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|  | TITLE: **Factor XIII Antigen - ACL TOP 750** | **DEPT OF LAB MEDICINE****CLINICAL HEMATOLOGY****Policy and Procedure Manual** |
| **DOCUMENT # HEM**  |
| **WRITTEN BY:**Parveen Bahel, MT(ASCP) | **EFFECTIVE DATE:** | **REVIEW/REVISION**H-1;(New) 10/2019 | **Pages 1-9** |

1. **Intended Use**

 Automated latex enhanced immunoassay for the quantitative determination of Factor XIII Antigen (FXIII Ag) in human citrated plasma on IL Coagulation Systems.

1. **Purpose**

This procedure provides instructions for the analysis of Factor XIII using HemosIL Factor XIII Antigen on the ACL TOP® Family

1. **Summary and Principles**

Factor XIII, also called fibrin-stabilizing factor, is a zymogen composed of two potentially active A subunits and two B subunits (carrier protein) that circulates in plasma as a tetramer. In the terminal phase of the clotting cascade, by the action of thrombin and Ca2+, the A subunits dissociate from the B subunits assuming an enzymatically active configuration.

FXIII is essential for maintaining hemostasis due to its role in fibrin stabilization and in the protection of fibrin from proteolytic degradation by the fibrinolytic system.2

Patients with inherited FXIII deficiencies exhibit severe bleeding diathesis and, in most cases, require life-long supplementation therapy. In addition, FXIII is also involved in maintaining pregnancy and in wound healing.

Acquired FXIII deficiency may occur in several diseases, including inflammatory bowel diseases and acute leukemia.

Increased plasma FXIII activity has been reported in patients with obliterative atherosclerosis and diabetic angiopathy, and in chronic leukemia patients with increased megakaryocytic activity.

Recently, a polymorphism of the FXIII A subunit, has been associated with a protective effect against occlusive vascular diseases.

 The FXIII Ag Latex Reagent is a suspension of uniform size polystyrene latex particles coated with rabbit polyclonal antibodies, highly specific for the A-subunit of FXIII. When a plasma containing the active A-subunit of FXIII is mixed with the Latex Reagent and the Buffer included in the kit, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of FXIII Ag in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

1. **Interpretation of Results**

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1. **Specimen Type**

Mix nine parts of freshly collected blood with one part of 3.2% sodium citrate anticoagulant.

Invert the tube gently three or four times immediately after venipuncture to ensure proper mixing of blood and anticoagulant.

A syringe or evacuated tubes (blue top) may be used for collection. If multiple specimens are collected; the coagulation sample should be the second or third tube collected. If only coagulation testing is to be performed, a red-top tube, which has no additives, should be drawn first and discarded prior to drawing the blue-top coagulation tube.

The patient cannot be on anti-coagulants when the test specimen is collected. Sufficient time after discontinuance of heparin should be allowed for heparin to be cleared from the patient’s blood, usually 6 hours.

If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 mL saline and the first 5 mL of blood or six dead space volumes of the catheter discarded or used for other laboratory tests.

The citrate concentration must be adjusted in patients who have hematocrit values above 55%.

Specimens that are clotted, collected in the wrong tube or serum, overfilled or have less than the 90% expected fill should be rejected.

1. **Handling Condition and Stability**

The whole blood specimen is checked for clot formation by gentle inversion and observation. Centrifuge the capped blood specimen to produce platelet-poor plasma (platelet count <10x109/L for **10 minutes at 4000 g.** Patient plasma should be tested within 4 hours. If immediate testing is to be done, the plasma may remain on the packed cells. For special coagulation testing, spin samples 20 minutes at 4000 g, separate plasma into plastic tubes, label and freeze all aliquots at –70C located in the Special coag area until ready to use. Always check samples for clots after aliquoting. Always track aliquots in BEAKER under YH Coag Hold before freezing them. A frost-free freezer should not be used. Frozen plasma samples must be rapidly thawed at 37°C while gently mixing and tested immediately after thawing. If testing is delayed, the sample may be held for 2 hours at 4°C until tested immediately after thawing. If testing is delayed, the sample may be held for 2 hours at 4°C until tested.

 **Specimen stability** at ambient temperature: 4 hours; Frozen at -70° C 6 months.

**Specimen Labelling:** Specimen should be properly labeled with at least 2 unique patient identifiers

1. **Environmental Operating Conditions**

The instrument functions correctly in an ambient temperature of 15° C to 32° C (59° F to 89° F) with a relative humidity of 15% to 85% (non-condensing).

In accordance with the IEC regulations, no instrument failures occur in the presence of short-term ambient temperatures as low as 5° C or as high as 40° C.

The ACL TOP Family 50 Series is compliant with IEC 60068-2-40 to 2000 meters. The instrument should not be used at an altitude greater than 2000 meters.

The instrument should be placed in an area free from dust, fumes, vibrations and excessive variations of temperature.

The heat generated by the instrument during normal operation is exhausted from the bottom, the front-right and the left side of the unit.

According to IEC 61010-1, the maximum audible noise emission should be 80 dBA. The ACL TOP Family 50 Series is compliant with IEC 61010-1 Third Edition.

The room temperature and humidity percent are monitored and documented on the Routine Coagulation checklist.

1. **Equipment and Materials**
	1. **Supplies**
		* Nerl Water: pH 7.0
		* Gauze
		* Citrated blue top tubes
		* Frosted tubes for aliquots
		* Cuvettes
		* ACL Top sample cups
		* ACL TOP 750
	2. **Reagents**
		* HemosIL Calibrator Plasma
		* HemosIL Normal control Assayed
		* HemosIL test Control level 2
		* HemosIL **FXIII Ag** Kit which contain Latex Reagent, Buffer, Diluent
		* HemosIL Cleaning solution Clean A and Clean B
		* HemosIL Rinse and waste
		* HemosIL Factor Diluent
2. **Product Information**

The **HemosIL FXIII Ag** kit (PN 0020201300) consists of:

 **Latex Reagent:** 2 vials of a suspension of polystyrene latex particles coated with a rabbit polyclonal antibody directed against the A-subunit of FXIII. The reagent contains buffer, stabilizer, and preservative.

 **Buffer**: 2 vials of TRIS buffer containing bovine serum albumin, stabilizers and preservative.

**Diluent:** 2 vials of TRIS buffer containing bovine serum albumin, stabilizers and preservative

1. **Reagent Preparation**

**Invert to mix all reagents before use.**

**Note:** Avoid foam formation when mixing reagents. Bubbles on top of the liquids may interfere with the instruments liquid sensors.

**Cleaning Agent** (Clean B Diluted): Make fresh Clean B Diluted every day, 1 Part Clean B + 7 parts of Nerl water.

**HemosIL Calibrator Plasma (Lyophilized):** Reconstitute with 1 mL of Nerl water. Used for calibration, if needed.

**HemosIL Normal control Assayed** **(Lyophilized):** Reconstitute with 1 mL of Nerl water.

 **HemosIL Test control Level 2 (Lyophilized):** Reconstitute with 1mL of Nerl water.

1. **Reagent Storage and Stability**

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

For optimum stability, remove reagents and calibrator from the system and store them at 2-8oC in the original vial.

Opened Factor XIII Antigen kit reagents are stable

1 month at 2-8˚C in the original vial or

4 days at 15˚C on the ACL TOP. **Do not freeze**

**Calibrator, Control Storage:** Unopened calibration plasma and controls are stable until the expiration date shown on the vial when stored at 2-8˚C.

Stability of HemosIL Calibrator after reconstitution is 8 hours at 2-8˚C in the original vial. Use reconstituted calibrator within 2 hours for assay calibration

Stability of Normal and abnormal controls after reconstitution are 24 hours at 15°C on the instrument.

1. **Calibration Details**

Calibration or recalibration frequency is based on Policy # HEM 179 (Calibration and Analytical measurement Policy).

Calibration and storage of a valid Factor XIII Antigen calibration curve are required to obtain FXIII Antigen 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

**Refer to test feasibility screen for loading of reagents, calibrators, and controls.**

 **Steps to follow calibration:**

1. **ALWAYS** check maintenance log before calibration and make sure all maintenance is current (not overdue) and replace Factor diluent with fresh from a new bottle.
2. **Always load 3 bottles of Clean B while calibrating Factor XIII Antigen assays.**
3. Choose **Setup, Materials List, Click Scan** and Scan to 2D barcode on the top of the box of the calibrator if a new lot. This will upload all the information about lot number, expiration date, and assay values **(Skip step c – g below).** Repeat for all reagents if the 2D barcode is not on the box, double-click on the **FXIII Ag** calibrator to open the **Materials** **Definition** screen.
4. Choose the **Lot Specific Information** tab and enter the Calibrator Lot Number and Expiration Date.
5. Enable **Lot Management** from the Lot Specific Information tab.
6. Select the **Save** icon to store the lot number. Once the lot number is saved, the **Assign Values** icon becomes available.
7. Select the **Assign Values** icon.
8. Enter the calibration value from the package insert. Press **OK**.
9. Choose the **Previous Screen** icon to exit.
10. Load the FXIII Latex, Buffer, Diluent reagents, Calibration Plasma, and 3 Clean B vials onto the instrument.

**Note: Refer to test feasibility screen for loading of reagents/ calibrator and controls.**



1. Select **Calibration, Status List**.
2. Double-click on the FXIII Ag test code to be calibrated to open the **Calibration Details** screen.
3. Choose the **Run** icon.
4. Select **OK** at the “Do you confirm the operation?” prompt.
5. Choose the **Previous Screen** icon to exit.
6. Verify the Job Status for the FXIII Ag test code says **Active.**
7. Once the calibration is complete, review calibration results. The Instrument will fail the calibration if the r2 value is less than 0.980.
8. Choose the Calibration Information tab to ensure that no errors or warnings. If there are no errors/failures or flag and the calibration is acceptable, choose the **Validate icon** to validate the calibration curve.
9. Always **print Calibration Curve** and put it in the ACL TOP 2 Calibration binder with initial and date.

**Note: The FXIII Ag H calibration curve must also be validated.**

1. **Once calibration is done, do regular daily maintenance before running any other test samples.**
2. **Quality Control**
	1. Load all appropriate Reagents FXIII Latex, Buffer, Diluent reagents, Normal and Special test Control onto the instrument. Before loading the reagent rack, make sure the analyzer is in Ready mode.

**Refer to test feasibility screen for loading of reagents/ calibrator and controls.**

* 1. Place normal and abnormal controls with the barcodes facing out in a Diluent Rack and load on the instrument in a Diluent track D1 or D2.
	2. Choose **QC** from the Main Menu and select **Test Status List**.
	3. Double-click on a test code to reveal the Test Materials Definition tree in the **QC statistics screen**.
	4. Select the box in front of the FXIII Ag box test and choose the **Program QC** icon. This will run all QC levels for that test.
	5. To Review QC, single click on **Previous screen (back arrow)**  will return to **QC Result list.**
	6. If the control is acceptable, click on the **data** point, click on the **comment icon** , and type your initials in the comment box. If control is outside the acceptable range, the Status of the QC in red ‘failed’ and QC alarm at the bottom will alert you. Take an appropriate QC corrective action below.
	7. Controls should be prepared and tested once each 8-hour shift and tested again whenever reagents are added or changed and after each new calibration curve. Tech has to review shift control and placed an initial in the comment box under each control.
	8. Controls should be run in the same manner as the test samples, and by all techs that perform special coagulation testing.
	9. Control tolerance limits--the range is calculated based on +/-2SD from the mean control value.

 **Corrective action when tolerance limits are exceeded**:

* + 1. Rerun control after swirling QC and reagents.
		2. If still out, check reagent expirations; make new if indicated. Perform Enhanced clean for all the probes.
		3. If control still out, prepare new controls or reagents depending on one level of QC is out or both levels, allow to sit for 20 minutes, mix gently, and rerun.
		4. If controls still out, Recalibrate the assay and notify the supervisor.
		5. Verify reagent performance.
		6. Check instrument performance
		7. Document actions taken to identify and correct the problem before reporting any patient data.
		8. Remove the results that are outside the acceptable range by clicking on the unacceptable point and then clicking the omit icon. On the next data point**, indicate** the corrective action that was performed in the comment box along with your initials. The control results are recorded in the ACL TOP 750 QC files and are reviewed monthly by the supervisor.
		9. If the problem cannot be resolved, call for Service if necessary and properly document in troubleshooting log.Notify supervisor.

**Note: Factor XIII Antigen controls for the ACL TOP 750 are not formatted in the BEAKER QC program but set up and reviewed in the instrument QC Software file.**

1. **Procedure:**
2. Load reagents onto the instrument. 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 instrument Diluent track.
4. Choose **QC** from the Main Menu and select **Test Status List.**
5. Double-click on the **FXIII Ag** test code item to reveal the **Test Materials Definition tree.**
6. Select the box in front of the FXIII Ag QC Control and choose the **Program QC** icon. This will run all QC levels for that test. See
7. Place sample tubes in a sample rack with barcodes facing outwards.
8. Select an available sample track and load the sample rack when the barcode reader is in position.
9. Verify the samples **have been identified and have a test ordered. If not, program the sample ID ma**nually and/or order the test manually from the test and programming window.
10. Choose the **Run** icon if the instrument is not currently running.

**To Run Patient Samples without barcode**

* + - * Place sample cup in sample rack and label with sample name.
			* Click on the sample area icon. Double click on the rack to the left.
			* Enter the sample ID.
			* Double click on the box to the right. Choose the **FXIII Ag** tab in the Tests and Profiles box.
			* Click the **insert rack** icon. Load into an available track, S1-S12.
			* If the instrument is currently running and the run icon is greyed out, the sample(s) will be added to the active list and will be run. If the run icon is purple, click it to start the test(s).

**Note: After patients run, do regular daily maintenance before running any other test samples.**

1. **Reporting Results**

Factor XIII Ag results are reported in activity (%).

Linearity for this assay is 3.8 - 150%.

The upper limit of reporting is 150%; if the result is higher than >150%, it will flag as “result above linear range” report “>150%” and any result lower than 3.8 will be reported as <3.8%.

Record results in the computer system; Post results through the outstanding list / manual reporting referring to the Beaker bench manual as needed.

Hemolyzed, lipemic, or icteric samples must be noted with the result.

**Note:** If the linear range (FXIII Ag) is exceeded at the lower end (i.e. FXIII Ag results below 15%), then samples will be re-assayed triggering the Diluted Rerun test. The instrument performs this diluted assay and result correction automatically thereby expanding the test range down to 3.8%. If the linear range (FXIII Ag) is exceeded at the higher end (i.e. FXIII Ag results above 150%) then samples will be re-assayed by the Factor XIII Ag High (FXIII H) assay, expanding the test range up to 300% on the instrument.

**Reference Interval:** Normal range data for adult population was validated by the hematology lab from hospital and non-hospital patients while the age specific ranges are from the literature available by “*Age dependency for coagulation parameters in paediatric populations-Pierre Toulon, Micheline Berruyer,Marie Brionne-Francois Grand, Dominique Lasne, Caroline Telion, Julien Arcizet, Roberta Giacomello, Neila De poote:Thromb Haemost 2016; 116; 9-16”*

*Development of the hemostatic system in the neonate and infants. Am J pediatr Hematol Oncol 12:95.1990*

**Adult Normal Range: 63 – 138%**

**Age-specific reference ranges from Literature;**



**Reflex Criteria**: All abnormal results reflex MD Interpretation.

1. **Critical Results:** No critical result for the procedure.
2. **Procedural note:**

The overall performance of Factor XIII Antigen testing is dependent on reagent and instrument performance. Acceptable variability (imprecision) should be such that the total coefficient of variation (CV) of the analytic system is less than <=12% on the same lot of Normal control plasma and <=14% on the same lot of abnormal control plasma.

The measuring range is defined by the concentration of the calibrators used and the extrapolation limits set

1. **Specific Performance Characteristics**

Within-run and total (run to run and day to day) precision was assessed over multiple runs using both normal and abnormal control samples with a specific lot of Factor XIII Antigen reagent.

Please refer to the appropriate package insert for precision study results.

1. **Limitations and Interference substances**

FXIII Ag results are not affected by:

* Hemoglobin up to 500 mg/dL
* Bilirubin up to 18 mg/dL
* Triglycerides up to 1280 mg/dL
* Rheumatoid Factor up to 500 IU/mL.
1. **References**
2. HemosIL Factor XIII Antigen package insert
3. ACL TOP® Family On-Line Help Manual
4. 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. Age dependency for coagulation parameters in paediatric populations-Pierre Toulon, Micheline Berruyer,Marie Brionne-Francois Grand, Dominique Lasne, Caroline Telion, Julien Arcizet, Roberta Giacomello, Neila De pooter, Thromb Haemost 2016; 116; 9-16
6. Development of the hemostatic system in the neonate and infants. Am J pediatr Hematol Oncol 12:95.1990
7. **History**

This procedure was written by P Bahel on 10/9/2019