Boston Medical Center
Boston MA 02118
Department of Pathology and Laboratory Medicine

BARC PRO 013 BARC PRO 013 Factor Assay-VIII IX XI XII v1.1

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BOSTON MEDICAL CENTER		Thrombosis in Cancer Patients Factor Assay – VIII, IX, XI, XII	
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1.0 **PURPOSE**

1.1. The purpose of this standard operating procedure (SOP) is to describe the Factor assay used in the Pathology and Laboratory Medicine Department of Boston Medical Center (BMC).

2.0 **SCOPE**

2.1. This SOP contains all text from the clinical document control software.

2.2. PRINCIPLE: Coagulation factors in the blood are measured for various reasons as listed below:

2.2.1. To identify a deficiency of a specific factor that would cause a bleeding disorder.

2.2.2. To detect the presence of inhibitors that might interfere with the normal function of the clotting factors.

2.2.3. To monitor the factor levels in a patient who has a known factor deficiency.

2.2.4. The percent of factor activity in plasma can be determined by the extent to which the patient plasma can correct the aPPT using specific factor deficient plasma. We use specific factor deficient plasma and add the patient plasma to this, and determine the percentage activity of the “test patient” plasma. Results are compared to a calibration curve. Factors VIII, IX, XI and XII are involved in the intrinsic pathway, therefore, the aPPT in seconds is the basis of this test system.

2.3. **Specimen Requirement**

2.3.1. 1 ml aliquot of plasma from whole blood that was mixed with 3.2% sodium citrate. The plasma aliquot will be placed in the -80°C freezer until weekly batch testing. Each aliquot must have a patient barcode label attached.

3.0 RESPONSIBILITY

3.1. Principal Investigator. It is the responsibility of the Principal Investigator (PI) at BMC to ensure that project personnel have been trained in accordance with this SOP, that the training is documented, and that this procedure is followed.

3.2. Project Personnel. It is the responsibility of the project lab personnel to ensure he/she has read, understands, and follows the SOP when working with blood samples and the data.

3.3. It is the responsibility of the project staff designated by the PI or biospecimen source site (BSS) to ensure that all the required case report forms (CRFs) in the Comprehensive Data Resource (CDR) are completed.

3.4. Any planned deviation or change from this SOP, known prior to a collection, should be pre-approved by the Biospecimen Research Group-Quality Management (BRG-QM and the Technical Project Manager (TPM), and well-documented by the site following the QM-0006 and submitting Change Request Form, QM-0006-F2.

3.5. Any unplanned deviation that is unexpected or identified during or after a collection should be well documented by the site. Such deviations should be submitted to BRG QM and the TPM, following QM-0006, and submitting Deviation Report Form, QM-0006-F3.

4.0 **DEFINITIONS**

4.1. Definitions

4.2. Acronyms

4.2.1. **aPPT** Activated Partial Thromboplastin Time
4.2.2. **BMC** Boston Medical Center
4.2.3. **BRG-QM** Biospecimen Research Group – Quality Management
4.2.4. **BSS** Biospecimen Source Site
4.2.5. **CDR** Comprehensive Data Resource
4.2.6. **CRF** Case Report Form
4.2.7. **PI** Principal Investigator
4.2.8. **PPE** Personal Protective Equipment
4.2.9. **RPM** Revolutions Per Minute
4.2.10. **SDS** Safety Data Sheets
4.2.11. **SOP** Standard Operating Procedure
4.2.12. **TPM** Technical Project Manager

5.0 **ENVIRONMENTAL HEALTH & SAFETY**

5.1. Universal Precautions (CDC-1987) shall be used for blood handling.
5.2. Comply with institutional policies regarding blood borne pathogens and the use of appropriate Personal Protective Equipment (PPE) at all times.
5.3. Dispose of all contaminated supplies in the appropriate biohazard and sharps containers.
5.4. Handle all chemicals appropriately according to Safety Data Sheets (SDS).

6.0 **MATERIALS/EQUIPMENT**

6.1. **Equipment**

6.1.1. ACL TOP 500 Coagulation System
6.1.2. ACL TOP Cuvettes (# 0029400100)
6.1.3. ACL TOP Rinse Solution (# 0020009700)
6.1.4. ACL TOP Cleaning Agent A (# 0009831700)

6.2. **Reagents**

6.2.1. **HemosIL SnthASil** (Cat # 0020006800, 10ml). A high quality synthetic phospholipid reagent for the in vitro determination of activated Partial Thromboplastin Time (aPTT) in human citrated plasma.

6.2.1.1. **aPTT Reagent.** 5x10 ml vials of buffered synthetic phospholipid reagent containing a colloidal silica activator, stabilizers and a preservative. Opened reagent is stable for 10 days at 15°C on the ACL TOP and 30 days at 2-8°C in the original vial.
6.2.1.2. **Calcium Chloride.** 5x10 ml vials of an aqueous solution of calcium chloride (0.020 Mo/L) and a preservative. Opened reagent is stable 30 days at 2-30°C.

6.2.2. **Calibration Plasma** (Cat # 0020003700). Dissolve the contents of vial with 1 mL of deionized water. 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.

6.2.3. **Factor Deficient Plasmas.** Dissolve the contents of each required vial with 1 mL of deionized water. 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.

6.2.3.1. HemosIL Factor VIII Deficient Plasma (Cat # 8466450)
6.2.3.2. HemosIL Factor IX Deficient Plasma (Cat # 8466550)
6.2.3.3. HemosIL Factor XI Deficient Plasma (Cat # 8466650)
6.2.3.4. HemosIL Factor XII Deficient Plasma (Cat # 20201200)

6.2.4. **Reagent grade deionized water**. For QC & reagent preparation (Fisher Scientific product # 751-628, 20L/5Gal)

6.2.5. **Factor Diluent** (Cat # 0009757600)

6.2.6. **HemosIL Normal Control Assayed** (Cat # 0020003110). Lyophilized preparation of human citrated plasma from healthy donors. Values for analytes are within the normal range. Normal Control Assayed is stable for 4 hours on board the TOP.

6.2.7. **HemosIL Special Test Control Level 2** (Cat # 0020012000). Lyophilized preparation of human citrated plasma from healthy donors and modified by means of a dedicated process to simulate an abnormal coagulation sample. Special Test Control Level 2 is stable for 4 hours on board the TOP.

7.0 **PROCEDURE**

7.1. **Quality Control (QC)**

7.1.1. Preparation of Control Material and Calibration Plasma (if needed)

7.1.1.1. Reconstitute Normal Control 1, Special Test Control Level 2 and Calibration Plasma (if need for lot change) each with 1 mL reagent grade deionized water using a clean 1ml serological pipette.

7.1.1.2. Restopper vials, and allow to stand until dissolved. Minimum time before testing is 30 minutes.

7.1.1.3. Invert gently to mix. Do not shake. Label vial with date, time reconstituted and initials.

7.1.1.4. Stability after reconstitution: Normal Control Assayed is stable for factor assays for 4 hours on board the TOP.

7.1.1.5. Special Test Control Level 2 is stable for 4 hours on board the TOP.

7.1.1.6. Performance Parameters: Indication of deterioration: no evidence of vacuum in vial upon opening, difficulty in reconstituting reagent, control values outside of determined range.

7.1.1.7. Safety WARNING: Human source material. Treat as potentially infectious. The materials from which these products have been produced were tested and found non-reactive for the presence of HbsAg, anti-HCV and to HIV antibodies. No known test method can offer complete assurance that hepatitis B virus, anti -HCV, HIV or other infectious agents are absent.

Therefore, all human blood-based products should be handled with proper laboratory practices using appropriate precautions.

7.1.1.8. Calibration Curve:

7.1.1.8.1. Calibration and storage of the specific Factor Assay calibration is required to obtain Factor results. Calibration is performed:

7.1.1.8.1.1. With a change of reagent lot numbers;

7.1.1.8.1.2. To satisfy local regulatory requirements;

7.1.1.8.1.3. At laboratory discretion.

7.1.1.8.2. Place the Calibration Plasma and Factor Diluent in a Diluent rack and load onto track D1. Place the Factor Deficient Plasma(s) in a Diluent rack and load onto track D2. Place the SynthASil and Calcium Chloride in a Reagent rack and load onto a Reagent track.

NOTE: *When calibrating Factor VIII, remove diluent Clean B and all other reagents that are not needed for the assay. Place straight Clean B in the rack that is in the D1 track and place another straight Clean B in any rack that is in an R track. Run Factor VIII calibration by itself. All other Factor calibrations may be done simultaneously.*

7.1.1.8.3. Select Calibration, Status List. Double-click on the appropriate Factor to open the Calibration Details screen. Choose the Run icon.

7.1.1.8.4. Select OK at the “Do you confirm the operation?” prompt. Verify the Job Status for the Factor Assay test code says Active

7.1.1.8.5. To review and validate the calibration, open the Calibration Details screen for the factor being calibrated.

7.1.1.8.6. Review the Calibration Details screen of the Multiple Math Model View for any significant errors and warnings.

7.1.1.8.7. Choose Actions ► Configuration ► Math Model High View and verify the R2 value is >0.980.

7.1.1.8.8. Repeat step 3 above for the Math Model Low View.

7.1.1.8.9. Validate  .

7.1.1.8.10. Print Worklist:

7.1.1.8.10.1. Print a coagulation Factor Worksheet using Misys SmarTerm in order to know what tests have been ordered and to serve as a record of the testing being performed.

7.1.1.8.10.1.1. FUNCTION: WO

7.1.1.8.10.1.2. PRINTER: 227 or 229

7.1.1.8.10.1.3. Select Option # 1 for Incomplete

7.1.1.8.10.1.4. Type A for ALL

7.1.1.8.10.1.5. At Cut-Off Date: Press ENTER to default to the current date

7.1.1.8.10.1.6. At Cut-Off Time: Press ENTER to default to the current time

7.1.1.8.10.1.7. Press ENTER 4 times – chooses all the defaults

7.1.1.8.10.1.8. At WORKSHEET: FACT
7.1.1.8.10.1.9. At WORKSHEET: press ENTER
7.1.1.8.10.1.10. A for Accept

7.1.2. TEST RUN:

7.1.2.1. Place QC vials with the barcodes facing out and Factor Diluent in a Diluent Rack and load onto the TOP in the D1track

7.1.2.2. Place the Factor Deficient Plasma(s) in a Diluent rack and load onto the TOP in the D2 track. Place Synthasil and CaCl2 in a Reagent rack and load onto a Reagent track.

7.1.2.3. Choose QC from the Main Menu and select Test Status List. Double-click any QC name to show Test Materials Definition tree.

7.1.2.4. Select the appropriate Factor Assay QC and click the Multiple Run box. This will run all QC levels for that test. Ensure that results are within range and record in Special Coag QC book.

7.1.2.5. Corrective action: Results of quality control must always be within acceptable range prior to reporting results. If results exceed expected range, begin troubleshooting reagent and instrument including but not limited to, preparing new reagent and/or QC material, performing instrument maintenance, replacing de-ionized water aliquot, observing reagents for bacterial growth, etc. If results still exceed expected limits, discontinue testing and notify supervisor immediately. Review all patient results (if applicable) since the last acceptable QC. Refer to QC Manual for detailed quality control instructions. Always record all values and document corrective action taken on the TOP QC Review Problem Log.

7.2. **Sample Processing**

7.2.1. Place barcoded 12x75 plastic aliquot sample tubes in a sample rack WITHOUT a blue bar that has the ADAPTERS INSERTED. Ensure that aliquot tube barcodes are facing outward. If only a minimal amount of plasma is available, pour it in a TOP sample cup and place in sample rack WITHOUT a blue bar. Sample cup is placed in this rack WITHOUT ADAPTER.

7.2.2. Select an available sample track and load the sample rack when the barcode reader is in position.

7.2.3. Verify the samples have been identified and have a test ordered. If not, go to the test box and add the specific Factor Assay.

7.2.4. Choose the Run icon if the TOP is not currently running. Repeat any result <50%. Dilute any plasma with a result over 150% and rerun. See procedure note for result over 150%.

7.2.5. Print out Parallelism Report as follows: Double click on any of the dilution results to see the parallelism screen. Click on Actions. Click on Parallelism Report. Click on Print.

7.2.6. Review results of all dilutions and check for error codes. See a supervisor with any questions. Result is generally but not always the result listed as the Mean of 100% located on the top left of the Parallelism Report.
7.2.7. Record results on the coagulation Factor Worksheet.
7.2.8. Attach TOP printout to worksheet and enter result in Misys SmarTerm using the MEH option. Refer to the "Using the MEH Option" procedure in the Routine Hematology Manual.

7.3. **Reporting Samples**
7.3.1. Normal value: 50-150%.
7.3.2. The patient test result is reported as percent rounded to a whole number.
7.3.3. Any patient test result that is less than 5% is reported as <5.
7.3.4. Use the following comment to result a factor that doesn't give a result because the inhibitor is so strong: UDIH. UDIH translates to: Unable to determine due to the presence of an inhibitor that cannot be reliably diluted out. Recommend a mixing study for characterization of the inhibitor.

7.4. **Computer Notes- In Misys SmarTerm**
7.4.1. Function: MEH
7.4.2. Worksheet: FACT
7.4.3. Test: F8, F9, F11, F12
7.4.4. Report as: % (whole number)
7.4.5. Release Result: Use LHR and RHR functions

7.5. **Procedure Notes**
7.5.1. Standard curve for all factors is linear to 150%. If a plasma gives a result of over 150%, it must be diluted with Factor Diluent so that it will fall within the limits of linearity for the curve. Multiply the diluted result by the dilution factor for the final result.
7.5.2. Multiple dilutions are run to detect the presence of a circulating anticoagulant if present. If results from each dilution do not agree with one another (approximately 10%), and the values become more normal as the sample dilution increases, a circulating inhibitor is most likely present. The most diluted sample is the most accurate but it may not be possible to report any result. These should be brought to the pathologist for interpretation.
7.5.3. When evaluating the Parallelism Report:
7.5.3.1. Parallelism r2 should be >0.985. This parameter is an indicator of the presence of an inhibitor and will be much lower if an inhibitor is present. It may be slightly lower than 0.985 with factor values that are near 100 and higher. Results may be reported out when the r2 value is slightly lower if dilutions are in agreement.
7.5.3.2. %CV of CR (corrected result) 100% should be <15
7.5.3.3. Mean of 100% = the result of the 100% dilution

7.5.3.4. Mean CR = average of the 50% and 25% dilutions
7.5.3.5. Mean CR 100% = average of all 3 dilutions
7.5.3.6. Generally, if all 3 dilutions look valid it is the Mean of 100% that should be reported. This value has the least amount of dilution and therefore the least amount of error.

8.0 **REFERENCES**

8.1. HemosIL™ SynthASil Cat # 20006800 package insert issued 04/2004, Instrumentation Laboratory.
8.2. ACL TOP On-Line Help Manual Rev 2.2, Instrumentation Laboratory.
8.3. Collection, Transport, and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays – 4th Edition; Approved Guideline. CLSI/NCCLS document H21-A4, Vol.23, No. 35. Clinical and Laboratory Standards Institute/NCCLS, 2003.
8.4. Westgaard JO, and Barry PL. Cost-Effective Quality Control; Managing the Quality and Productivity of Analytical Process, AACC Press, 1986.
8.5. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test; Approved Guideline. CLSI/NCCLS document H47-A4. Clinical and Laboratory Standards Institute/NCCLS, 1996.
8.6. HemosIL™ Factor VIII Deficient Plasma Cat # 8466450 package insert issued 06/2004, Instrumentation Laboratory.
8.7. HemosIL™ Factor XI Deficient Plasma Cat # 8466650 package insert issued 09/2004, Instrumentation Laboratory.
8.8. HemosIL™ Factor XII Deficient Plasma Cat # 20201200 package insert issued 06/2005, Instrumentation Laboratory.

9.0 **ATTACHMENTS**

INITIATION/REVISION HISTORY			
REV #	**DESCRIPTION OF CHANGE**	**AUTHOR**	**EFFECTIVE DATE**
1.0	Reformatting of current clinical protocol into BARC format	Liz Duffy	0.3/27/2017