Beaumont HEALTH

Beaumont Laboratory
Royal Oak

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Related Documents:
RC.HM.CG.PR.001 Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only)
RC.HM.CG.PR.002 Coagulation Tests: Reportable Limits and Normal / Therapeutic Values

PROTIME – START 4 METHOD

RC.HM.CG.PR.070.r02

Principle

This is a mechanical clot detection method.

The Prothrombin Time (PT) is the time required for recalcified plasma to clot after incubation with tissue thromboplastin. It is a measure of the rate at which thrombin is formed via the extrinsic pathway of coagulation. Tissue thromboplastin and calcium are added to citrated plasma so that the action of factors VIII, IX, XI, XII and platelets in the coagulation mechanism is bypassed. Tissue thromboplastin, however, reacts with factor V, VII and X to form prothrombinase which converts prothrombin to thrombin. Thrombin then acts on fibrinogen to form fibrin. In the presence of a normal fibrinogen level, a prolonged Prothrombin Time, therefore, indicates deficiencies of one or more of factors II, V, VII, X or the presence of a coagulation inhibitor.

The detection on the START 4 is based on the increase in viscosity of the plasma being tested as a clot is formed. Increases in viscosity are measured through the perpendicular motion of a steel ball. There are two coils on opposite sides of a cuvette that produce an electromagnetic oscillation of the steel ball. When the appropriate start reagent is added, the detection starts immediately. The ball starts oscillating left and right, activating the chronometer. As a clot appears, plasma viscosity increases and the amplitude of the ball's oscillation decreases.

Specimen Collection and Handling

Refer to Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only) procedure.

Supplies

EQUIPMENT / MATERIALS:

Diagnosica Stago START 4
Cuvettes
Metal balls with dispenser
100mcL and 200mcL pipettes
Small pipette tips
Plastic tubes

REAGENTS:

1. **HemosIL RecombiPlasTin 2G –** 5 x 20 mL vials of lyophilized recombinant human tissue factor, synthetic phospholipid with stabilizers, preservative and buffer. Allow each vial of RecombiPlasTin 2G and RecombiPlasTin 2G Diluent to equilibrate at 15-25ºC for at least 15 minutes before reconstituting the lyophilized reagent with the diluent. **Pipette** exactly 20 mL of diluent into the vial of reagent. **Do not pour the contents of the diluent vial into the vial of RecombiPlasTin 2G.** Following reconstitution, replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25ºC for 15 to 20 minutes and invert to mix before use. Stability after reconstitution: 10 days at 2-8°C, 5 days at 15-25°C in the original vial or 10 days at 15°C on the ACL TOP® Family

2. **HemosIL RecombiPlastin 2G Diluent- 5 X 20 vials of an aqueous solution of Calcium Chloride, polybrene and a preservative.**
3. **Distilled Water** – Reagent grade, distilled, deionized water.

CONTROLS:

1. **HemosIL Normal Control 1:** Lyophilized human plasma containing buffer, stabilizers and preservatives. Store unopened vials at 2-8ºC and use by the expiration date printed on the label. Dissolve the contents of each vial with 1 mL of deionized (DI) water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25ºC for 30 minutes and invert to mix before use. Stability after reconstitution is 24 hours at 2-8°C or 15-30°C on board the ACL TOP.
2. **HemosIL Abnormal Control 3:** Lyophilized human plasma containing buffer, stabilizers and preservatives. Store unopened vials at 2-8ºC and use by the expiration date printed on the label. Dissolve the contents of each vial with 1 mL of DI water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25ºC for 30 minutes and invert to mix before use. Stability after reconstitution is 24 hours at 2-8°C or 15-30°C on board the ACL TOP.

Maintenance

Refer to Attachment A.

Quality Control

START 4 QC is required only when patient samples are run on the instrument. Document QC results on START 4 QC log.

HemosIL Normal control 1 and HemosIL Abnormal control 3 are run once per shift when the instrument is used.

Procedure

1. Pour the amount of RecombiPlasTin 2G needed into a plastic tube. (200µL per sample; all samples are run in duplicate). Label the tube ”RecombiPlasTin 2G” with time and date. Incubate at 37^{0}C.
2. Place a 4-cuvette strip in incubation column #1. Add a metal ball to each cuvette. Let the cuvettes incubate for at least 3 minutes.

3. From the Main Menu, select *TEST MODE* by pressing the [1] key and confirm with [ENT] key. Select Protime by pressing the [1] key.
4. Enter the patient ID number. Only 2 samples per run. A maximum of 7 digits is allowed for each ID number. Only enter the ID once. The START 4 will display your ID in duplicate. After the ID numbers have been entered, press [ENT] and a working list will print out and the screen displays the working screen.
5. Pipette 100mcL of sample or control to each cuvette.
6. Immediately press incubation timer key #1 at bottom of incubation column. The column timer #1 starts to run.
7. Ten seconds before the 120 second incubation time is reached, the instrument starts to beep. At this sound, quickly transfer the cuvette strip from incubation column to test column. Exactly when the incubation timer #1 reaches the 120 seconds mark, dispense 200μL of RecombiPlasTin 2G into cuvette channel #1 and press the PIP key; repeat into the successive cuvette channels #2, #3, and #4. Each time RecombiPlasTin 2G is added press the PIP key simultaneously.
8. When a test in a cuvette channel has reached its end-point, the clotting time is displayed on the screen.
9. When all the tests in the 4-cuvette channels have reached their end-points, their respective clotting times are displayed and the results are printed out. If there is an "*" next to the result, repeat the sample due to a >5% CV.
10. Duplicates for PT should agree within 5% of the shorter clotting time. If duplicates do not agree within these criteria, the test must be repeated. Three of the four times should then agree within 10% of the shortest clotting time observed.
11. Record the control results on the QC log and the patient results on the START 4 report form.
12. Enter the results in LIS. If the test performed by START 4 confirms a questionable result from an automated instrument, then enter the result from the automated instrument and add the comment "verified by alternate method". If the result from the START 4 does not match the result from the automated instrument within 10%, then enter the result from the START 4 and add the comment "performed by alternate method".

Limitations

1. Many commonly administered drugs may affect the results obtained in prothrombin time testing. This should be kept in mind especially when unusual or unexpected abnormal results are obtained.

Expected Values

REPORTABLE RANGE:
PT <9.0 seconds - >100.0 seconds
INR <0.9 - >8.0

TAT:

Routine Protime	2 hours
STAT Protime	30 minutes

References

1. Start 4 Operator's Manual, Diagnostica Stago, France, June 2002.
4. Palkuti, HS: *Clinical Hemostasis Review,* Coagulation Questions and Comments, January 1993.
5. Palkuti, HS and Jensen R: *Clinical Hemostasis Review,* Coagulation Questions and Comments, February 1993.

Authorized Reviewers

Chair, Pathology and Laboratory Medicine
Medical Director, Coagulation

ATTACHMENT A – DIAGNOSTICA STAGO START 4 / PREVENTATIVE MAINTENANCE

MONTHLY: Cleaning

1. Clean the work-surface and the reagent storage wells with absorbent paper soaked with deionized water.
2. Use cotton-tips soaked in deionized water to clean the incubation and test wells.

SIX-MONTHS: System Check

1. From the “Main Menu” display, press the [4] key and confirm with [ENT]. Then from the “System check” display, select “Diagnostic Tests” by pressing the [2] key and confirm with [ENT]. Press [ENT] to initiate the Self-Check.
2. Follow the instructions displayed on the screen. If any of the tests do not check out notify the supervisor.
3. When the “Self Test” is completed, the “System check” Menu is displayed again.
4. Press the [ESC] key to go back to the “Main Menu”.

PROTIME – START 4 METHOD

Document Control

Location of Master: Coagulation Procedure Manual
Master electronic file stored on the Beaumont Laboratory server under S:\HEMACOAG\ Document Control\Coagulation\Procedure\Master Document\ProTime Start4.doc
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Karri Henderson MT(ASCP)	04/30/2012			
Approved by: Marc Smith, MD	12/11/2012			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Marc D. Smith, MD	12/11/2012	00	New Procedure.	OK
Mark D. Kolins, MD	12/12/2012			
Marc Smith, MD	12/22/2014		No change	OK
Marc Smith, MD	05/20/2016		No change	OK
Elizabeth Sykes, MD	02/22/2018			
Marc Smith, MD	10/02/2018	**01**	Updated logo. Changed reagents and controls used.	OK
Peter Millward	09/27/2018			
Marc Smith, MD	11/18/2019	**02**	Added Reportable limit	