Boston Medical Center
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Department of Pathology and Laboratory Medicine

BARC PRO 023 BARC PRO 023-Blood Processing-Storage-Shipping SOP

Copy of version 6.0 (approved and current)

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Organization	Boston Medical Center

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Comments for version 6.0

Additional NCI storage aliquot designated for timepoint <1hr to reflect additional required plasma with new biomarkers; note about thawing samples at 37°C, according to assay SOP

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Administrative Director	3/20/2018	6.0	Chris Andry, PhD Chris Andry	
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Version	Status	Type	Date Added	Date Effective	Date Retired
6.0	Approved and Current	Major revision	3/16/2018	3/20/2018	Indefinite
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BOSTON MEDICAL CENTER		Thrombosis in Cancer Patients Blood Sample Processing, Storage and Shipping	
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1.0 **PURPOSE**

1.1. The purpose of this standard operating procedure (SOP) is to provide instructions to biospecimen source sites (BSS) for blood sample processing, storage, and shipping. This SOP is to be followed by all Leidos Biomed subcontracted BSSs for the Thrombosis in Cancer Patients (TCP) project. Blood will be collected for the preparation of blood derivatives from all study donors for downstream marker analyses.

2.0 **SCOPE**

2.1. This procedure encompasses all activities required to properly process blood at all BSSs for the Thrombosis in Cancer Patients (TCP) study. This procedure is to be followed by all personnel performing the collection and processing of blood biospecimens.

3.0 **RESPONSIBILITY**

3.1. Principal Investigator. It is the responsibility of the Principal Investigator (PI) at each BSS to ensure that the phlebotomy and blood-processing lab personnel have been trained in accordance with the relevant SOPs, that the training is documented, and that this procedure is followed.

3.2. Blood Processing Lab Personnel. It is the responsibility of the blood processing lab personnel to ensure he/she has read, understands, and follows the SOP when processing blood samples.

3.3. It is the responsibility of the project staff designated by the PI or 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

4.1.1. **Case ID Donor ("case") identification (ID)** The Case ID is an 8-character identification (e.g., TCP-XXXXX) which is obtained from the kits and assigned to the donor by the BSS at the time of specimen blood draw.

4.1.2. **Specimen ID** Identifies each blood biospecimen from a study subject and is used on all tubes, including blood collection tubes, clinical assay tubes, and cryovial aliquots.

4.1.3. **"*g*" force** A unit of inertial force on a body that is subjected to rapid acceleration or gravity, equal to 32 feet per second at sea level; $g = 1.12r\ (rpm/1000)^2$; g = "g" force, r = radius of rotor in mm, rpm = revolutions per minute.

4.2. Acronyms

4.2.1. **BMC** Boston Medical Center
4.2.2. **BRG-QM** Biospecimen Research Group-Quality Management
4.2.3. **BSS** Biospecimen Source Site
4.2.4. **CDR** Comprehensive Data Resource
4.2.5. **CRF** Case Report Form
4.2.6. **CRL** Coagulation Research Laboratory
4.2.7. **ELISA** Enzyme-linked Immunosorbent Assay
4.2.8. **ID** Identification
4.2.9. **LN2** Liquid Nitrogen
4.2.10. **PI** Principal Investigator
4.2.11. **PPE** Personal Protective Equipment
4.2.12. **SDS** Safety Data Sheet
4.2.13. **SOP** Standard Operating Procedure
4.2.14. **TCP** Thrombosis in Cancer Patients
4.2.15. **TPM** Technical Project Manager

5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Precautions (CDC-1987) shall be used for all phases of blood collection and 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 Required at Collection Site**

Item Number	Description
1	Centrifuge capable of 3100 x *g*
2	Micropipettes, P-1000, P-200
3	Blood tube rack (open/wire)
4	Cryovial racks (minimum of 2)
5	Serological pipette pump (manual or electronic)
6	-80°C ± 10°C freezer with storage boxes
7	4°C cooler or refrigerator
8	Vapor phase LN2 freezer

6.2. **Materials Required**

6.2.1. The BSS will be responsible for any additional materials/equipment to be utilized during a case collection that is not detailed below.

6.3. **Materials for processing**

Description	Quantity per Blood Draw	Vendor	Recommended Catalog Number
12x75mm test tubes for clinical assay aliquots	Minimum of 6	Fisher Scientific	14-959-5
12x75 test tube caps	Minimum of 6,	Fisher Scientfic	14-376-73
Sterile plastic, disposable Serological Pipettes, 5 ml	Minimum of 6	USA Scientific	1405-9710
1000 ul sterile filtered Pipette tips	Minimum of 15	USA Scientific	1126-7810
200 ul sterile filtered Pipette tips	Minimum of 15	USA Scientific	1120-8810
15 ml sterile conical tubes with caps	Minimum of 6	Cardinal Health	C3976-15A

6.4. **Materials needed by module per blood draw**

6.4.1 Bolded items required for this procedure.

Experimental Design	Components of Kit(s)/Overpacks(s)
Design 1: Module 1- Delay to Centrifuge	• **27 pre-labeled cryovials** • **6-12x75mm test tubes for clinical assays** • **Extra tubes of all types for use in case of defective tubes in kits** • **Extra labels for conical vials and other tubes as needed**
Design 2: Module 2&3- Freeze/Thaw & Delay to Testing	• **30 pre-labeled cryovials** • **6-12x75mm test tubes for clinical assays** • **Extra tubes of all types for use in case of defective tubes in kits** • **Extra labels for conical vials and other tubes as needed**
Design 3: All modules	• **56 pre-labeled cryovials** • **10-12x75mm test tubes for clinical assays** • **Extra tubes of all types for use in case of defective tubes in kits** • **Extra labels for conical vials and other tubes as needed**

Module 2: Freeze/Thaw	• **24 pre-labeled 1.2 mL cryovials** • **3-12x75mm test tubes for clinical assays** • **Extra tubes of all types for use in case of defective tubes in kits** • **Extra labels for conical vials and other tubes as needed**
Module 3: Delay to Testing	• 5 pre-labeled 1.2 mL cryovials • 3 12x75 mm test tubes for clinical assay • Extra tubes of all types for use in case of defective tubes in kits • Extra labels for conical vials or other tubes as needed

7.0 **PROCEDURE**

7.1. **Data Entry into the required CRFs in CDR database:**

7.1.1. **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** is required to be completed in the CDR within 96 hours of all biomarker testing.

7.1.2. **Biospecimen Labeling**

7.1.2.1. Each biospecimen should already be identified using a unique specimen ID. The complete specimen ID is composed of two elements - a Case ID (e.g., TCP-XXXXX) and a sequence number (e.g., ##) - that, together form the final alpha-numeric Specimen ID; e.g., TCP-XXXXX-##. Check that labeling is complete.

7.1.2.2. The TCP collection team members present at the blood collection are responsible for recording their initials and date on the blood tube or on the paper form and in each field in the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form**. This includes but is not limited to recording the date and time of blood draw, name of individual that performed the blood draw, a secondary person who observed the blood draw, and time and date of receipt in processing lab.

7.2. **Blood Collection**

7.2.1. **Sample Collection Tubes**

7.2.1.1. Each blood draw will collect the blood volume needed as outlined in the experimental design 1, 2, or 3 in the Research Plan (PM-0022). Blood draw priority will be assigned as per the Research Plan (PM-0022). Experimental module conditions, collection tube type, aliquot size, and number of resulting aliquots to be used for this study are shown below in Table 1.

Table 1: Summary of Experimental Module conditions, blood collection volume, and aliquot amounts.

Priority	Experimental Modules	Control Conditions	Experimental Conditions	Minimum Blood Draw Amounts
1	All Modules	• 3.2% sodium citrate tube ≤ 1+10min hour to centrifuge (Delay to testing is defined as the time elapsed between completion of the plasma preparation (plasma pooled) to the beginning of the assay (plasma pipetted for the assay) • 1 F/T cycle	• 2 hour delay ± 10 minutes • 4 hour delay ± 10 minutes • 2 F/T • 3F/T • 24 hour ±1hr delay to testing • 72 hour±1hr delay to testing	• 9-4.5mL 3.2% sodium citrate vacutainers • 1-3ml EDTA vacutainer
2	Delay to Centrifuge	3.2% sodium citrate tube ≤ 1+10min hour to centrifuge (4.5mL tube)	• 2 hour delay ± 10 minutes • 4 hour delay ± 10 minutes	• 6-4.5mL 3.2% sodium citrate vacutainers • 1-3ml EDTA vacutainer
3	Freeze/Thaw	• 3.2% sodium citrate tube ≤ 1 hour+10min to centrifuge (4.5mL tube)	• 2 F/T • 3F/T	• 4-4.5mL 3.2% sodium citrate vacutainers • 1-3ml EDTA vacutainer
-N/A	Delay to Testing	3.2% sodium citrate tube ≤ 1hr+10min delay to testing (Delay to testing is defined as the time elapsed between completion of the plasma preparation (plasma pooled) to the beginning of the assay (plasma pipetted for the assay))	• 24 hour ±1hr delay to testing • 72 hour ±1hr delay to testing	• 5-4.5mL 3.2% sodium citrate vacutainers • 1-3ml EDTA vacutainer

7.2.2. **Minimum blood collection requirements**:

7.2.2.1. The minimum volume of blood to be collected will be determined by the nature of the experimental module, in order to completely fulfill the module's blood volume requirements.

7.2.2.2. If the minimum number of aliquots are not collected for an experimental module, please contact your TPM immediately for approval to continue.

7.3. **Blood Processing**

7.3.1. **Preparation of Blood Processing Supplies and Cryovials**

7.3.1.1. Prior to blood processing, prepare necessary supplies. Preparation of processing supplies includes:

7.3.1.1.1. Place the labels pre-printed on the sterile conical centrifuge tubes for the module conditions being collected. Extra collection tube labels and conical tube labels will be included in each kit – and extra unlabeled collection tubes (stored in bulk at the site)

7.3.1.1.2. Place all pre-labeled cryovials needed for a collection module onto a tube rack. Extra cryovial labels will be included in each kit – and extra unlabeled cryovials (stored in bulk at the site).

7.3.1.1.3. Have the CDR database and/or paper CRF (**Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form**) ready for data entry.

7.3.2. **Plasma Preparation (One Centrifugation Step)**

NOTE: All modules will use the same centrifuge process regardless of pre-analytical factors to be studied.

7.3.2.1. Visually assess whether clotting has occurred in any blood collection tubes. The presence of clotting should be noted on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database.

7.3.2.2. Enter the time that processing began on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or paper CRF.

7.3.2.3. Centrifuge the blood collection tubes at 3,100x*g* for 10 min at room temperature (20°C to 25°C or 68°F to 77°F) with centrifuge brake setting "low".

7.3.2.3.1. Module 1 – Delay to Centrifuge: Delay to centrifuge is defined as the time when the last blood collection tube inverted to the time when the first centrifugation began.

7.3.2.3.1.1. Control Samples: to be centrifuged ≤1 hour from blood draw.

7.3.2.3.1.2. Experimental samples: to be centrifuged at 2 hours (±10min) and 4 hours (±10min) post-blood draw and kept on the bench at room temperature until centrifugation.

7.3.2.3.2. Modules 2 & 3 – Freeze/Thaw Cycles and Delay to Testing

7.3.2.3.2.1. All blood samples are to be processed ≤1 hour from blood draw and the aliquots are to be placed in the experimental conditions.

7.3.2.4. EDTA tube for CBC analysis will be brought up to BMC clinical laboratory with the first d-dimer and FVIII aliquots. (if sampled for research only; otherwise CBC data will be from medical record).

7.3.2.5. After centrifugation, visually assess whether gross hemolysis is present in the plasma sample. Gross hemolysis will be defined as bright pink or red plasma. The presence of gross hemolysis should be noted on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database.

7.3.2.6. After centrifugation, carefully transfer the resulting plasma supernatant to one labeled, sterile 15mL polypropylene conical tube using a P-1000 pipette set at 500 uL, being sure to not disrupt any material at the interphase of the blood tube. Plasma from multiple blood collection tubes for one time point are to be pooled in one conical tube. Do not pool samples if hemolysis is present. It is acceptable to leave some plasma in the original vacutainer tube (approximately 200-400 µl volume of leftover plasma) so that transferred plasma is not contaminated with any cellular debris. Document the volume of plasma transferred into the new conical tube on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in CDR database or corresponding paper form. Use this pooled plasma for aliquots.

7.3.2.7. If the cell layer was disturbed during transfer of plasma, collect all the plasma in the sterile 15 ml conical tube, cap the tube and centrifuge again at 3,100xg for 10 minutes at room temperature (20°C to 25°C or 68°F to 77°F) with centrifuge brake setting "low". Repeat the plasma transfer to a new sterile, labeled 15 mL conical tube and use this for aliquots. Document the volume of plasma transferred to a conical tube on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in CDR database or corresponding paper form.

7.3.3. **Plasma Aliquots**

7.3.3.1. Using a P-200 micropipette, aliquot citrated plasma from 15 ml conical tubes into pre-labeled cryovials for biomarker analyses as stated in Table 2.

Table 2: Number of aliquots needed by volume, centrifuge time, and priority

	Priority I: All Modules			Priority II	Priority III
	Mod I	Mod II	Mod III	Mod I	Mod II

Centrifuge time	<1 hour	2 hour	4 hour	<1 hour	<1 hour	<1 hour	2 hour	4 hour	<1 hour
50uL	1	5	5	15	2	5	5	5	15
100uL	0	1	1	3	0	1	1	1	3
200uL	0	1	1	3	0	1	1	1	3
1ml BMC	1	2	2	3	2	2	2	2	3
250uL NCI	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
500uL NCI	**3**	**4**	**4**	**0**	**0**	**4**	**4**	**4**	**4**

7.3.3.2. Priority 1-All modules

7.3.3.2.1. Aliquots of 50uL, 100uL, and 200uL will be put into 0.5mL cryovials

7.3.3.2.2. Aliquots for BMC clinical labs will be put into the 12x75mm test tubes.

7.3.3.2.3. Aliquots for NCI will be in 2mL cryovials.

7.3.3.3. Any remaining plasma from any time points will be aliquoted first in 5-100uL aliquots and beyond that in 500ul aliquots for back up to any assays as needed or for long term storage to the NCI.

7.3.3.4. Do not rely on markings on the cryovial; please aliquot according to the amount set on the manual pipettes.

7.3.3.5. Document the volume of plasma transferred into each aliquot on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or paper form. Place caps on the 1.2mL cryogenic vials containing plasma. Input specimen IDs of all cryogenic vials into the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in CDR database or document on the paper form. All plasma aliquots collected shall be used for experimental purposes.

7.3.3.6. Each 15mL conical tube should be discarded according to institutional policies.

7.3.3.7. Record the time that plasma processing was completed on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or paper form, and document/comment if there were any issues in the processing of a module.

7.3.4. **Storage**

Table 3A: Priority I-All Modules 9 citrate tubes collected									
Centrifuge time	**<1 hour**			**2 hour**			**4 hour**		
Storage	**RT**	**-80°C**	**4°C**	**RT**	**-80°C**	**4°C**	**RT**	**-80°C**	**4°C**
50uL	1	15	2	1	4	0	1	4	0
100uL	0	3	0	0	1	0	0	1	0
200uL	0	3	0	0	1	0	0	1	0

1ml BMC	1	3	2	1	1	0	1	1	0
250uL NCI	0	2	0	0	0	0	0	0	0
500uL NCI	0	3	0	0	4	0	0	4	0

	Table 3B: Priority II-Module I Delay to Centrifuge 6-8 citrate tubes collected								
Centrifuge time	**<1 hour**			**2 hour**			**4 hour**		
Storage	**RT**	**-80°C**	**4°C**	**RT**	**-80°C**	**4°C**	**RT**	**-80°C**	**4°C**
50uL	1	4	0	1	4	0	1	4	0
100uL	0	1	0	0	1	0	0	1	0
200uL	0	1	0	0	1	0	0	1	0
1ml BMC	1	1	2	1	1	0	1	1	0
250uL NCI	0	2	0	0	0	0	0	0	0
500uL NCI	0	3	0	0	4	0	0	4	0

Table 3C: Priority III-Module II Freeze/Thaw 4-5 citrate tubes collected			
Storage	**RT**	**-80°C**	**4°C**
50uL	1	15	0
100uL	0	3	0
200uL	0	3	0
1ml BMC	0	3	0
250uL NCI	0	0	0
500uL NCI	0	5	0

7.3.4.1. D-dimer and FVIII assay samples will be transported immediately after each time point to the BMC clinical laboratory. Record the time that plasma was given to the BMC clinical lab as well record the time when the d-dimer and FVIII assay was done in the BMC clinical lab on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or paper form.

7.3.4.2. **All Modules**

7.3.4.2.1. 5 citrate tubes will be processed ≤1 hour.

7.3.4.2.2. 2 citrate tubes will be processed at 2 hours and at 4 hours

7.3.4.2.3. At each time point, plasma will be pooled and then stored as in Table 3A

7.3.4.2.4. 50uL aliquots

7.3.4.2.4.1. Room temperature (RT) aliquot will be used immediately for DNA assay

7.3.4.2.4.2. -80°C storage aliquots are for DNA, sP, MPO, F1.2, PAP ELISAs for Modules 1 and 2. DNA frozen aliquots will only be frozen at the ≤1 hour time point.

7.3.4.2.4.3. 4°C aliquots will be used for DNA for delay to testing time points

7.3.4.2.5. 100uL stored at -80°C at all time points to be used for Nucleosome assay for Module 1 and Module 2.

7.3.4.2.6. 200uL stored at -80°C at all time points to be used for PAI-1 assay for Module 1 and Module 2.

7.3.4.2.7. 250uL and 500uL aliquots for storage will be frozen at the time points for Module 1.

7.3.4.2.8. Clinical aliquots

7.3.4.2.8.1. RT will be for d-dimer at all time points for Module 1

7.3.4.2.8.2. -80°C will be for FVIII for all time points for Module 1 and 2 more aliquots at ≤1 hour to be used for Module 2 cycles.

7.3.4.2.8.3. 4°C aliquots will be used for d-dimer for the Module 3 time points

7.3.4.2.9. Delay to testing will be stored at 4°C in the CRL until time point for DNA analysis and D-dimer assay. The DNA analysis assay and d-dimer assay will be done at following timepoints: 24hr±1hr, and 72hr±1hr for both biomarkers (plasma DNA and D-dimer). Delay to testing is defined as the time elapsed between completion of the plasma preparation (plasma pooled) to the beginning of the assay (plasma pipetted for the assay)

7.3.4.2.9.1.

7.3.4.3. **Module 1: Delay to centrifuge**

7.3.4.3.1. Citrate tubes will be split evenly between the 3 time points and processed at ≤1 hour, 2 hour and 4 hour (± 10 minutes).

7.3.4.3.2. At each time point plasma will pooled and stored according to Table 3B.

7.3.4.3.3. 50uL aliquots

7.3.4.3.3.1. Room temperature (RT) aliquot will be used immediately for DNA assay at each time point

7.3.4.3.3.2. -80°C storage aliquots are for sP, MPO, F1.2, PAP ELISAs at each time point

7.3.4.3.4. 100uL stored at -80°C at all time points to be used for Nucleosome assay.

7.3.4.3.5. 200uL stored at -80°C at all time points to be used for PAI-1 assay.

7.3.4.3.6. 1mL aliquots for storage will be frozen at each time point.

7.3.4.3.7. Clinical aliquots

7.3.4.3.7.1. RT will be for d-dimer at all time points.

7.3.4.3.7.2. -80°C will be for FVIII for all time points.

7.3.4.4. **Module 2: Freeze/Thaw Cycles**

7.3.4.4.1. All samples will be processed ≤1 hour from blood draw

7.3.4.4.2. Plasma will be pooled and then stored as in Table 3C

7.3.4.4.3. 50uL aliquots

7.3.4.4.3.1. Room temperature (RT) aliquot will be used immediately for DNA assay

7.3.4.4.3.2. -80°C storage aliquots are for DNA, sP, MPO, F1.2, PAP ELISAs.

7.3.4.4.4. 100uL stored at -80°C to be used for Nucleosome assay.

7.3.4.4.5. 200uL stored at -80°C to be used for PAI-1 assay.

7.3.4.4.6. 1mL aliquots for storage will be frozen.

7.3.4.4.7. Clinical aliquots will be stored at -80°C FVIII

7.3.4.5. Freeze/Thaw samples will be directly placed in -80°C freezer and left for a minimum of 6 hours.

7.3.4.5.1. 1-F/T cycle samples will remain until testing.

7.3.4.5.2. 2-F/T cycles and 3-F/T cycles will be placed in a 37°C water bath for up to 3min for F-VIII assay as per CLIA protocol; as quick as 1min for all other biomarkers.. Then place back in -80°C.

7.3.4.5.3. Repeat previous step one more time with 3-F/T cycle samples.

7.3.4.5.4. ELISAs samples will remain frozen until ready for batch testing, approximately seven subjects per batch that have fulfilled all time points for the modules. DNA samples can be run after the last time point has been reach for every subject.

7.3.4.5.5. FVIII samples will be transported to the BMC clinical lab after completion of final freeze/thaw cycle and stored at -80°C until batch assay. Record the time that plasma was transferred to the BMC clinical lab, as well record the time when Factor VII assay was done in the BMC clinical lab on the TCP Blood Collection and Processing Form, in the CDR database or paper form.

7.3.5. Thawing samples for appropriate biomarker assay

7.3.5.1. Samples to be assayed under instructions of a specific biomarker SOP will be thawed according to the biomarker assays instructions, not globally throughout the project. i.e. if BARC PRO 017 specifies plasma samples be placed in 37°C for a minimum of 1 minute, those samples will be subject to those standards, rather than a blanket statement for all assays.

7.3.6. When biomarker determination of a module sample is completed and no further sample is required, all remaining samples will be stored in the vapor phase of LN_2 for long-term storage before the final shipment.

7.3.7. **Whole Cell Pellet Preparation**

7.3.7.1. After removing the plasma from the original tube of blood collected, there should be volume left over (of plasma, buffy coat, and erythrocytes), that will be mixed and referred to as the whole cell pellet.

7.3.7.2. . Use a 5 mL sterile serological pipet with manual or automatic pump to gently mix the sample by aspirate/expel (2-3 times) any remaining plasma, buffy coat and erythrocytes, and transfer the whole cell pellet from each collection tube at every time point to a new conical tube.

7.3.7.3. Record the volume of the pooled whole cell pellet remaining (approximated using volume reference tube) in the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or on the corresponding paper form.

7.3.7.4. Aliquot the resulting mixture of whole cell pellet into sterile, pre-labeled cryovials. Use a P-1000 manual pipet with attached sterile filtered disposable 1000µL pipet tip to transfer 1000µL aliquots from a collection tube to 2 pre-labeled 1.2mL cryovials designated for whole cell pellet collection based on Table 1. Place caps on the 1.2mL cryogenic vials containing the whole cell pellet. Record the sample IDs of all cryogenic vials into the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or document on the paper form. Document the volume of whole cell pellet transferred into each aliquot on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form** in the CDR database or paper form.

7.3.7.5. Freeze the whole cell pellet aliquots directly in vapor phase of liquid nitrogen and transfer to the liquid nitrogen freezer. Record the time that the whole cell pellet aliquots were placed into the freezer on the **Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form**in CDR database or paper form, and document/comment if there were any issues in the preparation or storage of the whole cell pellet.

7.3.8. **Shipment**

7.3.8.1. Plasma should be stored at the appropriate storage temperature until assay and then in the vapor phase of LN2 for long-term storage and shipment.

7.3.8.2. Timing of Blood Shipping

7.3.8.2.1. Plasma and whole cell pellet aliquots should have data entry completed and shipped when approved by the TPM.

7.3.8.3. Pack and ship all blood biospecimens to the designated facility, or as directed by the TPM, according to institutional shipping standards.

8.0 **REFERENCES**

8.1. BD Instructions for Use-Preparing a Quality Sample (http://www.bd.com/Vacutainer® /products)

8.2. Common Blood Collection and Plasma Processing Protocol - Clinical Proteomics Technologies Assessment for Cancer (CPTAC) Biospecimen Working Group (2008)

8.3. http://www.preanalytix.com/~/media/PreAnalytiX/Files/Resources%20Blood%20DNA/Blood_DNA_Tube_ProdCir.ashx

8.4. QM-0006 Deviations

9.0 **ATTACHMENTS**

9.1. Thrombosis in Cancer Patients Blood Collection, Handling and Processing Form

9.2. Process Flow: Processing and Aliquoting Schema

Initiation/Revision History			
Rev #	Description of Change	Author	Effective Date
1.0	Draft	Chris Andry	
2.0	Draft	Liz Duffy, Debbie Stearns-Kurosawa	1/11/2017
2.1	Removal of CBR references and updating number of tubes needed	Liz Duffy	1/17/2017
3.0	BMC response to LBR edits	Liz Duffy	2/23/17
4.0	Additions on how to gather more data from DNA aliquots	Liz Duffy	3/29/2017
5.0	Updates from approved changes request regarding aliquot scheme, freeze-thaw cycle timing. Additional changes to freezing protocol for whole cell aliquots to conform to actual practice.	Liz Duffy	10/10/2017
6.0	Additional NCI storage aliquot designated for timepoint <1hr to reflect additional required plasma with new biomarkers; note about thawing samples at 37°C, according to assay SOP	Morgan Thompson	03/07/2018