

 Proc. #4840-CO-0600

**TITLE: Prothrombin Time – Sysmex CS-2500**

**PRINCIPLE**

The Prothrombin Time test (PT) is a global screening test with three major applications. These include 1) detection of single or combined deficiencies of the extrinsic coagulation pathway indicative of hereditary and acquired coagulation disorders, liver disease or vitamin K deficiency; 2) monitoring test for oral anticoagulant therapy; and 3) assay for specific extrinsic coagulation factors.

The coagulation cascade is activated by incubating plasma at 37°C with the optimal amount of thromboplastin and calcium; the PT is the time, in seconds, required for a fibrin clot to form.

**SPECIMEN COLLECTION**

**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.

 **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 24 hours when stored at 15-25°C. Do not store on ice or refrigerated. If testing is not complete within 24 hours, specimens can be frozen at -20°C or below for short term storage (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

SLD mini-cups

Reaction Tubes (only use Cuvette SUC-400A)

**Materials:**

Dade® Innovin®

Control material:

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

CA Clean™ I and Clean II

 Preservative -free distilled or deionized water

**Preparation**

1. **Dade® Innovin®**—lyophilized recombinant human tissue factor and synthetic phospholipids (thromboplastin); calcium ions; a heparin neutralizing compound; buffer; and stabilizers (Bovine Serum Albumin).

Reconstitute each vial of lyophilized Innovin with sterile deionized water using the volume stated on the vial label.

Immediately after addition of water, mix contents of vial well before use to ensure a homogenous solution. If allowed to stand, mix contents of vial before. Continuous mixing is not necessary. Store at 2-8°C. **Do not freeze.**

 **Stability after reconstitution**:

 On board 72 hours

2-8°C 10 days at (closed vial)

 15-25°C 5 days in (closed vial)

 37°C 24 hours at (closed vial)

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

**3. Dade® Ci-Trol® Coagulation Control Level 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.*

**4. CA Clean I is liquid and ready for use.**

On-board stability is 120 hoursStore 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.

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

On-board stability is 120 hours

 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:

 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 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. Enter the results of QC 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 analyzed.

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**.

***NOTE:*** *The cause of any printed error code must be investigated and the appropriate corrective action taken prior to reporting results.*

**REPORTING RESULTS**

PT results are reported in seconds. These results should be related to the reference interval. All PT results are reported with the INR. The INR is calculated from the lot-specific and instrument specific ISI of Innovin (see package insert) and the geometric mean of the normal reference interval. Review values on the analyzer printout, monitor with any error codes or flagging, and in the computer system with any previous history. Review and verify quality control values in the LIS. Results will auto-verify if QC has been run, is within limits and there are no instrument error codes, flags or critical results.

Hemolyzed, lipemic, or icteric samples must be noted as a comment 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.

Refer to instrument Quick Guides for examples of curve errors.

***TO REPORT INR RESULTS***

In order to report INR results enter the Innovin reagent lot specific ISI value and mean of the reference interval.

1. Enter PT lot and ISI value in Reagent Lot Master using the 2D barcode from the PT ISI sheet. Follow the directions for entering reagent information.

2. Load PT reagent onto reagent table.

3. Press **Calibration Curve**.

4. Press **Change**.

5. Press **PT**.

6. Select specific lot number.

7. Select **Edit**.

8. Select **Lot Master** key to enter ISI value.

9. Select **Yes.**

10. Manual entry of Normal Patient Mean: Place cursor in **Normal Value** field and enter value using keypad. Press Enter. ISI Value can be entered manually if needed.

11. Select **OK**.

12. Select **Validate**.

13. Select **OK**.

***NOTE:*** *An INR will automatically be calculated if the ISI and geometric mean of the normal reference interval have been entered under the PT Standard Curve.*

 **Reference Ranges:**

**Reference interval values will be based on the ISI value for the current lot of Innovin.**

**Analytical Measuring Range (AMR):** **8.7 – 130.0**. **Results >130 seconds or an INR of >10.0 should be rerun and the specimen integrity of the sample should be evaluated.** **If the PT is <9.3 on analyzer the INR will populate <0.9 into LIS, this is correct and results should be released. If the PT is close to 9.3 then the INR sometimes calculates to a number less than 0.9 and <0.9 will populate into result field which is correct and results should be released.**

**Reference Range: PT 9.3-11.7 sec**

**Critical Values: INR: Outpatient: INR > 4.0**

 **Inpatient: INR >5.0 or no clot detected**

 **NICU:**  >**16.0 sec. or INR >5.0**

All Critical Values must be phoned to the patient’s nurse or physician as a Critical Value. Document in the LIS with the (RBTO) comment: RN’s full name, time/date and your initials.

As part of a routine check, the Sr. Tech or designee checks random printed patient reports as they are faxed to verify the following: the patient value, INR and that the correct Reference Range has printed.

**PROCEDURE NOTES**

Overall performance of PT 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 **Administrator 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**

Oral anticoagulants depress the production of factors II, VII, IX and X in the liver by inhibiting the action of Vitamin K. The PT is sensitive to the levels of factor II, VII and X and is used to monitor patient therapy with oral anticoagulants. Many commonly administered drugs may affect the PT results. These should be considered when unusual or unexpected abnormal results are obtained. Unexpected abnormal results should be followed by further coagulation studies to determine the source of the abnormality.

**INTERFERENCES:**

No interference up to:

|  |  |
| --- | --- |
| Triglycerides | 203 mg/dL |
| Hemoglobin  | 200 mg/dL |
| Bilirubin  | 60 mg/dL |

**REFERENCES**

1. Dade® Innovin® package insert, Siemens Healthcare Diagnostics Inc., Newark, DE, May 2008
2. Dade® Ci-Trol® Coagulation Controls, Levels 1, 2 and 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. Clinical Laboratory Standards Institute, One-stage Prothrombin Time Test (PT) and Activated Partial Thromboplastin Time (APTT) Test Approved Guideline: H47-A. Wayne, PA, June 1996.

 5. Application sheet for PT Innovin® on CS-2500

 6. Sysmex® CS-2500 series Operator’s Manual

S:\Laboratory Policies and Procedures\ Coagulation