Document Title: Factor Assays, Extrinsic on the ACL TOP 500
SH.CP.AU.coa.0043.0003

Author	Effective Date:	Supersedes Procedure #
Robert Miller	3/15/2017	SH.CP.AU.coa.0043.0002

Revised by:	Date Revised:	Effective Date(NEW)
Mary Johnson	10/15/2017	

Version #	Approval Signature	Approval Date
.0003	Majed Refaai, MD, Medical Director	

Distributed to	# of Copies	Distributed to	# of Copies
Lab Bench	1		
QC Office	1		
Sharepoint	1		

REVISION HISTORY

Procedure/Policy SH.CP.AU	Revision Date	Reason for Revision
Coa.0043.0001	12/5/2012	NEW
Coa.0043.0002	3/15/2017	Update to calculations and result reporting (RJM)
Coa.0043.0003	10/15/2017	Change all references to PT reagent Recombiplastin to Readiplastin due to reagent change; clarify Result Reporting section

TITLE: Factor Assays, Extrinsic on the ACL TOP 500

I. PURPOSE

This procedure provides instructions for the quantitative determination of Factor II, V, VII or X in citrated plasma based on the Prothrombin Time (PT) assay using HemosIL reagents on the ACL TOP® Family.[1,6,7,8,9,10]

II. PRINCIPLE

Factor II, V, VII or X activity in a patient's plasma is determined by performing a modified PT. Patient plasma is diluted and added to plasma deficient in the appropriate factor. Correction of the clotting time of the deficient plasma is proportional to the concentration (% activity) of that factor in the patient plasma interpreted from a calibration curve.[6,7,8,9]

IV. SCOPE

This procedure will be used by the UR Medicine Labs, Strong Memorial Hospital, Hematology and Chemistry Laboratory staff.

IV. RESPONSIBILITIES

TITLE	RESPONSIBILITY
Laboratory Director	Approval of Procedure
Supervisory Staff	Implementation of procedure
Staff	Adherence to procedure

V. SPECIMENS

A. Citrated blood 9:1 (blood to anticoagulant) 3.2 % sodium citrate. Follow NCCLS guideline H21-A2 and H3-A3. No other anticoagulant is acceptable.
B. Centrifugation: 12 minutes at 4,000 rpm (RCF = 3756g) or 2 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge).
C. Plasma Storage: 4 hours at 20 ºC, 4 months at - 20ºC and at -70 ºC. Frozen plasma should be thawed only once at 37 ºC for 5 minutes.[C]
D. Unacceptable Specimens: Samples that are short draws, clotted or hemolyzed may yield incorrect results.

E. Sample volume is CRITICAL to obtain accurate coagulation results. A 90% draw is the minimum volume acceptable for accurate testing (refer to the minimum volume indicator on the tube or see the BD Tube draw volume guide). Do not run or report these samples. Place ".ND" in the result field with the canned text comment "Sample volume inadequate, unable to perform required testing" In Order Entry add the SPROB test by following procedure Documentation of Patient/Specimen (SH.CP.SM.loe.0180), enter reason in the comment field and call for redraw.

F. Elevated hematocrit specimens (specimens determined to have a hematocrit >55%) may require the preparation of a special collection tube: Refer to General Operating Procedure for the ACL TOP 500, Section IV (SH.CP.AU.coa.0036 or see SH.CP.AU.jad.0136).

G. Clotted samples: Each specimen is checked visually for the presence of clots prior to analysis. If a clot is suspected, the tube is uncapped, and checked with a pair of applicator sticks. *ANY* clot present in the specimen makes it inadequate for ALL coag testing. Clotted specimens are rejected and a new specimen should be requested.

H. Hemolyzed samples: Grossly hemolyzed samples should be rejected. If sample has been centrifuged and hemolysis is present, check for a clot. If a clot is present, follow procedure for reporting clotted samples.

VI. QUALITY CONTROL

Normal and abnormal controls are recommended for a complete quality control program. HemosIL Controls for Prothrombin Time are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift and with reagent change in accordance with good laboratory practice. Refer to Westgard *et al* for identification and resolution of out-of-control situations.

QC data is transmitted to the LIS. Remedial action for out-of range values is documented in the LIS and on the QC log for that instrument. When a value is out of range, the operator should repeat the control using the same control material – if acceptable document in LIS.

A. If still not acceptable reconstitute a new control and rerun – if acceptable document in LIS.
B. If still not acceptable, run the Enhanced Probe Clean procedure and rerun the same well mixed control and well mixed reagent. If acceptable, document and continue processing samples.
C. If still not acceptable, reconstitute/use new reagent and rerun. If acceptable, a lookback of the last five samples analyzed (or an appropriate number of samples) for that assay on that analyzer should be done. These samples should be from the time when the last reagent vial was in use and the last QC point was in range, in accordance with the Quality Control Lookback Policy SH.CP.AU.gen.0002. If the samples do not agree, continue to look back at samples analyzed on that analyzer since the last successful QC run. Notify supervisor or technical specialist.

D. In the case of any if >4.0 SD QC failure (R1:XS in Soft QC), rerun the QC and evaluate according to Quality Control Policy SH.CP.AU.gen.0001. Perform a lookback as necessary according to the aforementioned policy if any patient samples were analyzed.
E. If still not acceptable, document and notify Hematology Supervisor or a Tech Specialist. Use backup analyzer for STAT/routine testing. Call service for further guidance and troubleshooting help.

VII. SPECIAL SAFETY PRECAUTIONS

All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC (Center for Disease Control) recommendations and in compliance with the Federal OSHA (Occupational Safety and Health Administration) Blood-borne Pathogen Standard, 29 CFR (Code of Federal Regulations) part 1910.1030. Follow specimen handling as outlined by Laboratory Safety Policy, (SH.CP.AU.gen.0005.0001).

VIII. MATERIALS

A. Equipment

1. ACL TOP 500 analyzer
2. centrifuge

B. Supplies

1. Pipettes
2. Pipette tips
3. Plastic test tubes
4. TYPE –I Reagent Grade Water

C. Reagents

1. **HemosIL ReadiPlasTin kit** (PN 0020301300/10mL or PN 0020301400/20mL) :Contains 5 vials of a **Reagent** solution of recombinant human tissue factor, synthetic phospholipids with stabilizers and 5 vials of an aqueous solution of calcium chloride, polybrene and a preservative (**Diluent**). Refer to reagent package insert sheet for more details.

2. **HemosIL Factor II Deficient Plasma** (PN 0020012200): 10 x 1mL of lyophilized human plasma that has been artificially depleted of FII, containing buffer and stabilizers. The residual FII activity < 1%, whereas all other factors have normal levels.6

3. **HemosIL Factor V Deficient Plasma** (PN 0020011500): 10 x 1mL of lyophilized human plasma that has been artificially depleted of FV, containing buffer and stabilizers. The residual FV activity < 1%, whereas all other factors have normal levels.7

4. **HemosIL Factor VII Deficient Plasma (**PN 0020011700): 10 x 1mL of lyophilized human plasma that has been artificially depleted of FVII, containing buffer and stabilizers. The residual FVII activity < 1%, whereas all other factors have normal levels.8

5. **HemosIL Factor X Deficient Plasma** (PN 0020010000): 10 x 1mL of lyophilized human plasma that has been artificially depleted of FX, containing buffer and stabilizers. The residual FX activity < 1%, whereas all other factors have normal levels.9

Please refer to the package inserts for further detail.[1,6,7,8,9,10,11]

The following are not supplied with the kits and those required may be purchased separately.[1,6,7,8,9]

HemosIL Calibration Plasma	00020003700
HemosIL Normal Control ASSAYED	00020003110
HemosIL Special Test Control Level 2	00020012000
HemosIL Factor Diluent	00009757600
HemosIL Cleaning Agent	PN 9832700
HemosIL Cleaning Solution	PN 9831704
HemosIL ACL TOP Rinse Solution	PN 20302400

IX. PROCEDURE – (STEP/ACTION)

A. Reagent Preparation

1. **ReadiPlasTin :** Pour the entire contents of the HemosIL ReadiPlasTin Diluent vial into the HemosIL ReadiPlasTin Reagent vial. Invert to mix before use. Can be used immediately

2. **Factor Deficient plasmas:** Dissolve the contents of each required vial with 1 mL of CLSI CLR water or equivalent.[11] Replace the stopper and swirl gently. Ensure complete reconstitution of the product. Keep at 15-25˚C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.[6,7,8,9]

3. **Cleaning Agent** (Clean B Diluted): PN 0009832700 - Dilute Clean B solution 1:8 with CLSI CLR (or equivalent) water (1 +7)

B. Reagent Storage and Stability

1. Unopened **ReadiPlasTin** reagent and diluent are stable until the expiration date shown on the vial, when stored at 2-8°C. Once prepared for use, the reagent is stable for 10 days at 2-8°C in closed original vial, or for 10 days at 15°C on the ACL TOP Family in the original vial with no stirring. For optimal stability remove the reagent from the system and store it closed at 2-8°C in the original vial. Do not freeze.

2. **Factor Deficient plasmas:** Stability after reconstitution: 24 hours at 2-8˚C in the original vial or 24 hours at 15˚C on the ACL TOP® Family.[6,7,8,9] For optimal

stability remove reagents from the system and store them at 2-8°C in the original vial.[1,6,7,8,9,10,11]

C. Calibration

Calibration and storage of a valid specific Factor Assay calibration are required to obtain Factor results. Calibration is performed:

- With a change of reagent lot numbers
- With a change of major instrument components
- To satisfy local regulatory requirements
- At laboratory discretion

Method for Calibration (if necessary):

1. Add appropriate PT Reagents for specific factor Test (II, V, VII or X), appropriate Factor Deficient Plasma(s), Calibration Plasma and Diluted Clean B to the Materials Programming Window if necessary (**Setup, Display, Materials Programming Window**).
2. Define Materials if necessary (**Setup, Material List**). Select the appropriate materials from the Material List. Add appropriate Factor assay reagents (), Factor Deficient Plasma(s), Calibration Plasma and Factor Diluent to the Materials Programming Window if necessary (**Setup, Display, Materials Programming Window**).
3. Define Results Units and Rerun Rules in the Specific Factor Test Definition if necessary (**Setup, Test List, Test Code, Result Units** and **Rerun Rules**).
4. Choose **Setup, Materials List.**
5. Double-click on the appropriate calibrator to open the **Materials Definition** screen.
6. Choose the **Lot Specific Information** tab and enter the Calibrator lot number and Expiration Date.
7. Enable **Lot Management** from the Lot Specific Information tab.
8. Select the **Save** icon to store the lot number. Once the lot number is saved, the **Assign Values** icon becomes available.
9. Select the **Assign Values** icon.
10. Enter the calibration value from the package insert. Press **OK**.
11. Choose the **Previous Screen** icon to exit.
12. Load the appropriate Factor assay reagents (PT Reagent, Factor Deficient Plasma(s)), Calibration Plasma and Factor Diluent onto the ACL TOP® Family instrument.
13. Select **Calibration, Status List**.

14. Double-click on the appropriate Factor Assay test code to open the **Calibration Details** screen.
15. Choose the **Run** icon.
16. Select **OK** at the "Do you confirm the operation?" prompt.
17. Choose the **Previous Screen** icon to exit.
18. Verify the Job Status for the Factor Assay test code says **Active.**
19. Once the calibration is complete, review calibration results. The instrument will fail the calibration if the r^2 value is less than the value in the chart below:

Extrinsic Factors Assay	**Math Model High (r^2 ≥)**	**Math Model Low (r^2 ≥)**
Factor II RDP	0.985	0.980
Factor V RDP	0.985	0.985
Factor VII RDP	0.985	0.950
Factor X RDP	0.985	0.950

20. If there are no errors/failures and the calibration is acceptable, the curve will automatically validate. If review is necessary and the calibration is acceptable, make the appropriate changes and choose the **Validate** icon to validate the calibration curve.[2]

D. Test Procedure

1. Create/Edit QC files, if necessary (**Setup, QC List**, **Test Code** to access the **QC Definition Screen**). Add FII, FV, FVII and/or FX test code(s) to the Test/Profiles Programming Window, if necessary **(Setup, Display, Test Programming Window**).
2. Add appropriate Factor assay reagents (PT Reagent, Factor Deficient Plasma(s)), and Factor Diluent to the Materials Programming Window if necessary (**Setup, Display, Materials Programming Window**).
3. Define Parallelism parameters, if necessary (**Setup, Test List, Test Code, Parallelism**).
4. Load the appropriate Factor assay reagents (PT Reagent), Factor Deficient Plasma(s), and Factor Diluent on to the ACL TOP® Family instrument.

5. Place QC materials with the barcodes facing out in a Diluent Rack and load onto an ACL TOP® Family instrument in a Diluent track. (If running the QC from the sample rack, refer to **Quality Control, Performing a QC Test** in the ACL TOP® On-Line Help Manual).
6. Choose **QC** from the Main Menu and select **Test Status List**.
7. Double-click on a test code show Test Materials Definition tree.
8. Select the appropriate Factor Assay QC Control and choose **Program QC** icon. This will run all QC levels for that test.
9. Verify that all factor QC has passed before proceeding with testing.
10. Place sample tubes in a sample rack with barcodes facing outwards.
11. Select an available sample track and load the sample rack when the barcode reader is in position.
12. Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually from the test and programming window.
13. Choose the **Run** icon if the ACL TOP® Family instrument is not currently running.[2]

For instructions on loading samples without barcodes or LIS, please refer to **Samples Analysis, Managing Patient Samples, Programming Bar Coded Samples** and **Programming Non-Bar Coded Samples** in the ACL TOP® Family On-Line Help Manual[2] or SH.CP.AU.coa.0036, TOP 500 General Operating Procedure.

X. LIMITATIONS

A. PT results may be affected by many commonly administered drugs and further studies should be made to determine the source of unexpected abnormal results.

B. The assay shows no interference on the ACL TOP Family up to:

Assay	UFH	LMWH	HGB	TRIG	Bilirubin
PT	1.0 IU/mL	1.4 IU/mL	500 mg/dL	1000 mg/dL	50 mg/dL

C. Samples with excessive hemolysis, icterus, or lipemia, should not be used. Refer to the ReadiPlasTin package insert for relative interference claims.[1,6,7,8,9,11]

XI. CALCULATIONS

Patient samples are run at multiple dilutions. The analyzer calculates the % activity for each dilution from a calibration curve. Additional checks are performed including the variance between dilutions and comparison between the slope of the calibration curve and the patient sample dilution curve (parallelism). If the checks are outside prescribed limits, analyzer flags

are generated and sent to the LIS (see Result Reporting section). In the absence of flags, if the result is ≤ 20%, the 100% sample dilution result (Mean of 100%) is reported. This correlates with the "FT_10" on the LIS interface. If the result is >20%, the average of the 50% and 25% sample dilution corrected results (Mean CR) is reported. This correlates with the "FT_20" on the LIS interface.

XII. INTERPRETATION

Decreased levels of Factor II, V, VII or X may be found in congenital deficiency of each Factor or may be acquired secondary to other diseases such as liver disease, hyperfibrinolysis or Disseminated Intravascular Coagulation (DIC) or due to a specific Factor Inhibitor.[6,7,8,9]

FII: Congenital deficiency of FII is a very rare inherited disorder that causes in general a mild to moderate bleeding tendency. Patients receiving oral anticoagulant therapy or with a Vitamin K deficiency due to intake or absorption abnormalities will have reduced plasma levels of FII, a Vitamin K dependent clotting factor. Please refer to FII Deficient Plasma (0020012200) insert sheet for further details.[6]

FV: Congenital deficiency of FV leads to Owren's disease (or parahemophilia), which is a rare inherited disorder that causes from mild to severe bleeding. Please refer to FV Deficient Plasma (0020011500) insert sheet for further details.[7]

FVII: Congenital deficiency of FVII is a rare inherited disorder that causes in general mild bleeding. Patients receiving oral anticoagulant therapy or with a Vitamin K deficiency due to intake or absorption abnormalities will have reduced plasma levels of FVII, a Vitamin K depending clotting factor. Please refer to FVII Deficient Plasma (0020011700) insert sheet for further details.[8]

FX: Congenital deficiency of FX is a rate inherited disorder that may cause bleeding after dental extractions and other surgery. FX deficiencies may also be acquired secondarily due to systemic amyloidosis, liver diseases, hyperfibrinolysis and Disseminated Intravascular Coagulation (DIC). Patients receiving oral anticoagulant therapy or with a Vitamin K deficiency due to intake or absorption abnormalities will have reduced plasma levels of FX, a Vitamin K depending clotting factor. Please refer to FX Deficient Plasma (0020010000) insert sheet for further details.[9]

Current laboratory reference range for ACL TOP® Family:

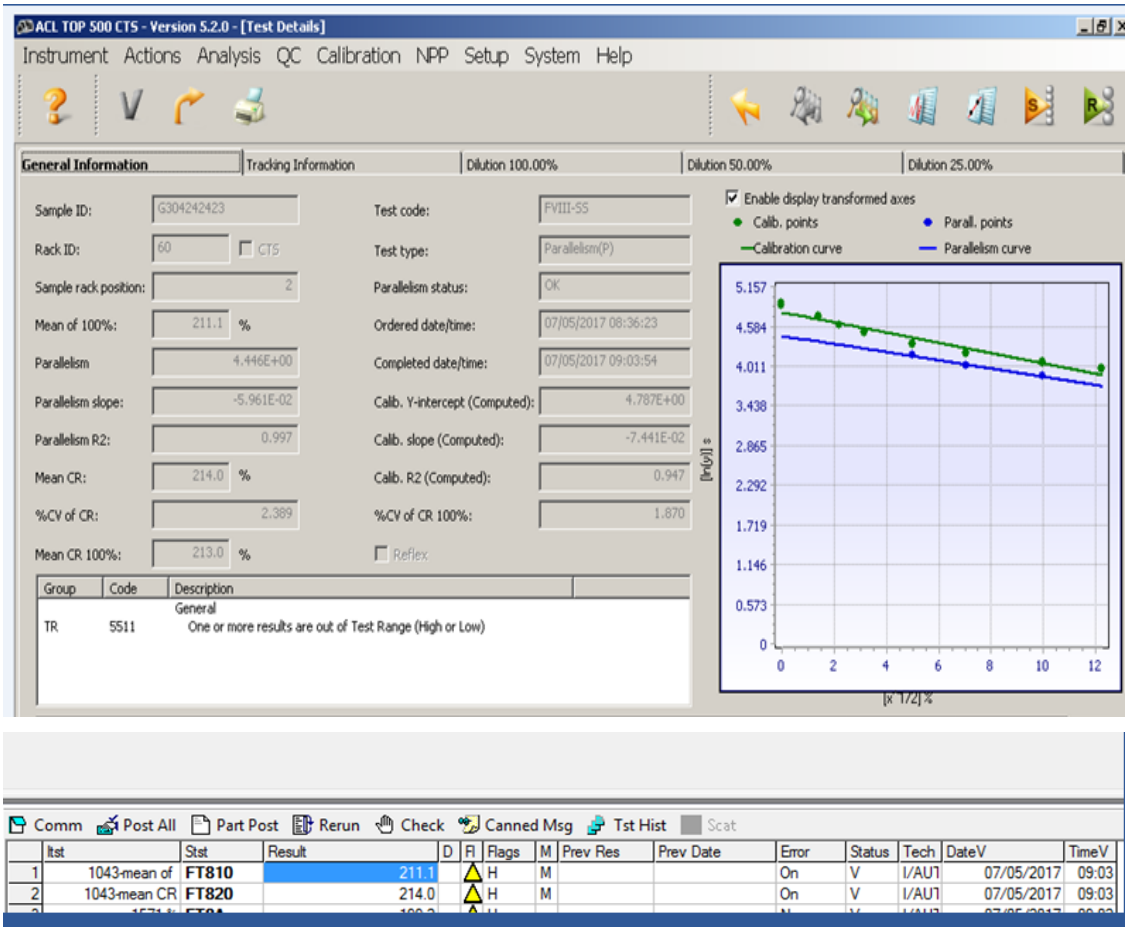
HemosIL Factor Deficient Plasma	Normal Range (% Activity)
FII	89 -136
FV	67 – 139
FVII	66 -159
FX	78 - 147

XIII. RESULT REPORTING

A. Analyzer results with no instrument or parallelism errors are auto-verified and released through the LIS (see Calculations, Section XI).

B. Results with instrument flags will hold on the LIS interface:

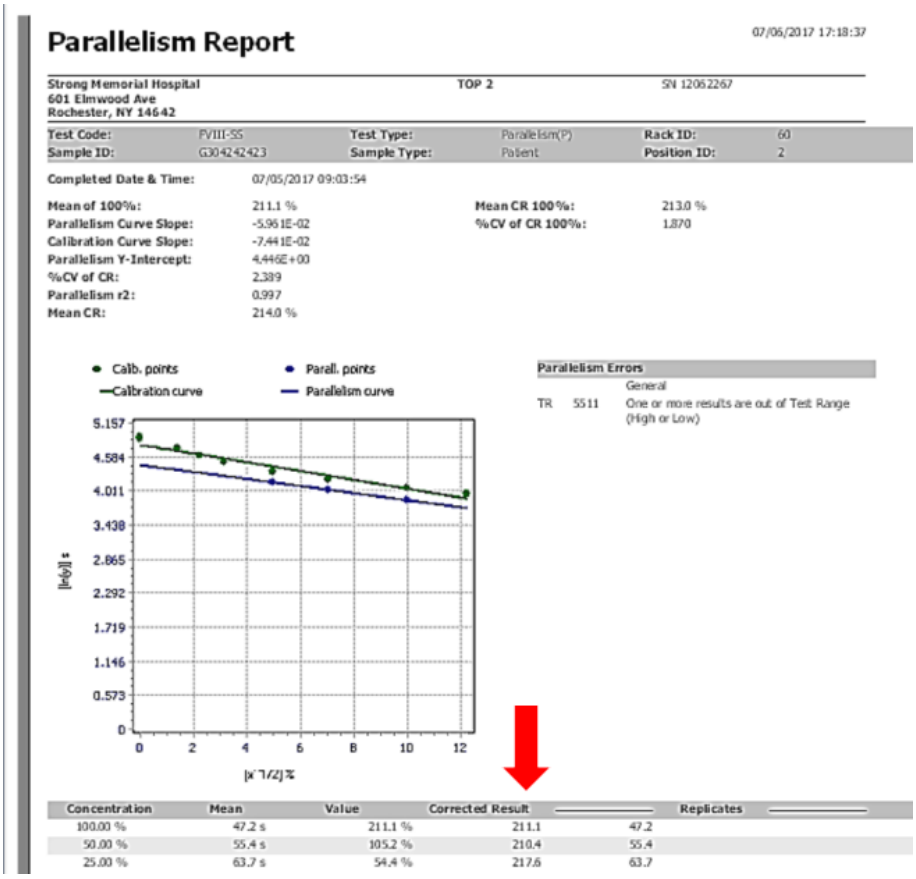
1. "One or more results are out of Test Range (High or Low)"



a. If result is High (above linearity for assay): Print and review parallelism report. If dilution results match (no max variance flag), report "Mean CR", average of the 50% and 25% sample dilution corrected results, represented by "FT**_20**" result on LIS interface.

1) To print a parallelism report: Select the **Sample List** icon on the top bar and select/place a check next to the patient that needs the parallelism report printed.
2) Select **Actions→Print→Print Parallelism Report→OK**

b. If the dilution results do not match, rerun the sample.



Parallelism Report

07/06/2017 17:18:37

Strong Memorial Hospital
601 Elmwood Ave
Rochester, NY 14642

TOP 2 SN 12062267

Test Code:	FVIII-SS	Test Type:	Parallelism(P)	Rack ID:	60
Sample ID:	G3042423	Sample Type:	Patient	Position ID:	2

Completed Date & Time: 07/05/2017 09:03:54

Mean of 100%:	211.1 %	Mean CR 100%:	213.0 %
Parallelism Curve Slope:	-5.961E-02	%CV of CR 100%:	1.870
Calibration Curve Slope:	-7.441E-02		
Parallelism Y-Intercept:	4.446E+00		
%CV of CR:	2.389		
Parallelism r2:	0.997		
Mean CR:	214.0 %		

Calib. points
Parall. points
Calibration curve
Parallelism curve

Parallelism Errors

		General
TR	5511	One or more results are out of Test Range (High or Low)

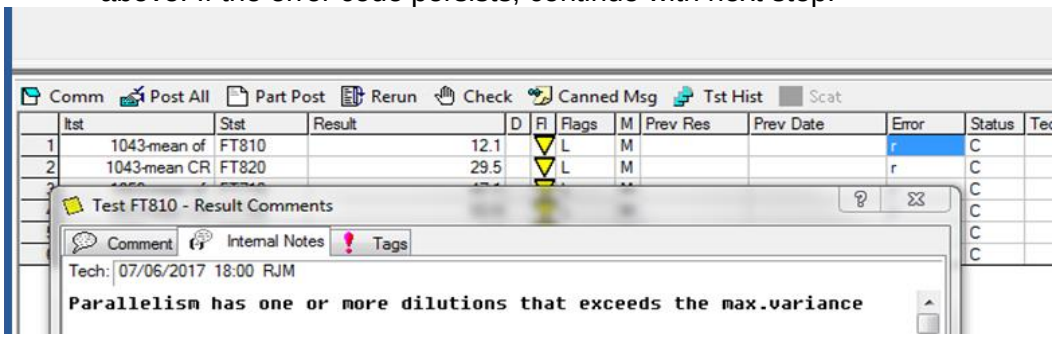
Concentration	Mean	Value	Corrected Result	Replicates
100.00 %	47.2 s	211.1 %	211.1	47.2
50.00 %	55.4 s	105.2 %	210.4	55.4
25.00 %	63.7 s	54.4 %	217.6	63.7

c. If result is Low (below linearity for assay): Print and review parallelism report. If dilution results match (no max variance flag), report “Mean of 100%”, which is 100% sample dilution result, represented by “FT_**10**” on LIS interface.

d. If the dilution results do not match, rerun the sample.

2. “Parallelism has one or more dilutions that exceeds max variance”:

a. This error message means the corrected results are significantly different. The first step should be to rerun sample. If error code disappears, report as above. If the error code persists, continue with next step.



Comm | Post All | Part Post | Rerun | Check | Canned Msg | Tst Hist | Scat

	Itst	Stst	Result	D	Fl	Flags	M	Prev Res	Prev Date	Error	Status
1	1043-mean of	FT810	12.1			L	M			r	C
2	1043-mean CR	FT820	29.5			L	M			r	C

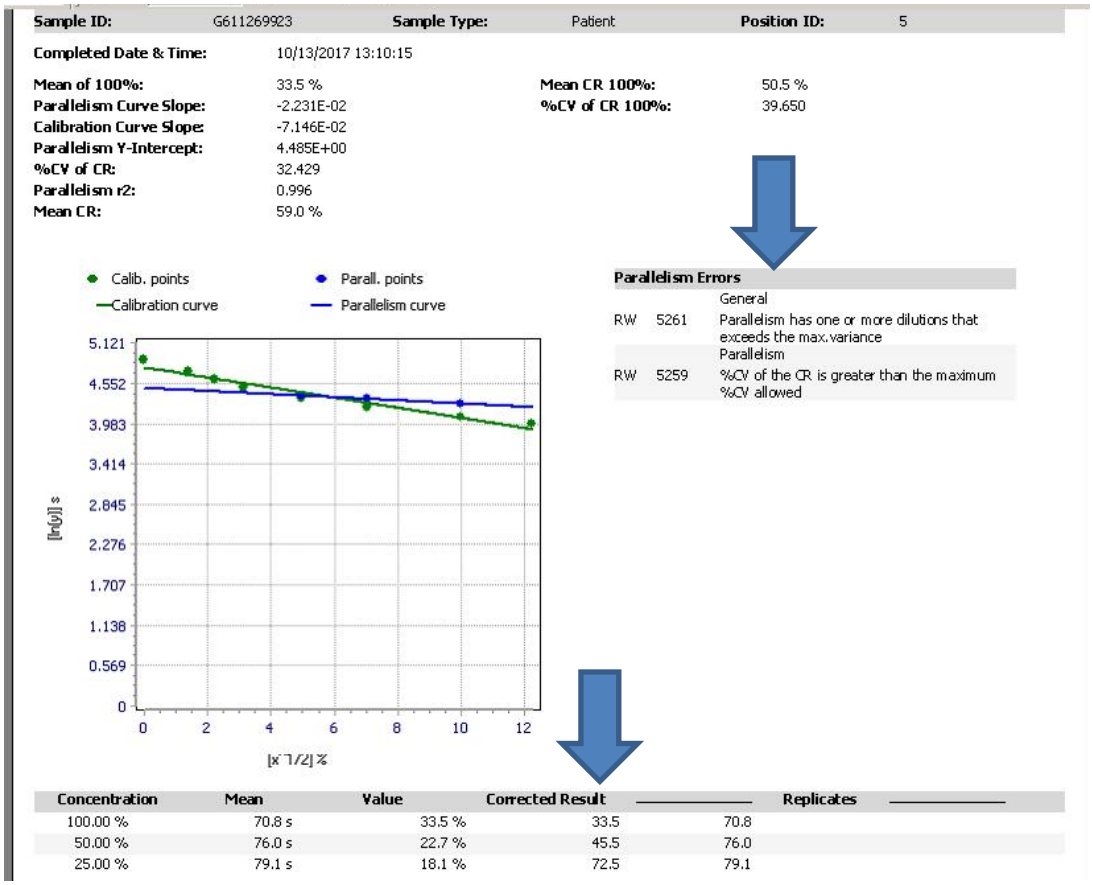
Test FT810 - Result Comments

Comment | Internal Notes | Tags

Tech: 07/06/2017 18:00 RJM

Parallelism has one or more dilutions that exceeds the max.variance

b. A parallelism report should be printed and reviewed for indications of interference from an inhibitor. If a factor inhibitor is present, the factor activity increases as the dilution increases.



c. Interpretation of the parallelism reports should be performed by coagulation faculty, resident, supervisor or technical specialist. **DO NOT** report the presence of an inhibitor without approval of one of the aforementioned personnel.

d. If it is determined that an inhibitor is present, the results from the highest dilution (25% or higher) should be reported with the canned comment, “Factor dilutional analysis indicates possible inhibitor”.

e. If it is determined that there is not an inhibitor present, follow reporting procedure in Section XI – CALCULATIONS.

NOTE: For more information, please refer to SH.CP.AU.coa.0054 Factor Inhibitor Assay on the ACL TOP 500.

XIV. TRAINING

Staff are trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

XV. REFERENCES

A. HemosIL ReadiPlasTin kit (PN 0020301300/10mL or PN 0020301400/20mL): PN 20301400 package insert

B. ACL TOP® Family On-Line Help Manual

C. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition, CLSI Document H21-A5; Vol. 28 No. 5

D. Westgard JO, and Barry PL. Cost-Effective Quality Control; Managing the Quality and Productivity of Analytical Process, AACC Press, 1986

E. Clinical and laboratory Standards Institute. One Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline – Second Edition, CLSI Document H47-A2; Vol. 28 No. 20

F. HemosIL Factor II Deficient plasma (PN 0020012200) package insert

G. HemosIL Factor V Deficient plasma (PN 0020011500) package insert

H. HemosIL Factor VII Deficient plasma (PN 0020011700) package insert

I. HemosIL Factor X Deficient plasma (PN 0020010000) package insert

J. HemosIL Calibration Plasma (PN 0020003700) package insert

K. Reference Clinical and Laboratory Standards Institute.Preparation and Testing of Reagent Water in the Clinical Laboratory;Approved Guideline. Fourth,Edition, CLSI Document C3-A4;Vol.26 No.22

Factor Assays, Extrinsic on the ACL TOP 500
Knowledge Check

In the event of a question answered incorrectly: Single-line through the incorrect answer, initial & date, then select the correct answer.

ALWAYS HAVE CHANGES INITIALED BY YOUR TRAINER.

Circle True or False for each of the following statements.

1. True or False — Samples with extrinsic factors ordered may be processed by centrifuging specimens for 7 minutes at 4000 r.p.m..
2. True or False — Interpretation of the parallelism reports should be performed by coagulation faculty, resident, supervisor or technical specialist. Report the presence of an inhibitor without approval of one of the aforementioned personnel.
3. True or False — If the result is ≤ 20%, the 100% sample dilution result (Mean of 100%) is reported.
4. True or False — If there are no errors/failures and the calibration is acceptable, the curve will automatically validate. If review is necessary and the calibration is acceptable, make the appropriate changes and choose the **Validate** icon to validate the calibration curve.
5. True or False — In most cases, the first step an operator should take when QC fails is to repeat the control using the same control material.

Any incorrect answers I may have initially written have been discussed and corrected. I now understand the answers I may have gotten wrong.

PASSING GRADE IS 75% OR GREATER

_____
Employee name (print)

_____ _____
Employee signature **(Date)**

_____
Supervisor/Manager name (print)

_____ _____
Supervisor/Manager signature **(Date)**