TITLE: Factor X Activity, Chromogenic assay on the ACL TOP 500

I. PURPOSE

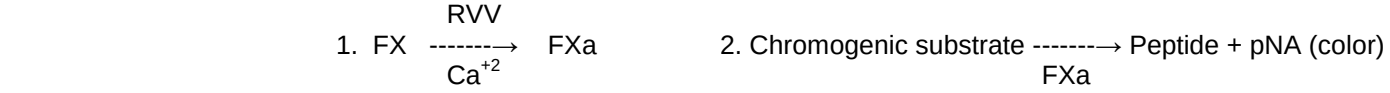
The Diapharma Factor X Chromogenic Assay Kit is an in vitro diagnostic test kit for the quantitative determination of Factor X activity in human citrated plasma. Factor X activity is useful for monitoring patients on oral anticoagulant (OAC) therapy (e.g. warfarin).[1] The Factor X Chromogenic assay kit allows the accurate prediction of therapeutic warfarin monitoring when INR values are unreliable, such as in the presence of lupus anticoagulants (LAC) or when direct thrombin inhibitors (DTI) (e.g. Argatroban) are discontinued (bridging from DTI to warfarin), due to the issues of varying sensitivities of different thromboplastins. This assay is unaffected by LAC because the assay end point is not a phospholipid-dependent clotting time.[2] The chromogenic method directly measures Factor X activity via the reaction explained below, thereby is unaffected by the inhibition or low activity level of other clotting factors or the presence of LAC.[8] The DiaPharma Factor X Chromogenic Assay Kit is also useful for screening for Factor X deficiencies.[1]

II. PRINCIPLE

Coagulation Factor X (Stuart Prower Factor, FX) is a vitamin K dependent protein produced by the liver. It has a central position in the coagulation cascade. Factor X is activated both by the extrinsic and intrinsic systems before exerting its effect on the conversion of prothrombin to thrombin.

Patients who need anticoagulation therapy can be treated with warfarin, which is a vitamin K antagonist. This treatment leads to a decrease in all vitamin K dependent clotting factors (X, IX, VII & II) and thus a prolonged clotting time. The anticoagulation therapy requires careful monitoring since it is essential to find an optimal balance between risk of thrombosis and risk of bleeding for each patient.

The method is based on a two-stage principle. In stage one, Factor X is activated in the presence of calcium to Factor Xa (FXa) by the activator Russell's Viper Venom (RVV). In stage two, the generated FXa hydrolyses the chromogenic substrate, thus liberating the chromophoric group, pNA. The color is then measured with a spectrophotometer at 405nm. The intensity of color is proportional to the FX activity in the sample.[1]



III. SCOPE

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

IV. RESPONSIBILITIES

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

V. SPECIMENS

A. Nine parts of freshly drawn venous blood collected into one part 3.2% trisodium citrate (Na Cit.). Refer to the most recent Clinical and Laboratory Standards Institute (CLSI) Document H21-A5, and GP for further instructions on specimen collection, handling, and storage.[3] No other anticoagulant is acceptable.

B. Centrifugation: 12 minutes at 4,000 rpm (Hettich) (RCF=3756g) **or 2 minutes at 16,000 RPM (Eppendorf High Speed Centrifuge).** Refer to NCCLS document H21-A5 for further instructions on specimen collection, handling, and storage.[3]

C. Plasma Storage: Four hours at 20°C, one month at -20ºC and 6 months at -70°C. Frozen plasma should be placed in 37ºC water bath just until completely thawed and mixed well.

D. Unacceptable Specimens: Samples that are short draws, clotted, grossly lipemic or hemolyzed may yield incorrect results.

VI. QUALITY CONTROL

A. Normal and abnormal controls are recommended for a complete quality control program. The HemosIL Normal Assayed and Special Test Controls are designed for this program. Quality control is used to monitor the accuracy and precision of the IL ACL TOP 500 by processing the control fluids for each analyte at least once every 8 hours in accordance with good laboratory practice. Any controls that are outside of the Laboratory established range should be rerun with proper troubleshooting and documentation as stated below.

B. QC data is transmitted to the LIS. Remedial actions for out-of-range values (>2.5 SD) are documented in the LIS and in the QC Exception log for that instrument.[6] When a value is out of range, the operator should:

1. **Repeat** the well mixed control using the same control material – if acceptable, document and continue processing samples.

2. If not acceptable**, reconstitute a new control** with new distilled/deionized water (dH_2O) and rerun. If acceptable, document and continue processing samples.
3. If 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.
4. If 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.
5. If QC is still not acceptable, **recalibrate** the assay. See the **IX. B. Calibration Procedure** portion of this document.
6. **In the case of any >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.
7. 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 the Laboratory Safety Policy, SH.CP.AU.gen.0005.

VIII. MATERIALS

A. Equipment

1. IL ACL TOP 500 analyzer
2. Centrifuge

B. Supplies

1. Pipettes
2. Pipette tips
3. Plastic test tubes
4. TYPE – I Reagent Grade Distilled/Deionized Water
5. IL bottles, 4 mL and 10 mL sizes
6. Reagent labels

C. Reagents

1. The **DiaPharma Factor X** Kit contains[1]:

    a. **Chromogenic Substrate:** 25 mg lyophilized FXa chromogenic substrate with mannitol added as a bulking agent. Reconstitute substrate with 20 mL sterile Type I reagent grade water. Reconstituted substrate is stable for 6 months at 2 – 8°C.

    b. **Russell's Viper Venom (RVV):** FX activating protein from Russell's Viper Venom. Reconstitute the RVV with 15 mL sterile Type I reagent grade water. Reconstituted activator is stable for 1 month at 2 – 8°C.

    c. **$CaCl_2$:** 20 mL of 0.1 mol/L calcium chloride solution. Before use, mix 1 volume of RVV with 1 volume of $CaCl_2$. Mixture is stable for 48 hours at 2 – 8°C. The $CaCl_2$ solution is stable at 2 – 8°C until the expiry date printed on label.

    d. **Buffer:** 100 mL buffer solution containing 0.05 mol/L Tris, pH 7.8 and 20 mg/L Polybrene® (hexadimethrine bromide). Ready for use. The buffer is stable at 2 – 8°C until the expiry date printed on label.

    e. The sealed reagents are stable at 2 – 8°C until the expiry date printed on label. Contamination by organisms should be avoided once vials are opened.

    f. The following reagents are not supplied with the kits and may be purchased separately[1]:

        1) HemosIL Calibration Plasma 0020003700
        2) HemosIL Normal Control, ASSAYED 0020003110
        3) HemosIL Special Test Control Level 2 0020012000

IX. PROCEDURE – (STEP/ACTION)

A. Reagent Preparation[1,5]

1. **Chromogenic substrate:** Reconstitute substrate with 20 mL CLSI CLRW Type I reagent grade sterile water. Transfer 2 mL to a 4 mL bottle and label accordingly. Aliquot remainder of substrate into 2 mL aliquots, label and place in refrigerator. Reconstituted substrate is stable for 6 months at 2 – 8°C.

2. **Russell's Viper Venom (RVV):** Reconstitute with 15 mL CLSI CLRW Type I reagent grade sterile water. Remove 1 mL for current day's testing and label accordingly. Aliquot remainder of RVV into 1 mL aliquots, label and place in - 70°C freezer for later use. When using frozen aliquots for testing, freeze rapidly in 37° water bath. Reconstituted activator is stable for 1 month at 2 – 8°C.

3. **$CaCl_2$:** Solution is liquid, invert to mix before use. Mix 1:1 RVV:$CaCl_2$ i.e. 1 mL of RVV and 1 mL of $CaCl_2$ in a 4 mL bottle, mix and label accordingly.

4. **Buffer:** Solution is liquid, invert to mix before use. Transfer 5-10 mL into a 10mL bottle and label accordingly.

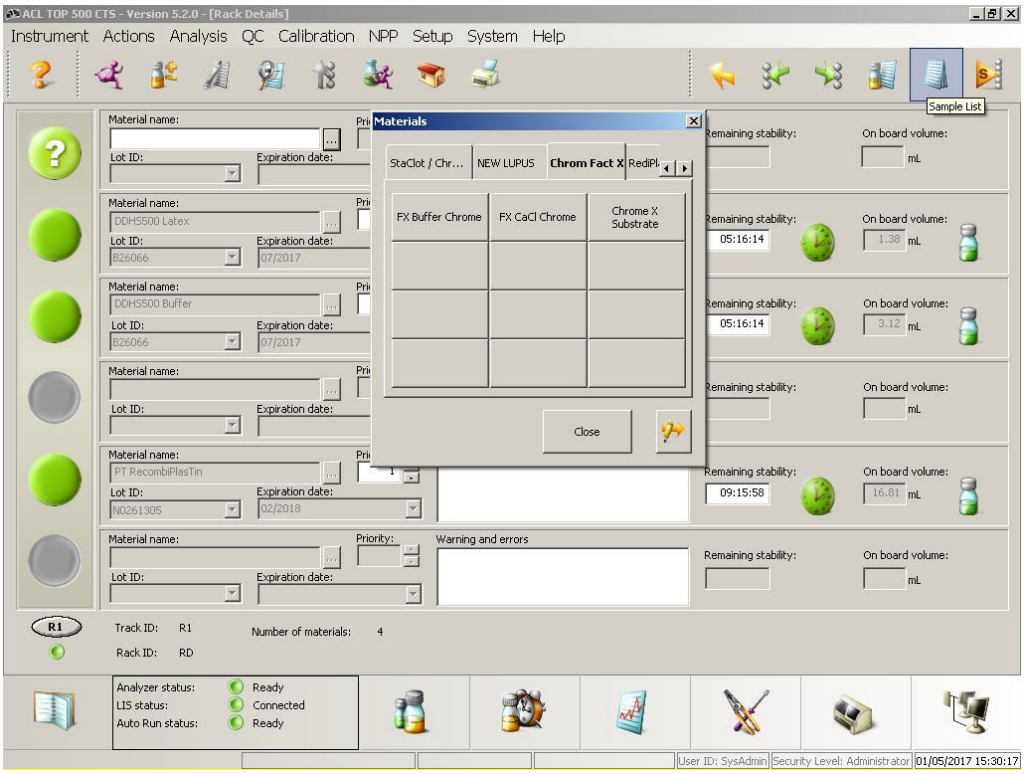
B. Manually Loading of Reagents

1. An off line or virtual rack is available that allows you to program the rack and maintain that information while the rack is not placed, providing you do not exit the application.

2. Once the rack is inserted, the information moves with the inserted rack and is removed from the off line rack, providing the information read by the bar code reader matches what you have manually identified on the rack or if the bar code reader has been disabled or there is no barcode.
   Note: If the bar code reader is enabled but the scanned information does not match your off line rack information, the off line rack information is not used.

3. Program the Off line rack:

   a. Select the menu options **Analysis, Material Area** (Diluent or Reagent)
   b. Double-click a position on the off line rack (located on the left hand side of the screen) to obtain the **Rack Details** screen.
   c. Select the position where the material is located.
   d. Double-click the ellipsis next to the Material Name field and select the material from the Materials window under the **Chrom Fact X** tab.



e. Select the **Insert Rack** icon  on the toolbar and insert the rack.

Note: Once the material is in use, manual identification is disabled until the rack is removed.

C. Calibration Procedure (if necessary)[7]:

NOTE: See SH.CP.AU.jad.0143 for details on Calibration Verification and AMR Verification for this analyte.

1. Define Materials if necessary (**Setup, Material List)**. Select the appropriate materials from the Material List.
2. Add Factor X Chromogenic reagents and HemoSil Calibrator to the Materials Programming Window if necessary **(Setup, Display, Materials Programming Window**).
3. Define Results Units and Rerun Rules in the Factor X Chromogenic Definition if necessary (**Setup, Test List, Factor X Chromogenic 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 Calibration Plasma 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.[4] Press **OK**.
11. Choose the **Previous Screen** icon to exit.
12. Load the FX Chromogenic substrate, 1:1 $CaCl_2$:RVV solution, buffer, and Calibration Plasma onto the IL ACL TOP® 500.
13. Select **Calibration, Status List**.
14. Double-click on the Factor X Chromogenic 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. Once the calibration is complete, review calibration results. An acceptable r^2 value is $\geq$0.980. If there are no errors/failures and the calibration is acceptable, choose the **Validate** icon to validate the calibration curve, if needed.

D. QC Procedure

1. Create/Edit QC files, if necessary (**Setup, QC List**, **Test Code** to access the **QC Definition Screen**). Add Factor X Chromogenic code (**FT10C**) to the Test/Profiles Programming Window, if necessary **(Setup, Display, Test Programming Window**).
2. Load reagents onto the ACL TOP® Family instrument. Identify reagents as loaded by their rack position. Calibrate, if necessary (see calibration section of this procedure).
3. Place QC materials with the barcodes facing out in a Diluent Rack and load onto an ACL TOP® Family instrument Diluent Track 1. (If running the QC from the sample rack, refer to **Quality Control, Performing a QC Test** in the ACL TOP® Family On-Line Help Manual[7]).
4. Choose **QC** from the Main Menu and select **Test Status List**.
5. Double-click on the **FX Chrom** test code item to reveal the Test Materials Definition tree.
6. Select the box in front of the Factor 10 Chromogenic QC controls(**FX Chrom**) and click the **Program QC** icon. This will run both QC levels for that test.[7]
7. When the QC comes across to the LIS Instrument menu, the operator will upload the results to the QC program, ensuring failed results are documented and passing results are obtained.

E. Sample Procedure

1. Place sample tubes in correct sample rack (open tube or closed tube) with barcodes facing out.
2. Select an available sample track and load the sample rack when barcode reader is in position, ensuring no analyzer alarms occur.

3. 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.
4. Choose the **Run** icon if the ACL TOP® Family instrument is not currently set to AutoRun.

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.[7]

F. Procedure for making sodium citrate tubes for patients with a HCT greater than 55%:

For information on the procedure for making special sodium citrate tubes for patients with high hematocrits, please refer to the TOP 500 General Operating procedure, SH.CP.AU.coa.0036.

X. LIMITATIONS

Chromogenic Factor X results on the ACL TOP® Family are affected by[1]:

- Heparin (UF) over 30 U/mL
- Bilirubin, Hemolysis and Lipemia may interfere with absorbance readings

XI. CALCULATIONS

N/A

XII. INTERPRETATION

Congenital deficiency of FX is a rare 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.

XIII. RESULT REPORTING

Chromogenic Factor X results are reported in **%** activity[1]:
Normal Range: 72% – 148%
Reportable Range: 12% - 200%
Any results below or above this range should be reported out as “<12” or “>200” **%,** respectively. Refer to the ACL TOP® Family On-Line Help Manual for additional information. If any flags or alarms are present, refer to ACL TOP® Family On-Line Help Manual for details[7].

XIV. TRAINING

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

XV. REFERENCES

1. DiaPharma Factor X Kit (KDPGFX) Package insert
2. DiaPharma online brochure: http://diapharma.com/wp-content/uploads/2015/10/ML-01-00001_DiapharmaFX_Brochure.pdf
3. Clinical and Laboratory Standards Institute/CLSI. Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation and Molecular Haemostasis Assays; Approved Guideline – Fifth Edition, CLSI Document, H21-A5; Vol. 28 No. 5
4. HemosIL Calibration Plasma package insert
5. 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.
6. Westgard JO, and Barry PL. Cost-Effective Quality Control; Managing the Quality and Productivity of Analytical Process, AACC Press, 1986
7. ACL Help On-line manual
8. Mayo Medical Laboratories: Test Catalog - Factor X Chromogenic Assay http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/89042

Factor X Activity Chromogenic Assay on 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: A result of 9.5% stopped on the interface. It is reported out as is.
2. True or False: $CaCl_2$ must be mixed 1:1 with the reconstituted Russell's Viper Venom reagent. The solution is stable 48 hours at 2 – 8°C.
3. True or False: The chromogenic factor assay is preferential to the clotting factor assay (e.g. in cases of possible lupus anticoagulant) because the assay end point is not a phospholipid dependent clotting time.
4. True or False: Factor X is a Vitamin K dependent clotting factor and is activated by both the intrinsic and extrinsic factor cascade.
5. True or False: In the event of a QC failure and the reagent(s) is/are changed, a lookback does not need to be performed.

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)**

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Supervisor/Manager name (print)

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Supervisor/Manager signature **(Date)**