use.

(2) Controls should be run in the same manner as the test samples.

1. Control tolerance limits--the range is calculated based on +/-2.0 to +/-2.5 standard deviations (SD) from the mean control value.

(4) Corrective action when tolerance limits are exceeded:

1. Rerun out-of-range control material.

(b) Verify reagent performance.

 (c) Check instrument performance.

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

PROCEDURES FOR SETUP:

 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/ time.

Loading QC:

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.

1. **Dispose of Used Reaction Tubes from the Trash Box.**

Reset the trash counter.

\*Loading consumables and discarding waste should be done along with the daily maintenance. See the SOP for complete directions for performing maintenance.

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**.

 11. Once complete the QC results must be entered in the LIS

SAMPLE PROCESSING

After QC is performed and found to be acceptable, 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:**

Results of the PTT testing should be reported as the PTT in seconds. These results should be related to the reference interval for the PTT. Verify results in the computer system. Hemolyzed, lipemic, or icteric samples must be noted with the result. In cases of extreme lipemia, icteric or hemolysis a result may not be possible if a valid curve is not obtained. Hemolyzed specimens should be re-collected. Lipemic or icteric specimens may have to be sent out to a reference lab for testing.

**Reference Interval:**

 **Reference interval values determined for each lot of Actin® (+/- 2.0 SD). See reference range binder for lot specific values.**

**Reference Range: 22.4-35.8 seconds**

**Analytical Measuring Range: 20.0 – 205.0 report as <20 or >205**

**If results are <20, check the sample for clots, rerun sample to verify <20 or >205, Document in comment field that sample was rerun and was checked for a clot. If clot is found request redraw and cancel test.**

**For Samples with a “No Coagulation” use the @NCRX in the result field for the PTT. Call the nurse and document as we do for critical values. Repeat “No Coagulation” on extend PTT mode. If repeated result is “No Coagulation” result as @NCRX.**

**Critical Results: PTT > 75.0 seconds or no clot detected**

 **NICU (0-3 months): >60.0 seconds**

**Critical values must be phoned to the patient’s nurse or physician as a Critical Value. Document with (RBTO) comment; RN’s full name, time/date called and your initials.**

 **PROCEDURE NOTES:**

Overall performance of PTT testing is dependent on reagent and instrument performance.

Acceptable variability (imprecision) should be such that the total day-to-day coefficient of variation (CV) of the analytic system is less than 5% on the same lot of control plasma.

**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 will need to be repeated.

**LIMITATIONS OF THE PROCEDURE:**

Plasma collection and storage conditions must be carefully controlled. Avoid small plasma volumes prior to testing which may cause pH changes in the plasma leading to inactivation of specific components of the coagulation system. Testing may be affected by a number of commonly administered drugs. Short PTT values during conjugated estrogen therapy in males and oral contraceptive administration in females may occur. Diphenylhydantoin, heparin, warfarin, naloxone and radiographic agents may increase the PTT. Hemolyzed, lipemic, chromogenic specimens may also affect results. Blood clotting factor deficiencies, which should produce prolonged PTT values may be compensated and appear normal by elevated levels of one or more different clotting factors. The presence of active intermediates, which would normally shorten the clotting time may also mask conditions that would lead to prolongation of the PTT. Mild or minor deficiencies in several factors may have an additive effect on increasing the PTT. Unexpected abnormal PTT results should be followed by additional coagulation studies to determine the cause of abnormal results.

**INTERFERENCES:**

 No interferences up to:

|  |  |
| --- | --- |
| Triglycerides | 544 mg/dL |
| Hemoglobin  | 1000 mg/dL |
| Bilirubin unconjugated | 60 mg/dL |
| Bilirubin conjugated | 40 mg/dL |

**REFERENCES:**

1. Dade® Actin® FSL Activated PTT Reagent package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, June 2008.
2. Dade® Ci-Trol® Coagulation Controls, Levels 1, 3 package inserts, Siemens Healthcare Diagnostics Inc., Newark, DE, May 2008.

3. Clinical Laboratory Standards Institute. Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays: Approved Guideline-Fifth Edition. CLSI Publication H21-A5. Wayne, PA, January, 2008.

4. Sysmex CS-2500 Operators Manual

*S:\Laboratory Policies and Procedures \Coagulation 7/04/20 TML*