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

BARC PRO 021 PAP ELISA

Copy of version 1.7 (approved and current)

Last Approval or Periodic Review Completed 3/20/2018

Next Periodic Review Needed On or Before 3/20/2019

Effective Date 8/20/2018

Controlled Copy ID 149101

Location SharePoint SOPs

Organization Boston Medical Center

Description
Not final format

Comments for version 1.7
Typos and clarifications

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	QA Review	8/20/2018	1.7	ERDuffy Elizabeth Duffy	
Approval	Primary Investigator	8/23/2017	1.6	Chris Andry, PhD Chris Andry	
Approval	Quality Approval	8/23/2017	1.6	ERDuffy Elizabeth Duffy	
Approval	Quality Approval	8/23/2017	1.5	ERDuffy Elizabeth Duffy	
Approval	Quality Approval	8/23/2017	1.4	ERDuffy Elizabeth Duffy	
Approval	Quality Approval	8/23/2017	1.3	ERDuffy Elizabeth Duffy	

Approval	Quality Approval	8/23/2017	1.2	 Elizabeth Duffy	
Approval	Quality Approval	8/23/2017	1.1	 Elizabeth Duffy	
Approval	Lab Director	3/23/2017	1.0	Chris Andry	Recorded when document uploaded to MediaLab
Periodic review	Designated Reviewer	3/23/2017	1.0	Chris Andry	Recorded when document uploaded to MediaLab

Approvals and periodic reviews that occured before this document was added to the MediaLab Document Control system may not be listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.7	Approved and Current	Minor revision	8/20/2018	8/20/2018	Indefinite
1.6	Retired	Minor revision	8/23/2017	8/28/2017	8/20/2018
1.5	Retired	Minor revision	8/23/2017	8/23/2017	8/28/2017
1.4	Retired	Minor revision	8/23/2017	8/23/2017	8/23/2017
1.3	Retired	Minor revision	8/23/2017	8/23/2017	8/23/2017
1.2	Retired	Minor revision	8/23/2017	8/23/2017	8/23/2017
1.1	Retired	Minor revision	8/23/2017	8/23/2017	8/23/2017
1.0	Retired	First version in Document Control	3/23/2017	3/23/2017	8/23/2017

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1.0 PURPOSE AND SCOPE

1.1. The purpose of this SOP is to provide standardized instructions and guidance for measurement of PAP Complex 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. This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human Plasmin-α2-antiplasmin (PAP) complex has been pre-coated onto a microplate. Standards, samples 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: Plasmin-α2-antiplasmin (PAP) complex is formed from the inhibition of plasmin by its principle inhibitor, alpha2-antiplasmin. This provides a measure of fibrinolytic activity. Elevated levels of PAP complex have shown to indicate thrombotic states. Patients with cancer are at an increased risk for thrombotic events and may benefit from the predictive nature of elevated PAP complex in plasma.

2.3. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin or EDTA anticoagulant). A minimum of 40 microliters (40 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
PAP	Plasmin-α2-antiplasmin complex
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
HRP	Horseradish Peroxidase
TMB	3,3',5,5'-Tetramethylbenzidine

4.2 Assay Procedure Summary For ELISAs

Prepare all reagents, samples, controls and standards.

Add 100 µl of **Sample, Standard, Control or Blank** to each well and Incubate for 90 minutes at 37 °C.
Aspirate and do not wash wells

Add 100 µl of **Biotinylated Detection Antibody**
Incubate for 1 hour at 37 °C.

Aspirate and wash 3 times.

Add 100 µl of **HRP Conjugate**
and Incubate for 30 minutes at 37 °C.

Aspirate and wash 5 times.

Add 90 µl of **TMB Substrate** solution
and Incubate for 15 minutes at 37 °C.

Add 50 µl of **Stop Solution**

Read immediately at 450 nM

5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Safety Precaution 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 0023 (Blood sample processing, storage, and shipping). Samples can be anticoagulated with citrate, heparin or EDTA from blood obtained in standard vacutainer collection tubes.

6.2. Critical reagents- Human Plasmin-Antiplasmin Complex ELISA kit (LifeSpan Biosciences, Inc., Seattle, WA 98121), Catalog number LS-F21825. To prepare BMC Control samples, two separate kits are purchased to provide antigen. 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 -20°C. Use once, then discard.
Normal human	Sigma-Aldrich	P9523-	2-8°C, sterile	Prepare BMC

pooled plasma in 4% trisodium citrate		5ML		Controls
PAP Complex ELISA standard from 2 separate kits	LifeSpan BioSciences, Inc. Seattle, WA	LS-F21825	2-8°C, sterile	Prepare BMC Controls
Sample Diluent from separate kits	LifeSpan BioSciences, Inc. Seattle, WA	LS-F21825	2-8°C, sterile	Prepare BMC Controls
Coated 96-well Strip Plate	LifeSpan BioSciences, Inc. Seattle, WA	LS-F21825	2-8°C Supplied in ELISA kit	1 plate
Standard (Lyophilized)			2-8°C Supplied in ELISA kit	2 vials, lyophilized
Sample Diluent			2-8°C Supplied in ELISA kit	1 vial x 20 ml
Biotinylated Detection Antibody (100x)			2-8°C Supplied in ELISA kit	1 vial x 120 µl
Biotinylated Detection Antibody Diluent			2-8°C Supplied in ELISA kit	1 vial x 10 ml
HRP Conjugate (100x)			2-8°C Supplied in ELISA kit	1 vial x 120 µl
HRP Conjugate Diluent			2-8°C Supplied in ELISA kit	1 vial x 10 ml
Wash Buffer (25x)			2-8°C Supplied in ELISA kit	1 vial x 30 ml
TMB Substrate			2-8°C Supplied in ELISA kit	1 vial x 10 ml
Stop Solution			2-8°C Supplied in ELISA kit	1 vial x 10 ml

6.3. Reagent Comments

6.3.1. PAP is not available commercially. The PAP standard (antigen) from the additional ELISA kits are used to prepare the BMC Controls.

6.4. Consumables- See Table III

Table III. Consumables

Item	Range / Capacity	Quantity	Suggested Vendor / Catalog #
Pipet tips	100-1000 μL	1 box	
Pipet tips	20-200 μL	1 box	
Pipet tips	0.1-10 μL	1 box	
Volumetric pipette with dispenser or bulb	10ml	at least 2	
Polystyrene round bottom test tubes	12x75mm	about 20	
2.0-mL tubes, O-ring screw cap, conical bottom, sterile	2 mL		Sarstedt 72.692.005
0.5-mL tubes, O-ring screw cap, conical bottom, sterile	0.5 mL		
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			ThermoFisher 95128095

6.5. 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
37^{O}C Incubator, Dry				

6.6. Reagent storage and stability

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

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

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

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

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

6.6.2.4. 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. PAP is not available commercially. Additional kits are purchased and their stock standards are used as an antigen source for preparation of BMC Control aliquots. Sample diluent from the kit is used to reconstitute the antigen; all other components from the kits are discarded.

7.4.1.1. For this SOP, 2 additional kits are purchased to create the BMC control (total: 4 vials of standard)To each PAP ELISA kit standard stock vial, add 1 mL of sample diluent to re-constitute (stock 200ng/mL). Allow to sit for 10 minutes at room temperature with occasional gentle mixing to ensure complete resuspension. Pool the reconstituted standards into a 50mL conical tube. Label as “PAP Control.”

7.4.1.2. Reconstitute lyophilized human plasma in 5mL of deionized water. Allow to sit for 15 minutes at room temperature. Gently swirl to mix. Add 3.2 mL of plasma to PAP Control conical tube.

7.4.1.3. Add 24.8 mL of HBSS to PAP Control conical tube for a final volume of 32 mL (net 10% plasma). Gently swirl to mix.

7.4.1.4. Divide into 400 µl aliquots using 0.5 mL cryovials. This should make about 80 vials. The Control concentration of PAP will be approximately 25ng/mL of PAP higher than normal 1:10 diluted plasma.

7.4.1.5. For remainder of normal human pooled plasma, make 100 µL aliquots in screw cap tubes with O-ring. Label and put in -80°C to freeze rapidly.

7.4.1.6. Store frozen at -80°C. Document lot number of kit and standard used for preparation of the Control. 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

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

7.6.1. Reconstitute standard stock vials in 1 mL of sample diluent each (200 ng/mL). Allow to sit for 10 minutes at room temperature before pipetting for standards. Swirl to mix. Prepare standards according to Table V. These concentrations differ slightly from the manufacturer’s instruction. Standards concentrations were adjusted by BMC to improve the quality of the standard curve.

Table V. Preparation of Standards

Standard #	Concentration (ng/mL)	Volume Diluent (µL)	Volume PAP (µL)	Final concentration in assay (ng/mL)
1	200	-----	1000 of stock	200
2	150	210	630 of tube #1	150
3	100	250	500 of tube #2	100

4	50	400	400 of tube #3	50
5	25	400	400 of tube #4	25
6	12.5	400	400 of tube #5	12.5
7	6.25	400	400 of tube #6	6.25
8	0	400	----	0
	(Volume, µL)	(2250)		

7.7. Preparation of Unknowns (plasma samples)

7.7.1. Dilute plasma sample(s) 1:10 with HBSS/buffer/diluent

7.7.1.1. For each unknown sample, add 360 µL Diluent to a polystyrene tube. Add 40 µL plasma. Vortex briefly to mix.

7.8. Preparation of Wash Buffer

7.8.1. Dilute 30 mL of wash buffer concentrate in 720 mL of deionized water.

7.9. **Assay Procedure**

7.9.1. In each well of the 96 well plate, add 100µL of standard, control, or diluted plasma sample. Each is run in triplicate wells. Refer to Plate Map Design.

7.9.1.1. Seal plate with adhesive plate sealer.

7.9.1.2. Incubate plate at 37°C for 90 minutes. Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3A).

7.9.1.3. Aspirate the liquid from each well by flipping the plate, followed by tapping the plate on 5 layers of paper towels to remove residual buffer. Alternatively vacuum removal can be used. Do not wash and do not allow the plate to dry out.

7.9.2. Add Biotinylated Detection Antibody

7.9.2.1. Dilute 110 µL detection antibody in 10.89 mL detection antibody diluent (total volume = 11mL). Add to a disposable reagent reservoir.

7.9.2.2. Add 100 µL of diluted Biotinylated Detection Antibody to wells using a multichannel pipettor. Cover plate with adhesive tape to seal.

7.9.2.3. Incubate at 37°C for 1 hour. Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3B).

7.9.3. Wash

7.9.3.1. Aspirate the plate using a plate washer (for the BioTek Plate Washer, use the ELISA program). Immediately wash the plate 3 times with 350 µL Wash Buffer, aspirating the plate between each wash and being sure no residual liquid remains.

7.9.3.2. After the wash, tap the plate on paper towels to remove residual buffer. Proceed immediately to the next step; do not allow the plate to dry out.

7.9.3.3. For the BioTek Microplate Washer, the settings are:

METHOD	ELx405 Select	ELx405
Number of Cycles:	3 (First Wash) 5 (Second Wash)	3 (First Wash) 5 (Second Wash)
Soak/Shake:	Yes	Yes
Soak Time:	5 sec	5 sec
Dispense Volume:	300 µL/well	300 µL/well

7.9.4. Adding HRP Conjugates

7.9.4.1. Dilute 110 µL HRP conjugate in 10.89 mL conjugate diluent. Mix and add to reagent reservoir.

7.9.4.2. Add 100 µL of 1x HRP Conjugate to wells using a multichannel pipettor. Cover plate with adhesive tape to seal.

7.9.4.3. Incubate for 30 minutes at 37°C. Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3C).

7.9.5. Wash

7.9.5.1. Aspirate the plate using a plate washer (for the BioTek Plate Washer, use the ELISA program). Immediately wash the plate 5 times with 350 µL Wash Buffer, aspirating the plate between each wash and being sure no residual liquid remains.

7.9.5.2. After the wash, tap the plate on paper towels to remove residual buffer. Proceed immediately to the next step; do not allow the plate to dry out.

7.9.6. Adding TMB Substrate

7.9.6.1. Calculate the required amount needed before beginning the experiment. One 96-well plate needs 10 ml of the Substrate. No dilution is required. Use a sterile volumetric pipette to transfer the needed volume of TMB Substrate to a reagent reservoir.

7.9.6.2. Add 90 µL of TMB Substrate to each well using a multichannel pipettor. Cover the plate with a new plate sealer.

7.9.6.3. Incubate for 15 minutes at 37°C; start timer after addition of substrate to the last column. Protect the plate from light. Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3D).

7.9.7. Adding Stop Solution

7.9.7.1. Calculate the required amount needed before beginning the experiment. One 96-well plate needs 5 ml of the stop solution. No dilution is required. Transfer the needed volume of Stop Solution to a reagent reservoir.

7.9.7.2. Add 50 µL of Stop Solution to each well using a multichannel pipettor. The Stop Solution should be added to the wells in the same order and timing as the TMB Substrate solution.

7.9.7.3. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7.9.8. Determine Optical Density (O.D.)

7.9.8.1. Determine the optical density of the wells within 30 minutes using a microplate reader set to 450 nm.

7.9.8.2. 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.

7.10. 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 fluorescence of the “blank wells” from the fluorescence 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 comparing plates run on different days (CV<30%).

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. LSBio User Manual for Human Plasmin-antiplasmin 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	John Kim	
1.1	Draft	DSK, JK	3/7/2017
1.2	Draft	DSK, MPT	07/05/2017
1.3	Draft	DSK, MPT	07/26/2017
1.4	Draft; minor clarifications	DSK, MPT	08/02/2017
1.5	Draft; minor clarifications	DSK, MPT	08/16/2017
1.6	Draft; minor clarifications	DSK, MPT	08/22/2017
1.7	Minor Clarifications, typos, formatting	BET,DSK,ERD,MPT	8/1/2018

APPENDIX 1: PLATE MAP DESIGN: Patient samples from Module I and II may be assayed on the same plate (same design), but the pre-analytic variable grouping for each patient must be included on the same 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 Module I Plate Design (Time to Centrifuge): 37°C Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A		STDS		Blank (Diluent)								
B				S1T1			S7T1			S13T1		
C				S2T2			S8T2			S14T2		
D				S3T4			S9T4			S15T4		
E				S4T1			S10T1			BMC CTL	BMC CTL	BMC CTL
F				S5T2			S11T2					
G				S6T4			S12T4			Blank (Diluent)		
H				Blank (Diluent)								

A1.4 Module I Plate Design (Freeze-Thaw Cycles): 37°C Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A		STDS		Blank (Diluent)								
B				S1C1			S7C1			S13C1		
C				S2C2			S8C2			S14C2		
D				S3C3			S9C3			S15C3		
E				S4C1			S10C1			BMC CTL	BMC CTL	BMC CTL
F				S5C2			S11C2					
G				S6C3			S12C3			Blank (Diluent)		
H				Blank (Diluent)								

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
Pooled plasma	/ /		/ /
Coated 96-well plate	/ /		/ /
PAP Complex Standards for sample ELISA	/ /		/ /
Sample Diluent for sample ELISA	/ /		/ /
Biotinylated Detection Antibody (100X)	/ /		/ /
Biotinylated Detection Antibody Diluent	/ /		/ /
HRP Conjugate (100X)	/ /		/ /
HRP Conjugate Diluent	/ /		/ /
Wash Buffer (25X)	/ /		/ /
TMB Substrate	/ /		/ /
Stop Solution	/ /		/ /

PAP Complex Standards for BMC control	/ /		/ /
Sample Diluent for BMC control	/ /		/ /
Hank's Buffered Saline 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					

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

a. Add clinical samples, controls, and standards to the 96-well plate, cover plate, and incubate at 37°C for 90 minutes. Record below.

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

b. Add detection antibody to the 96-well plate, cover plate, and incubate at 37°C for 1 hour. Record below.

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

c. Add conjugate, cover plate and incubate at 37°C for 30 minutes. Record below.

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

d. Add substrate, cover plate and incubate at 37°C for 15 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
	Spectrofluorometer	Molecular Devices	Gemini XPS	XPS05453
	Refrigerator (2-8°C)			
	Freezer (-80°C)			

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 Plasmin-Antiplasmin ELISA.

↓

BARC PRO 021:

Thrombosis in Cancer Patients: Immunoassay of Plasmin-Antiplasmin in blood sample

- Perform ELISA with clinical samples, Plasmin-Antiplasmin standards, and Plasmin-Antiplasmin Controls
- Using Versa Max Microplate reader, determine relative signal of all samples