Boston Medical Center
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Department of Pathology and Laboratory Medicine

BARC PRO 026 Souble Tissue factor SOP

Copy of version 1.3 (approved and current)

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Comments for version 1.2 (last major revision)
Initial version

Comments for version 1.3 (this revision)
Typos and clarifications

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	QA Review	8/20/2018	1.3	ERDuffy Elizabeth Duffy	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.3	Approved and Current	Minor revision	8/20/2018	8/20/2018	Indefinite
1.2	Retired	Initial version	2/12/2018	2/13/2018	8/20/2018

1.0 PURPOSE AND SCOPE

1.1. The purpose of this SOP is to provide standardized instructions and guidance for measurement of Soluble Tissue Factor (sTF) in human plasma in the Pathology and Laboratory Medicine Department of Boston Medical Center (BMC).

1.2. This procedure applies to all personnel involved in the use of this assay during the study. The goal of the SOP and associated training is to ensure consistency in measurement across samples.

2.0 OVERVIEW

2.1. PRINCIPLE OF THE ASSAY:
This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human sTF has been pre-coated onto a microplate. Standards, samples, blank, and control are pipetted into the wells followed by a biotin-conjugated secondary antibody. An avidin-horseradish peroxidase conjugate and TMB substrate are the detection reagents and color is developed which is proportional to analyte concentration. The color development is stopped and the intensity of the color is measured. Assay quality control criteria are applied to the background, calibrator and control samples to validate the assay run. Quality control criteria are then applied to the unknown samples and data reporting guidelines are defined.

2.2. CLINICAL SIGNIFICANCE:
Tissue factor (TF) is a cell-bound receptor and the cellular initiator of the cascade that forms thrombin, the terminal enzyme of the coagulation pathway. TF activity contributes to malignancy by promoting fibrin deposition in the tumor microenvironment, and directly promotes angiogenesis, tumor growth, and tumor dissemination. Procoagulant TF antigen is found on microparticles (extracellular vesicles) shed from cancer cells and TF antigen detected in plasma of cancer patients reflect these sources.

2.3. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin or EDTA anticoagulant). A minimum of 200 microliters (200 µL) plasma is needed for each sample.

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 approved by the Biospecimen Research Group – Quality Management (BRG-QM) and Leidos Technical Project Manager (TPM) and ***well-documented by the site***.

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 the BRG-QM and TPM along with a corrective action description for documentation.

4.0 DEFINITIONS and ACRONYMS

4.1. Acronyms- see Table I.

Table I. Acronyms

Acronym	Name
Soluble Tissue Factor	sTF
CV	coefficient of variation
HBSS	Hank's balanced salt solution
ID	Identification/ Identifier
LLQ	lower limit of quantification
SD	standard deviation
SOP	standard operating procedure
UA	unanalyzable
ULQ	upper limit of quantification

4.2 Assay Procedure Summary

Prepare all reagents, samples and standards.

Add 100 µl of **assay diluent** to each well.

Add 100 µl of **Sample, Standard, Blank, or Control** to each well.

Cover and incubate at room temperature for 2 hours on an orbital shaker.

Wash the plate 1x with **wash buffer** four times.

Add 200µL of **1x HRP-Conjugated Antibody** to all wells.

Cover and incubate at room temperature for 2 hours on an orbital shaker.

Repeat previous wash step.

Add 200µL of **TMB Substrate Solution** to each well.

Incubate at room temperature for 30 minutes. Protect from light.

Add 50µL of **Stop Solution** to each well. Read within 30 min at 450nm with wavelength correction at 540 or 570nm

5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Safety Precautions will be followed

6.0 CRITICAL REAGENTS, MATERIALS, AND EQUIPMENT REQUIRED

6.1. Human platelet-poor plasma sample(s) handled as per SOP BARC PRO 023 (Blood sample processing, storage, and shipping). Samples can be anticoagulated with citrate, heparin or EDTA from blood obtained in standard vacutainer collection tubes. Serum is not a valid sample type for this assay.

6.2. Critical reagents

6.2.1. Human sTF ELISA kit (Catalog number: DCF300, R&D Systems Inc., Minneapolis, MN, 55413 USA). Store all kit components at 2-8°C. The substrate should never be frozen. Unused strip plate wells should be stored at 2-8°C in a sealed bag containing desiccant in order to minimize exposure to moisture. Do not use the kit beyond its expiration date. See Table II for kit components.

6.2.2. Other critical reagents see Table II.

Table II. Critical Reagents

Reagent	Vendor	Catalog #	Storage	Notes
Hank's balanced salt solution (HBSS)	ThermoFisher Scientific	14025-092	keep stock solution bottles at room temp (~25°C)	Store in sterile 10mL aliquots at -80 ± 5°C. Use once, then discard.
Normal human pooled plasma in 4% trisodium citrate	Sigma-Aldrich	P9523-5ML	2-8°C, sterile	Prepare BMC Control
Recombinant sTF	R&D Systems, Inc. Minneapolis, MN	R&D 2339-PA-010	-80 ± 5°C, Avoid repeat freeze thaw cycles	10 µg protein in Tris-saline buffer; variable concentrations; Prepare BMC Control
Coated 96-well strip plate	R&D Systems, Inc. Minneapolis, MN	DCF300	2-8°C, supplied in ELISA kit	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C. 1 plate included in kit.
Standard			2-8°C, supplied in ELISA kit	Recombinant human Coagulation Factor III in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume.
Assay Diluent RD1-89			2-8°C, supplied in ELISA kit	11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD5-20			2-8°C, supplied in ELISA kit	21 mL of a buffered protein base with preservatives.
HRP Conjugate			2-8°C, supplied in ELISA kit	21 mL of a polyclonal antibody specific for human Coagulation Factor III conjugated to horseradish peroxidase with preservatives.
Wash Buffer			2-8°C, supplied in ELISA kit	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. May turn yellow over time.
Color Reagent A			2-8°C, supplied in ELISA kit	12 mL of stabilized hydrogen peroxide.
Color Reagent B			2-8°C, supplied in ELISA kit	12 mL of stabilized chromogen (tetramethylbenzidine).
Stop reagent			2-8°C, supplied in ELISA kit	6 mL of 2 N sulfuric acid.
Plastic Plate Sealers			supplied in ELISA kit	4 sealers

6.3. Reagent Comments

6.3.1. High concentrations of sTF are detectable in saliva. Take care to prevent contamination of kit reagents while running this assay.

6.3.2. Consumables- See Table III

Table III. Consumables

Item	**Range / Capacity**	**Quantity**	**Suggested Vendor / Catalog #**
Pipet tips	200-1000 μL	1 box	
Pipet tips	50-200 μL	1 box	
Pipet tips	2-20 μL	1 box	

Volumetric pipette with dispenser or bulb	5ml	at least 2	
Polystyrene round bottom test tubes	12x75mm	about 20	
1.5-mL tubes, O-ring screw cap, conical bottom, sterile	1.5 mL		Sarstedt 72.692.005
Polypropylene tubes, sterile	15 mL		VWR 21008-918
Polypropylene tubes, sterile	50 mL		VWR 21008-951
Sealing tape for 96 well plates			Thermo Fisher 15036
Disposable reagent reservoirs			Thermo Fisher 95128095
aluminum foil			

6.4. Equipment – see Table IV

Table IV. Equipment

Equipment	Range/Capacity	Manufacturer	Model	Serial No
Pipettor	100-1000 μL			
Pipettor	20-200 μL			
Pipettor	0.5-10 μL			
Multichannel Pipettor	30-300 μL			
Microplate Washer		BioTek	ELx50	259186
Microplate Reader		Molecular Device	VersaMax	BNR06440
Refrigerator	2-8°C			
Orbital Shaker				
-80°C Freezer	-80 ± 5 °C			

6.5. Reagent storage and stability

6.5.1. Record the date of receipt, lot number, and provided reagent concentration and expiration date for all Critical Reagents in the Batch Record (Appendix 2, Section 1).

6.5.2. All critical reagents are to be labelled with date of receipt and stored under the specified conditions for no longer that the recommended duration.

6.5.2.1. Check dates on all vials and replace any that are expired.

6.5.2.2. Storage conditions and expiration dates for all Critical Reagents are provided on the package inserts.

6.5.2.3. Do not exchange reagents from one set of qualified Critical Reagents with a set of reagents qualified separately.

6.5.2.4. Do not use any materials past expiration date.

7.0 OPERATING PROCEDURE

7.1. Prior to beginning the assay, refer to the Plate Map Design and Batch Record to review all actions required for successful assay setup (Appendices 1 and 2).

7.2. Record the name and certification number of the Certified Assay Operator and the facility running the SOP in the Batch Record (Appendix 2). Include reference to 96-well plate ID, if applicable.

7.3. Plate Map Preparation

7.3.1. Based on the number of patient samples to be analyzed, generate a Plate Map (Appendix 1) to define the location and replicates of clinical samples, control samples, and standards. A single patient's **batched** samples should be contained on one 96-well plate, not split over two plates, to ensure consistent sample handling.

Important: The data analyses template is based on the 96-well sample designations in the Plate Map (Appendix 1). To prevent user errors, always load the plate according to the plate map well designations.

7.3.2. Once the number of wells is known, determine the amount of reagents required for the assay. Once these calculations are complete, check that sufficient reagents and supplies are on hand to complete the assay.

7.3.3. Record serial numbers of equipment in the Batch Record (Appendix 2, Section 5).

7.4. **Pre-Assay Reagent Preparation**

7.4.1. Prepare BMC Control for aliquot storage.

7.4.1.1. Re-suspend lyophilized plasma with 5mL DI water. Allow to sit for at least 15 minutes with gentle mixing. Do not shake.

7.4.1.2. Transfer 4mL of re-suspended plasma into a conical labeled "BMC sTF Control." Add 4ml of Calibrator diluent RD5-20 buffer. Mix gently by swirling.

7.4.1.3. Record concentration of stock recombinant sTF protein in Batch Record Appendix 2, section 1. The concentration from the manufacturer varies and needs to be recorded. Dilute to 80 ng/mL with HBSS buffer. For example,

for 440 µg/mL stock sTF, add 1µL to 5.5mL HBSS in a 15 ml conical tube. Label this vial "sTF working solution."

7.4.1.4. Add 10 uL of sTF working solution to the BMC sTF Control tube. The final concentration of sTF will be approximately 100pg/mL increase over initial levels present in plasma.

7.4.1.5. Aliquot sTF control solution in 400µL aliquots into 0.5mL cryovials labeled "BMC sTF Control." This should make approximately 20 aliquots.

7.4.1.6. For remainder of normal human pooled plasma, make 100 µL aliquots (about 8 or 9) in screw cap tubes with O-ring. Label and put in -80°C to freeze rapidly.

7.4.1.7. Store frozen at -80°C. Controls are used once and excess is discarded.

7.5. **Reagent Preparation on Assay Day:** All reagents should be at room temperature prior to assay

7.5.1. **Prepare Wash Buffer**

7.5.1.1. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

7.5.1.2. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

7.5.2. **Prepare Substrate Solution**

7.5.2.1. Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

7.6. **Preparation of Standards (for triplicates on each plate)**

7.6.1. Reconstitute the Standard with DI water. Refer to the vial label for reconstitution volume. This produces a stock solution of 5,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Add Calibrator Diluent RD5-20 to labelled polystyrene tubes, followed by the standard for 2-fold serial dilutions. (Table V).

Table V. Preparation of Standards

Standard #	Concentration (pg/mL)	Volume Calibrator Diluent RD5-20 (µL)	Volume Standard (µL)	Final concentration (ng/mL)
1	500	900	100 of stock	250
2	250	500	500 of tube #1	125
3	125	500	500 of tube #2	62.5

4	62.5	500	500 of tube #3	31.3
5	31.3	500	500 of tube #4	15.6
6	15.6	500	500 of tube #5	7.8
7	7.8	500	500 of tube #6	3.9
8	0	500	0	0

7.7. Preparation of Unknowns (plasma samples)

7.7.1. Dilute plasma sample(s) 1:1 with Calibrator Diluent RD5-20

7.7.1.1. Thaw plasma samples at 37°C

7.7.1.2. For each unknown sample, add equal parts plasma and diluent. For example, add 200 µL Calibrator Diluent RD5-20 to a polystyrene tube, then add 200 µL plasma. Vortex briefly to mix.

7.8. **Assay Procedure**

7.8.1. In each well of the 96 well plate, add 100µL of Assay Diluent RD1-89.

7.8.2. To each well add 100µL of standard, control, blank or diluted plasma sample. Each is run in triplicate wells. Refer to Plate Map Design. (Appendix 1).

7.8.3. Cover with an adhesive strip and incubate at room temperature for 2 hours on a horizontal orbital shaker set at 500 ± 50 rpm (0.12" orbit).

7.8.4. Wash for a total of 4 washes with Wash Buffer.

7.8.4.1. Remove the cover and wash the plate as follows:

7.8.4.1.1. Aspirate the liquid from each well.

7.8.4.1.2. Dispense 0.4 ml of 1x washing solution into each well.

7.8.4.1.3. Aspirate the contents of each well.

7.8.4.1.4. Repeat another three times

7.8.4.1.5. After the final wash, remove residual liquid by aspirating, then inverting the plate and blotting on clean paper towels. Do not let the plate dry.

7.8.5. For the Biotek Microplate Washer, the settings are:

METHOD	ELx405 Select	ELx405
Number of Cycles:	4	4
Soak/Shake:	Yes	Yes

Soak Time:	30 sec	30 sec
Dispense Volume:	400 µL/well	400 µL/well

7.8.6. Add 200 µL of Conjugate into all wells.
7.8.7. Cover with an adhesive strip and incubate at room temperature for 2 hours on the shaker.
7.8.8. Wash for a total of 4 washes
7.8.8.1. Repeat wash step in 7.8.5.
7.8.9. Add 200 µL of freshly prepared Substrate Solution into all wells. Cover with an adhesive strip and protect from light using aluminum foil.
7.8.10. Incubate in the dark for 30 minutes at room temperature on the benchtop.
7.8.11. Add 50 µL of Stop Solution into all wells.
7.8.12. Read the absorbance value of each well using 450 nm as the primary wavelength with correction at 540 nm or 570 nm if available.
7.8.13. Save the resulting readings in BIOMARKER NAME MM/DD/YEAR PLATE X format to a secure computer; recommended to label the file with the date and a unique assay identifier (Plate ID). Print a paper copy of the raw data for inclusion with the Batch Record.

7.9. Review and finalize the Batch Records (Appendix 2) and obtain required signature. Document ANY and ALL deviations from this SOP in the Batch Record (Appendix 2 Section 7).

8.0 DATA ANALYSIS

8.1. PRINCIPLE:

8.1.1. Signal data is converted to analyte concentration with a computer program, SoftMax Pro. Acceptable results are obtained with computer programs using a standardized curve-fitting four parameter logistic method, or a logistic/log regression analysis.

8.1.2. The protocol calls for an analyte analysis program which tells the calculation-program the location of samples, standards, controls, the initial dilution and any serial dilutions. Wells designated as Diluent Only in the Plate Map (Appendix 1) should be labeled as “blank wells” in the template. The program should subtract the average absorbance of the “blank wells” from the absorbance of other wells.

8.1.3. The analyte concentration for each sample is found by calculating the mean of the sample triplicate determinations based on the standard curve.

8.2. DATA INSPECTION RULES

8.2.1. Blanks: the signal of blank wells should be less than 0.2 units for all assay plates. If any blank wells are >0.2, the assay should be examined for inappropriate results and should be re-assayed if no apparent causes are found.

8.2.2. Triplicates: If the coefficient of variation (CV) of triplicate wells is >15% and two wells have a CV of ≤ 10%, then the outlier well value can be excluded from the calculation. This has to be documented in Appendix 2, section 7. If > 1 outlier well is observed, the assay should be examined for cause and re-assayed if no apparent causes are found.

8.2.3. Standards: The slope of the linear portion of the reference standard curve (e.g., OD 0.1 to 2.0) should be near 1.0 (0.9 – 1.1) when the log of the OD signal is graphed against the log of the standard concentration.

8.2.4. Sensitivity: Calculate the lower detection limit for the assay and confirm that the detection limit is within in the established range.

8.2.5. Quality Control: Control sample values must be within the established range for intra-assay variability (CV<15%; plates run on the same day) and inter-assay variability (CV<30%; comparing plates run on different days).

8.2.6. If a sample has readings greater than the highest standard used in the assay, the sample should be re-assayed after additional dilution.

8.2.6.1. If an unknown value is high and is diluted more than that defined in the assay procedure, then new controls should be made with normal human pooled plasma using the same dilution factor to replicate the amount of plasma in all the samples.

8.2.7. If the analyte concentration of the sample was calculated by averaging the data from multiple dilutions and the CV of the concentration exceeds 30%, then the data should be examined for inappropriate results and should be re-assayed if no apparent causes are found.

8.2.8. If the lower limit of detection is equal to or less than the lowest standard concentration and a sample has undetectable analyte concentration, report one half of the established assay lower limit as the concentration for the sample. If the lower limit of detection is more than the established value and a sample has undetectable analyte concentration, do not report the result for the sample and re-analyze the sample.

8.3. **DATA ANALYSIS.** Most software analysis packages, including SoftMax Pro, will perform curve fitting and data analysis to obtain concentrations.

8.3.1. Obtain average signal of Standards and each sample well groupings.

8.3.2. For each analyte concentration, obtain the 'signal' by subtracting the average signal of the background wells from the average signal value of the corresponding wells that contain standards or unknowns.

8.3.3. Plot the background corrected signal values on the Y-axis and the logarithm of standard concentration on the X-axis to obtain the standard curve.

8.3.4. Obtain unknown concentrations from the standard curve. Multiply by any dilution to obtain the final analyte concentration.

9.0 REFERENCES

9.1. R&D Systems User Manual for Human Soluble Tissue Factor ELISA kit.

9.2. National Clinical Target Validation Laboratory, Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc. by Frederick National Laboratory for Cancer Research.

10.0 ATTACHMENTS

INITIATION/REVISION HISTORY			
REV #	**DESCRIPTION OF CHANGE**	**AUTHOR**	**EFFECTIVE DATE**
1.0	Draft	MPT	01/03/2018
1.1	Draft	DSK	01/11/2018
1.2	Draft	DSK, MPT	01/26/2018
1.3	Minor Clarifications, Typos, Formatting	BET,DSK,ERD,MPT	08/01/2018

APPENDIX 1: PLATE MAP DESIGN: Patient samples from Module I, <1 hour will be assayed with similar samples from different donors on this plate.

- When only 1 or 2 patient samples (S) are run, the Plate Map Design can be adjusted, so long as triplicate wells are used for samples, standards and controls.
- Blank wells are loaded with Reagent Diluent only (no sample).
- Document the sample/patient IDs and other pertinent information in the Sample Calculation Table in the Batch Record (Appendix 2)

A1.1 Plate Design (Baseline Donor Sample): Room Temperature Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A		STDS		S1T1			S9T1			S17T1		
B				S2T1			S10T1			S18T1		
C				S3T1			S11T1			S19T1		
D				S4T1			S12T1			S20T1		
E				S5T1			S13T1			S21T1		
F				S6T1			S14T1			S22T1		
G				S7T1			S15T1			BMC CTL	BMC CTL	BMC CTL
H				S8T1			S16T1					

APPENDIX 2: BATCH RECORD

NOTE: Record times using **military** time (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Certified Assay Operator: _____ Certification Number:_____

Facility/Laboratory Running SOP: _____

Clinical Protocol Number: _____

Date Immunoassay Run: _____

Plate ID (optional): _____

1. Critical Reagents

Complete the table as designated. Be sure the lot numbers on each of the reagents match those cited in the product insert accompanying the reagents. Reagents from one kit **should not** be exchanged with reagents from another.

Reagent Name	Date Received	Lot No	Exp Date
96 well microtiter strip plate	/ /		/ /
Standard	/ /		/ /
Wash Buffer	/ /		/ /
Calibrator Diluent	/ /		/ /
Assay Diluent	/ /		/ /
Conjugate	/ /		/ /
Color Reagent A	/ /		/ /
Color Reagent B	/ /		/ /
Stop Solution			
Normal human pooled plasma in 4% trisodium citrate	/ /		/ /
Recombinant Human Tissue Factor Concentration:	/ /		/ /
Hank's Balanced Salt Solution	/ /		/ /

2. **Unknown Samples.** The first line gives an example with sample/patient ID, Module with Pre-analytic variable (PAV) and plasma dilution

Sample No	Sample/Patient ID	Module/PAV	Dilution (X)		
S Ex	TCP_0001	I / T2	20		
S1					
S2					
S3					
S4					
S5					
S6					
S7					
S8					
S9					
S10					
S11					
S12					
S13					
S14					
S15					
S16					
S17					
S18					
S19					
S20					
S21					
S22					

3. Plate Incubation: If not applicable, cross out.

a. Add clinical samples, controls, and standards, and conjugate to the 96-well plate, cover plate, and incubate at room temperature for 2 hours. Record below.

Date	Start	Stop	Incubation Temp (°C)
/ /	:	:	

b. Add Conjugate to the 96-well plate, cover plate, and incubate at room temperature for 2 hours. Record below.

Date	Start	Stop	Incubation Temp (°C)
/ /	:	:	

c. Add Substrate, cover plate and incubate at room temperature for 30 minutes. Record below.

Date	Start	Stop	Incubation Temp (°C)
/ /	:	:	

4. Software:

4.1. SoftMax Pro Version:_____

4.2. Name of original SoftMax Pro data file: _____

5. Equipment

Standard equipment is listed below. Check if used for the biomarker assay. If different equipment was used, document in Appendix 2, Section 7.

Check if used	Equipment	Manufacturer	Model	Serial No
	Microplate Washer	BioTek		ELx50
	Microplate Reader	Molecular Devices		VersaMax
	Refrigerator (2-8^{o}C)			
	Freezer (-80^{o}C)			
	Orbital Shaker			

6. Plate Map QC

a. Name of saved Excel data analysis workbook
_____

b. Plate Map Set Up QC

( ) Recommended Plate Map used. Circle one: A1 A2 A3 A4

( ) Alternative plate map used; cells copy and pasted individually to the Plate Layout QC worksheet.

Reason: _____

7. Notes, including any deviations from the SOP:
If assay fails QC, state the specific reason for assay failure and notify the Laboratory Director/Supervisor.

8. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____(Print)

_____(Sign)

9. Date: _____

APPENDIX 3: Work Process Flow

OVERVIEW OF IMMUNOASSAY SAMPLE PROCESSING

BARC PRO 012: Thrombosis in Cancer Patients: Blood sample Collection SOP	• Properly collect blood at all BSSs for the the Thrombosis in Cancer Patients Pre-Analytical Factors (TCP) study. • Immediately invert the tube slowly and gently. • Transport to blood processing laboratory.

BARC PRO 023: Thrombosis in Cancer Patients: Blood sample Processing, Storage and Shipping	Instruction to biospecimen source sites for blood sample processing, storage and shipping. • Blood will be processed for the preparation of blood derivatives from all study donors for downstream marker analyses. • Collected Plasma will be aliquoted to a pre-labeled cryovial for sTF ELISA.

BARC PRO 026: Thrombosis in Cancer Patients: Immunoassay of soluble Tissue Factor in blood sample	• Perform ELISA with clinical samples, sTF standards, sTF controls • Using Versa Max Microplate reader, determine relative signal of all samples