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

FIBRINOGEN
START 4 METHOD

RC.HM.CG.PR.072.r02

Principle

This is a mechanical clot detection method.

A standard solution of thrombin is added to the patient plasma and fibrinogen concentration is determined from standard clottable protein curve. The thrombin clottable protein method is specific for clottable fibrinogen. Antithrombins and anticoagulants are not likely to interfere with the method because of the dilution of the patient's plasma with buffer.

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:

Diagnostica Stago START 4
Cuvettes
Metal balls with dispenser
100-1000mcL adjustable pipette (or individual pipettes)
Small pipette tips
Large pipette tips
Plastic tubes

REAGENTS:

1. **HemosIL Q.F.A. Thrombin (Bovine)** – Lyophilized bovine thrombin containing buffers and antiheparin agent and a preservative. Store unopened vials at 2-8ºC and use by the expiration date printed on the label. Allow each vial of reagent to equilibrate at 20-25˚C for **at least 15 minutes before reconstitution**. Pipette the exact amount required 2 mL of DI water or equivalent. Replace the stopper and swirl gently make sure of the

complete reconstitution of the product. Let sit for 30 minutes at 20-25˚C and invert to mix before use. Stability after reconstitution is 7 days at 2-8ºC or 15-25°C on board the ACL TOP.

2. **Factor Diluent:** Store unopened vials at 15-25ºC and use by the expiration date printed on the label.

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 DI water or equivalent. Replace the stopper and swirl gently. Ensure the complete reconstitution of the product. 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 Low Fibrinogen Control:** Lyophilized fresh human citrated plasma containing a reduced level of fibrinogen with buffer and stabilizers. 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. Ensure the complete reconstitution of the product. Keep the control at 15-25ºC for 30 minutes and invert to mix before use. Stability after reconstitution is 24 hours at 15-30°C on board the ACL TOP.

STANDARD:

1. **HemosIL calibrated Plasma –** Lyophilized human plasma containing buffer, stabilizers and preservatives. Dissolve the contents of each vial with 1 mL of DI water or equivalent. Replace the stopper and swirl gently. Ensure the complete reconstitution of the product. Keep the control at 15-25ºC for 30 minutes and invert to mix before use. Stability after reconstitution: 24 hours at 2-8ºC in the original vial for fibrinogen, Antithrombin, Plasminogen, Plasmin Inhibitor, Protein C and Protein S.

Maintenance

Refer to Attachment A.

Quality Control

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

HemosIL Normal control 1 and abnormal low fibrinogen control are run once per shift when the instrument is used for this assay.

Procedure

1. Prepare 1:10 dilution of patient plasma and control plasma with factor diluent (900mcL factor diluent + 100mcL of plasma). Bring CA System buffer to room temperature before preparing dilution.
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 *Fib* by pressing the [3] key and then [ENT].
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 all the ID numbers have entered, press [ENT] and a working list will print out and the screen displays the working screen.
5. Pipette 200mcL of the sample or control to each cuvette. All patients and controls must be run in duplicate.
6. Immediately press incubation timer key #1 at bottom of the incubation column. The column timer #1 starts to run.
7. Ten seconds before the 180 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 180 seconds mark, dispense 100mcL of Q.F.A. thrombin reagent into cuvette channel # 1 and press the PIP key; repeat into the successive cuvette channels #2, #3, and #4. Each time Thrombin 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. 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". Enter START 4 as the instrument code.
11. Linearity limits of this procedure are 70 – 500 mg/dL
    a. If 1:10 dilution gives >70 seconds on START 4 printout (< 70 mg/dL), prepare 1:5 dilution of plasma, repeat test and divide result by 2.
    b. If 1:5 dilution gives value of >70 seconds on the START4 printout (< 70 mg/dL), report in LIS as <35mg/dL.
    c. If 1:10 dilution gives a fibrinogen value >500 mg/dL, prepare a 1:20 dilution of patient plasma, repeat test and multiply result by 2. If this 1:20 result reads > 500 mg/dL, report result as >1000 mg/dL.
12. Specimens with low fibrinogen values must be checked for clots before reporting.

PREPARATION OF A STANDARD CURVE:

1. Prepare dilutions of calibrator plasma and factor diluent

Test Tube#1 (1:5): 0.8mL factor diluent + 0.2mL calibrator plasma	reference value x 2
Test Tube#2 (1:10): 0.9mL factor diluent + 0.1mL calibrator plasma	reference value
Test Tube#3 (1:20): 0.5mL factor diluent + 0.5mL of #2	reference value / 2
Test Tube#4 (1:40): 0.5mL factor diluent + 0.5mL of #3	reference value / 4

2. From the Main Menu, select *Test Mode* by pressing the [1] key and confirm with [ENT]. Select *Fib* by pressing the [3] key and then [ENT]. The patient id screen is displayed. Assign an appropriate ID# to each dilution of the fibrinogen calibrator.
3. Pipette 200mcL of 1:5 dilution in each of cuvettes #1 and #2; pipette 200mcL of 1:10 dilution in each of cuvettes #3 and #4.
4. Immediately press the incubation column timer #1 at bottom of incubation column. The timer starts to run.
5. Ten seconds before the 180-second incubation time is reached, the instrument starts to beep. At this sound, quickly transfer the cuvette strip from incubation column to test column.
6. Exactly on the 180-second mark as indicated by the incubation column timer, pipette 100mcL of Q.F.A. thrombin reagent into each cuvette beginning with the top cuvette. Press the PIP key simultaneously with the addition of each Thrombin.
7. Press [ESC] to display the next working screen and repeat the same testing procedure for the two remaining calibration dilutions.
8. After all the calibration dilutions have been tested, the results are printed out. If there is an "*" by the result, repeat the dilution due to a >5% CV.
9. Press [ESC] key to go back to "Main Menu"
10. Select "Calibration" by pressing [2] key and confirm with [ENT] key. Next, press [3] for "Fib" and confirm with [ENT].
11. Enter the lot number for the Standard (SHP) and the Start Reagent (Thrombin).
12. Press any key to enter the calibration data. Enter Fibrinogen value of each dilution of Fibrinogen Standard (see table above) and its corresponding clotting time in duplicate starting with the 1:5 dilution. See **example** below:

STD1:	500.00 mg/dL	=	4.7 s	and	4.8 s
STD2:	250.00 mg/dL	=	9.1 s	and	9.2 s
STD3:	125.00 mg/dL	=	18.6 s	and	19.0 s
STD4:	62.50 mg/dL	=	62.5 s	and	63.0 s

13. After all the calibration data have been entered, the calibration parameters are displayed. Store the curve in the memory by pressing [ENT]. The calibration curve will automatically print out.

Expected Values

NORMAL RANGE:

Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.

REPORTABLE RANGE:

Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.

TAT:

2 hours

CRITICAL VALUE:

<100 mg/dL

References

1. Start 4 Operator's Manual, Diagnostica Stago, France, June 2002.
2. 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:

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”.

FIBRINOGEN – 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\Fibrinogen 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
Marc D. Smith, MD	12/12/2012			
Marc D. Smith, MD	12/22/2014		No change	OK
Marc D. Smith, MD	06/21/2016	01	Pg 3 #11 Instructions on when to make 1:5,1:10 and 1:20 dilutions.	OK
Elizabeth Sykes, MD	02/22/2018			
Marc D. Smith, MD	09/17/2018		Updated logo. Changed reagents and controls used. Changed critical value to <100.	
Peter Millward, MD	09/27/2018			