Manual of Procedures

BioBOOST

Sample Collection, Processing, and Storage

26 July 2022

- **You are receiving this manual of procedures because your site has agreed to participate in this BioBOOST study as an add-on to the BOOST III protocol.**

- **DO NOT BEGIN ENROLLING SUBJECTS In BioBOOST UNTIL YOUR INSTITUTION HAS RECEIVED THE NECESSARY IRB and HRPO APPROVAL.**

- **Only process, store, and ship buffy coat samples on subjects that have consented to possible future genetic testing.**

- **Biospecimen Sample Collection Schedule:**

Schedule of Sample Collection (from time of TBI)	*Blood Collection*	*CSF Collection*
Day 1, Enrollment	✓	✓
Day 1, 16 hours post injury	✓	✓
Day 1, 24 hours post injury	✓	✓
Day 2 AM	✓	✓
Day 2 PM	✓	✓
Day 3 AM	✓	✓
Day 3 PM	✓	✓
Day 4 AM	✓	✓
Day 4 PM	✓	✓
Day 5 AM	✓	✓
Day 5 PM	✓	✓
Post-cerebral hypoxic / hypoperfusion event	✓	✓
Day 7	✓	
2 weeks	✓	
6 months	✓	

****Note: Day 1 – 5. Morning(AM) and PM draws should be 12 (window: 8 - 16) hours apart.**

If a research blood sample is scheduled to be drawn within 2 hours or less of the last research blood sample draw, that research blood draw should be skipped (for example if the 24 hours post-injury draw is obtained at 7am and the Day 2 AM draw is scheduled for 8am, the Day 2 AM draw can be skipped).

BioBOOST Biospecimen Collection Summary

Tube Type	Sample Type	# of Cryovials/Caps Supplied in Kit	Processing/ Aliquoting	Specimens to NTBI-BR
6 mL Purple-Top K_3EDTA tube	**Whole blood for isolation of plasma**	6 cryovials + 6 purple screw tops	0.5 ml plasma aliquots per 1.5 ml cryovial	6
	Whole blood for isolation of buffy coat (for DNA* extraction)	1 cryovials + 1 clear screw top	0.5 ml buffy coat aliquots per 1.5 ml cryovial	1* (baseline visit only)
6 mL Red-Top SCA tube	**Whole blood for isolation of serum**	6 cryovials + 6 orange screw tops	0.5 ml serum aliquots per 1.5 ml cryovial	6
2.5 mL PAXgene tube	**Whole blood for isolation of RNA**	N/A	N/A	1* (whole tube)
Blood TOTAL	**14.5 mL**	13 cryovials with color-coded caps (6 purple, 6 orange, 1 clear)		13 cryovials + 1 PAXgene tube from baseline visit; 12 cryovials +1 PAXgene tube from follow-up visits

Tube Type	Sample Type	# of Cryovials/Caps Supplied in Kit	Processing/ Aliquoting	Specimens to NTBI-BR
15 mL polypropylene conical tube	**Whole CSF**	5 cryovials + 5 clear screw tops	0.5 ml CSF aliquots per 1.5 ml cryovial	5

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Ventilator respiratory rate

1.0 Abbreviations

EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
MTA	Material Transfer Agreement
NTBI-BR	National TBI Biospecimens Repository
PPE	Personal Protective Equipment
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
TBI	Traumatic Brain Injury

2.0 Purpose

The purpose of this Manual is to provide BioBOOST staff (PIs, study coordinators, lab personnel and phlebotomists) at the enrolling sites with instructions for collection of whole blood, fractionation of blood from vacutainer tubes, aliquoting, labeling, freezer storage and shipping frozen samples to the NTBI-BR located in Pittsburgh. The following samples will be collected:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)
- PAX-Gene (for RNA extraction)
- Cerebrospinal Fluid (CSF)

Note: PAXgene tubes will be frozen at the collection site without processing.

3.0 NTBI-BR Information

3.1 Laboratory Contacts

Neurotrauma Clinical Trials Center
University of Pittsburgh
Department of Neurological Surgery
200 Lothrop Street, Suite B-400
Pittsburgh, PA 15213

NTBI-BR Director: Ava Puccio, RN, PhD
Office: 412-648-9246
Mobile Phone: 412-298-7033
Email: puccioam@upmc.edu

NTBI-BR Administrator: Miri Rabinowitz, PhD
Office: 412-648-2031
Mobile Phone: 412-491-6199
Email: rabinowitzmk@upmc.edu

NTBI-BR Manager: Mike Mancinelli
Office: 412-648-2389
Mobile Phone: 484-885-8211
Email: mancinellimd@upmc.edu

3.2 Hours of Operation

The Laboratory operates from 9 AM to 4 PM EST, Monday through Friday.

Frozen samples must be shipped Monday – Wednesday only.

Frequency of shipments will depend on enrollment rate at each study site. Shipping volume may vary by site enrollment. Shipments should contain no less than four (4) cryoboxes, and no more than sixteen (16) cryoboxes. For common scenarios with packing and shipment, refer to Section 8 of this MOP.

Frozen samples should be shipped at least quarterly or as determined by the Study Monitor. Please ensure adequate storage at -80°C prior to shipment. Contact the NTBI-BR Manager prior to arranging any shipments.

Check the weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples.

3.3 lHoliday Schedules

Please be sure to verify with your courier's schedule prior to shipping close to a holiday.

Holiday Observations* – United States

Date	Holiday
New Year's Day	January 1
Martin Luther King Day	3rd Monday in January
President's Day	3rd Monday in February
Memorial Day	4th Monday in May
Juneteenth	June 19
Independence Day	July 4
Labor Day	First Monday in September
Columbus Day	2nd Monday in October
Veteran's Day	2nd Monday in November
Thanksgiving	4th Thursday of November
Day after Thanksgiving	4th Friday of November
Christmas Day	December 25

***Additionally, each year the University of Pittsburgh is officially closed from December 24 through January 2. Do not schedule any shipments during this time.**

4.0 Supplies and Equipment

4.1 Supplies and Equipment Provided by Enrolling Site

- The following items and equipment are to be **supplied by the local institution**:
  - Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
  - Tourniquet
  - Alcohol Prep Pad
  - Gauze Pad
  - Bandage
  - Butterfly needles
  - Microcentrifuge tube rack
  - Cryogenic Gloves
  - Sharps bin and lid
  - Biohazard disposal

- In order to process samples consistently, BioBOOST sites must have access to the following equipment:
  - **Centrifuge capable of ≥ 1500 rcf (1500 x g)**
  - **-80°C Freezer**

4.2 Specimen Collection Kit Contents

- The NTBI-BR will provide research specimen collection kits. The kits will include the vacutainer tubes needed for whole blood collection and cryovials for plasma/serum/buffy coat aliquots. Labels pre-printed with study information specific to the type of sample being drawn will also be provided, as will 81-slot cryoboxes for freezer storage.

- Collection kits contain the following supplies to collect samples from a given subject-timepoint. **Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from NTBI-BR Manager to do so.**

Kit Supplies

Blood Draw Kit Components

Quantity	Baseline Kit Components
1	PAXgene™ blood collection tube
1	EDTA (purple top) blood collection tube
1	Serum determination tube (red top)
13	1.5 mL Polypropylene cryovial tubes
6	Purple caps (for plasma)
6	Orange caps (for serum)
1	Clear cap (for buffy coat)
3	Disposable graduated transfer pipettes

CSF Draw Kit Components

Quantity	Baseline Kit Components
5	1.5 mL Polypropylene micro-cryovial tubes
5	Clear caps
1	15 mL conical polypropylene tube
1	Disposable graduated transfer pipettes

* The baseline visit includes aliquoting the buffy coat in cryovials using the clear caps; the buffy coat is NOT collected in visits after the baseline visit unless the sample was compromised or omitted. Then, a replacement can be obtained from the next obtained plasma sample.

4.3 Initial Supply to Study Sites

Each site will initially be supplied with 30 blood draw kits, 30 CSF collection kits, 10 cryoboxes, and sheets of pre-printed labels for 4 subjects (a total of 14 sheets of labels).

4.4 Resupply to Study Sites

Each site will be responsible for maintaining and requesting adequate inventory after the initial supply has been received. Regularly check your supplies (*including Vacutainer expiration dates*) and order additional kits and sheets of pre-printed labels before you run out, so you are prepared for both scheduled and unanticipated visits.

Email the NTBI-BR Manager to request a resupply of kits. Allow **14 days** for kit orders to be processed and delivered.

*Take note that Vacutainer expiration dates on the tubes are in the format: **YYYY-MM-DD**

See the “Bio-BOOST Site Lab Kit Receiving and Shipping” instructions within Toolbox > Project Documents in WebDCU for specific procedures for lab kit receiving, removing, labeling, specimen destruction, and shipping within the WebDCU database. Instructions for specimen shipping and specimen destruction in sections 8 and 11 must be followed prior to removing the specimen/shipping in WebDCU.

5.0 Blood Collection and Processing Procedures

Important Note

In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies, forms, and equipment out and prepared prior to drawing blood. Draw blood in the order of the most essential, i.e. first collect the purple top tube for plasma, then the red top tube for serum, and finally the PAXgene™ tube for RNA.

****This blood draw order differs from standard clinical practice.****

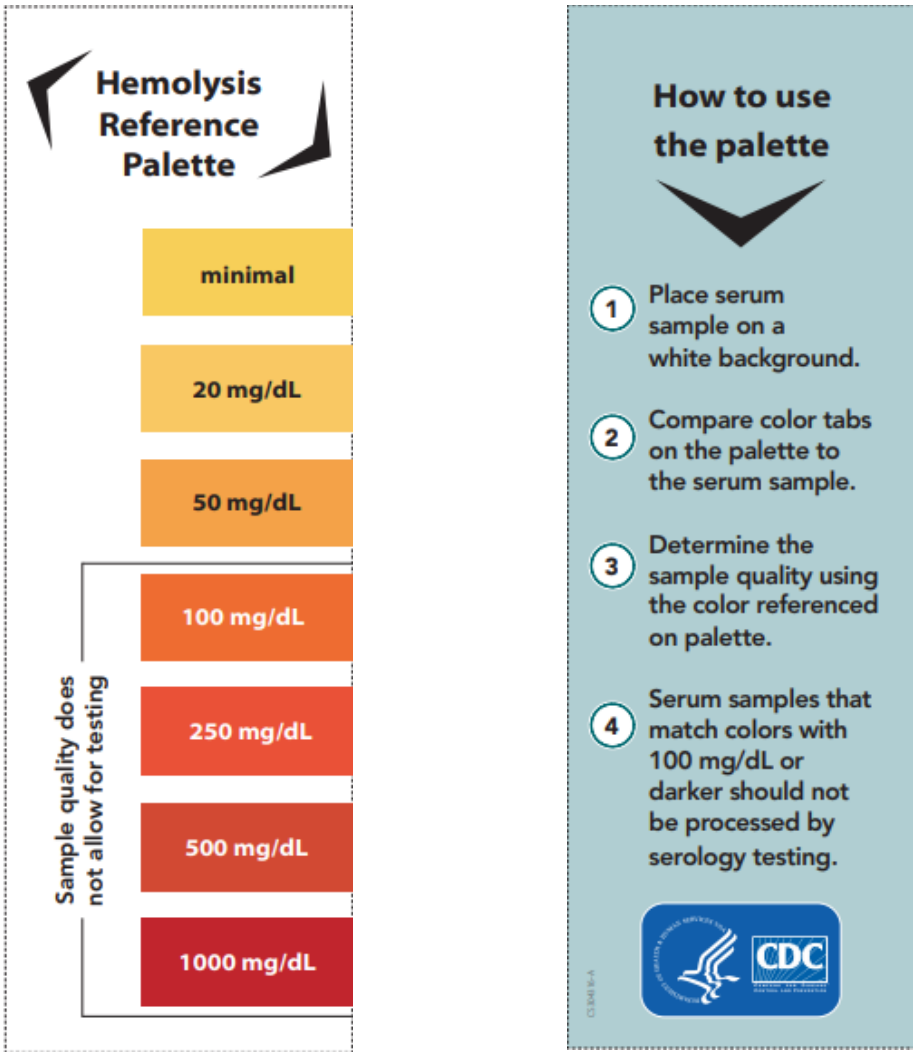
5.1 Sample Collection and Quality

Care must be taken during collection of study samples to prevent hemolysis and/or contamination.

The following techniques shall be used to prevent possible backflow and hemolysis:

- Place the donor's arm in a downward position.
- Avoid drawing blood from a hematoma
- If drawing from an existing IV using a syringe, avoid drawing the plunger back too forcefully.
- Avoid using very small needles.
- Make sure the venipuncture site is dry.
- Avoid a probing, traumatic venipuncture
- Hold the tube in a vertical position, below the donor’s arm during blood collection.
- Avoid prolonged tourniquet application or fist clenching
- Release tourniquet when the final tube is nearly filled.
- Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
- Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.
- Vacutainer tubes are designed to draw the correct volume of blood into the tube to mix with any additive.

Level of hemolysis must be assessed for plasma and serum specimens. The CDC Hemolysis Reference Palette (reproduced below) will be provided to every site to evaluate if the hemolysis is significant. After specimens have been labeled, aliquoted, and capped, one cryovial of each plasma and serum should be measured against the provided laminated palette. Specimens should be recorded as either “100 mg/dL or more” or “Less than 100 mg/dL” on the applicable CRF.



5.2 Labeling Specimens

Each kit is supplied with labels for the specimens shipped to the NTBI-BR (18 for enrollment sample collection and 17 for all subsequent sample collections, as DNA is collected only on the initial visit).

There are 15 potential timepoints for sample collection (11 scheduled during initial hospitalization, 1 unscheduled collection during the first hypoxic/hypoperfusion event, and 3 follow-up timepoints). The "Day 1" timepoints are determined by subject time of injury. The unscheduled timepoint "X" is only collected during the first hypoxic or hypoperfusion event. No more than 1 "X" timepoint should be collected per subject.

Each cryovial will be labeled with an alphanumeric string. For example: **BT-1502-001-A-P01** is the 1st plasma cryovial (P01), from the enrollment blood draw (A) on the first subject at BOOST III site 1502.

Each kit should be labeled upon subject enrollment with the Lab Kit ID sticker, included on provided label sheets prior to the related timepoint specimen labels. The Lab Kit ID is composed of the Subject ID and timepoint letter code.

The code used in the labeling is as follows:

Study	Site ID	Bio-BOOST Subject ID	Blood Draw Timepoints: A thru N, X	Specimen Type and Vial
BT	1502	001 to 999	**A** – Day 1, Enrollment	Serum: S01 to S06
			B – Day 1, +16 hours post-injury	Plasma: P01 to P06
			C – Day 1, +24 hours post-injury	Buffy Coat: D01*
			D – Day 2 AM	PAXgene: R01**
			E – Day 2 PM	CSF: C01 to C05
			F – Day 3 AM	
			G – Day 3 PM	
			H – Day 4 AM	
			I – Day 4 PM	
			J – Day 5 AM	
			K – Day 5 PM	
			L – Day 7	
			,	
			N – 6 Month	
			X - Hypoxic/Hypoperfusion Event	

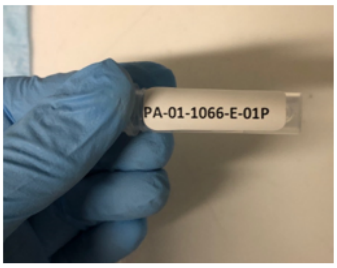
Lab ID + Blood Draw = Lab Kit ID

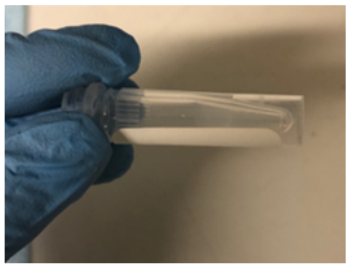
***D for DNA; DNA will only be collected at the baseline blood draw. Only samples with an "A" designation will have labels D01. ONLY DRAW IF CONSENT FOR GENETIC TESTING WAS OBTAINED.**

****R for RNA; ONLY DRAW IF CONSENT FOR GENETIC TESTING WAS OBTAINED.**

Note: There are 6 cryovials for serum, 6 cryovials for plasma, 1 cryovial for buffy coat, and 5 cryovials for CSF. The provided cryovial caps are color-coded. Use the **Purple caps** for plasma; **Orange caps** for serum; **Clear cap** for buffy coat; **Clear caps** for CSF.
In order to ensure the pre-printed label adheres properly to the cryovial, follow these instructions:

- Place labels on **ALL** cryovials **BEFORE** any sample processing/freezing. This should help to ensure the label properly adheres to the cryovial before exposure to moisture or different temperatures.





- Place label **vertically** on the cryovial.
- Take a moment to ensure the label is **completely adherent** to each cryovial. It may help to roll the cryovial between your fingers after applying the label.

- Be sure to only place **plasma** in
- labeled with the suffix **P01 to P06**
    - Depending on the amount of blood drawn you may fill less than 6 cryovials:
        - Use the lowest numbered labels, i.e. P01, P02 and P03 rather than P03, P04 and P05 if only 3 cryovials are used.
    - Cap with the **PURPLE** caps.

- Be sure to only place **buffy coat** in 1.5 mL cryovial labeled with the suffix **D01**

- Be sure to only place **serum** in cryovials labeled with the suffix **S01 to S06**
    - As before, you may fill less than 6 cryovials with serum - use the lowest numbered labels first.
    - Cap with the **ORANGE** caps.

- Be sure to only place **CSF** in cryovials labeled with the suffix **C01 to C05**
    - As before, you may fill less than 5 cryovials with CSF - use the lowest numbered labels first.
    - Cap with the **CLEAR** caps.

- Finally, label the **PAXgene** tube with the **R01** suffix label.

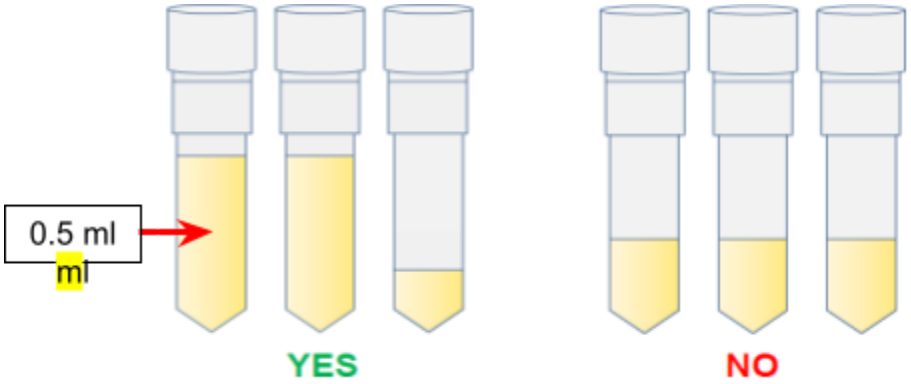
- In summary, only place **serum** in "**S**" labeled cryovials, **plasma** in "**P**" labeled cryovials, **buffy coat** in "**D**" labeled cryovials, and **CSF** in "**C**" labeled cryovials.

- **NO PHI OR IDENTIFIABLE INFORMATION SHOULD BE PLACED ON ANY SPECIMEN**

5.3 Filling Aliquot Cryovials (Plasma and Serum)

A micropipette with disposable tips should be used to aliquot plasma, serum, and CSF. Each kit is provided with 1 disposable transfer pipettes for transferring the buffy coat to its appropriately labeled cryovial. Cryovials should be filled with 0.5 milliliter of the respective biologic material. Over-filled cryovials may burst once placed in the freezer, resulting in a loss of that sample. You do not have to fill all cryovials provided; you should attempt to fill as many cryovials as possible with 0.5 ml of sample.

Example: if 2.7 ml of sample is obtained, you should fill 5 cryovials each with 0.5 ml, and one additional cryovial with the remaining 0.2 ml.



*****Important Note*****

Plasma, serum and buffy coat specimens must be placed in the -80° C freezer within 2 hours of whole blood collection.

5.4 Serum Collection

Whole Blood Collection for Isolation of Serum: 6 ml Red Top Tube (for processing of serum aliquots)

1. **Store 6 ml red top tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

2. Using a blood collection set and a holder, collect blood into a **6 ml red top tube** using your institution's recommended procedure for collection of whole blood by venipuncture or an indwelling catheter.

3. **Immediately after blood collection, gently invert/mix (180 degree turns) each tube 8-10 times.**

4. **Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes.**

5. After 30 minutes of clotting and within 60 minutes of collection, centrifuge the balanced vacutainer tube for 15 minutes at 1500 RCF. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation. (See worksheet in Appendix A to calculate RPM in your particular rotor)**

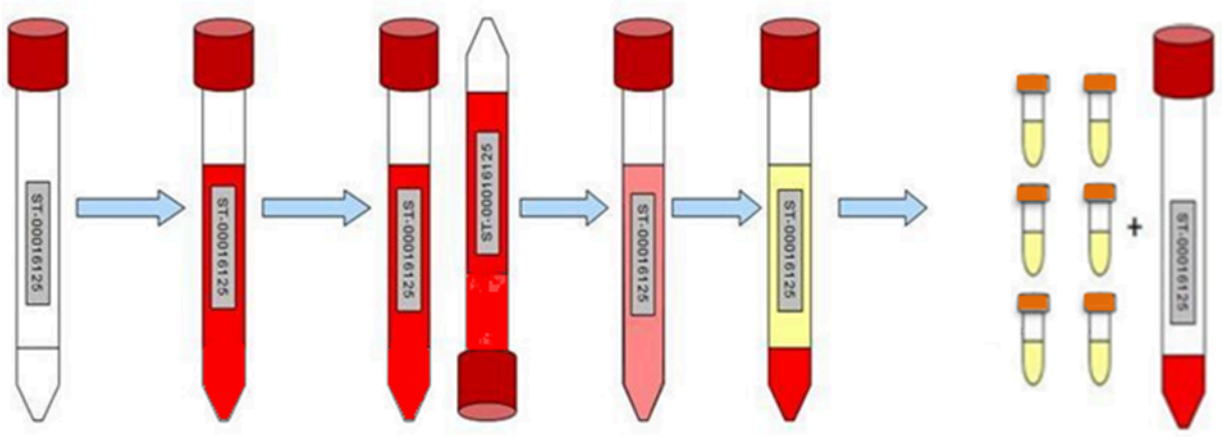
6. Place pre-printed "**SERUM**" (S01-S06) labels on the 1.5 ml cryovials (6).

7. Remove the serum, being careful not to agitate the clot at the bottom of the vacutainer tube.
    a. Tilt the tube and place the pipette tip along the lower side of the tube wall without touching the pellet.
    b. Using a micropipette with disposable tip, aliquot serum into the pre-labeled cryovials.
        i. The red top tube should yield, on average, 3 ml of blood serum. Aliquot 0.5 ml per cryovial (total vials = 5-6 with 0.5 ml each).
    c. Be sure to only place **serum** in cryovials labeled with the suffix **S01 to S06** (as before, you may fill less than 6 cryovials with serum- use the lowest numbered labels first).
    d. Place an ORANGE cap on each cryovial filled with serum.

8. Place cryovials in an 81 grid cryovial box and freeze samples immediately in **-80°C Freezer**.

9. Dispose of red top serum vacutainer tube with clotted blood in the bottom of the tube into a biohazard container.

10. Fill in the applicable CRF F181 during processing with:
    a. Blood Collection Date and Time
    b. Serum Aliquots Collected
    c. Time of Serum in Freezer
    d. Level of Hemolysis



1: Store tubes at room temp	2: Collect blood into red top tube, allowing blood to flow for 10 sec and ensuring blood has stopped flowing.	3: Immediately after blood draw, invert tube gently 8-10 times to mix sample.	4: Allow blood to clot for 30 - 60 mins. Then centrifuge tubes at 1500 x g for 15 minutes.	5: Label cryovials with BT "serum" labels. Use graduated pipette to aliquot 0.5 ml of serum into each cryovial. Cap serum cryovials with orange caps. Store at -80°C until shipment.

5.5 Plasma and DNA Collection

Steps for Whole Blood Collection for Isolation of Plasma and DNA: 6 ml EDTA Purple Top Tube (for processing of plasma and buffy coat aliquots)

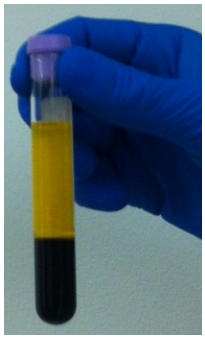
1. **Store EDTA Purple Top Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

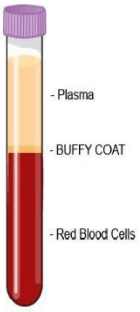
2. Using a blood collection set and a holder, collect blood into the **6 ml EDTA-Purple tube** using your institution's recommended procedure for collection of whole blood by venipuncture or an indwelling catheter.

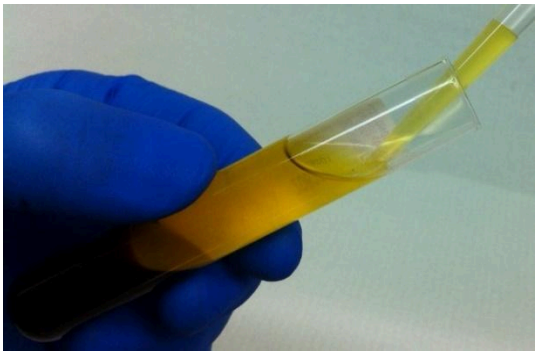
3. **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

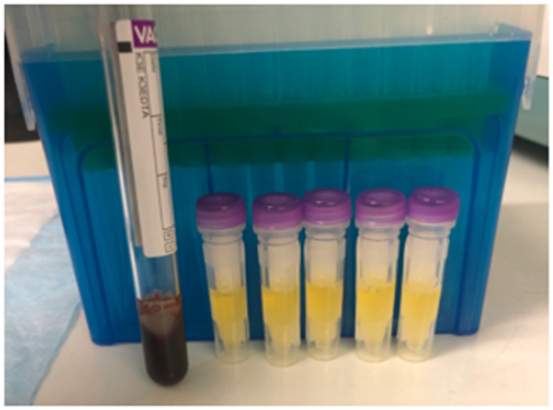
4. Within 60 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) with no brake.
    - **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**
    - Refrigeration prior to or during centrifugation is not recommended.

5. Place:
    - Pre-printed "**PLASMA**" labels (P01 – P06) on the 1.5 mL cryovials (6).
    - Pre-printed "**BUFFY COAT**" label (D01) on 1.5 mL cryovial.
6. Remove the plasma, being careful not to agitate the buffy coat layer or the packed blood cells at the bottom of the vacutainer tube
    a. Tilt the tube and place the pipette tip along the lower side of the tube wall without touching the buffy coat layer or the pellet below so that plasma is not contaminated by these materials (see below).
    b. Using a disposable graduated transfer pipette, transfer plasma into the pre-labeled cryovials.
        i. The EDTA vacutainer tube should yield, on average, 3 ml of blood plasma. Aliquot 0.5 ml per cryovial (total vials = 5-6 with 0.5 ml each).
    c. Be sure to only place **plasma** in cryovials labeled with the suffix **P01 to P06.**
    d. Place a PURPLE cap on each cryovial filled with plasma.





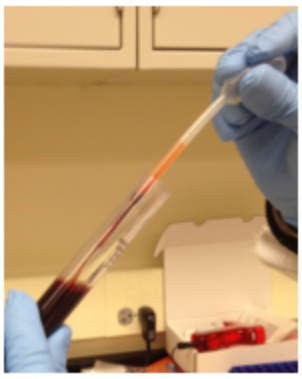
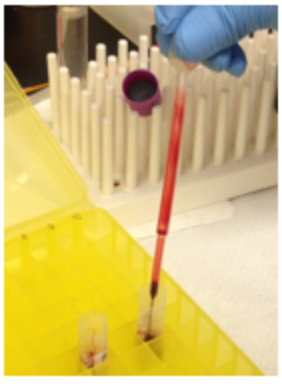




7. Place the labeled cryovials in the 81 grid cryovial box and freeze samples immediately following processing by transferring to **-80°C Freezer**. Store all samples at **-80°C until shipped** to NTBI-BR on dry ice.

> ***Important Note***
>
> BUFFY COAT MUST NOT BE COLLECTED IF THE SUBJECT HAS NOT CONSENTED TO POSSIBLE GENETIC TESTING

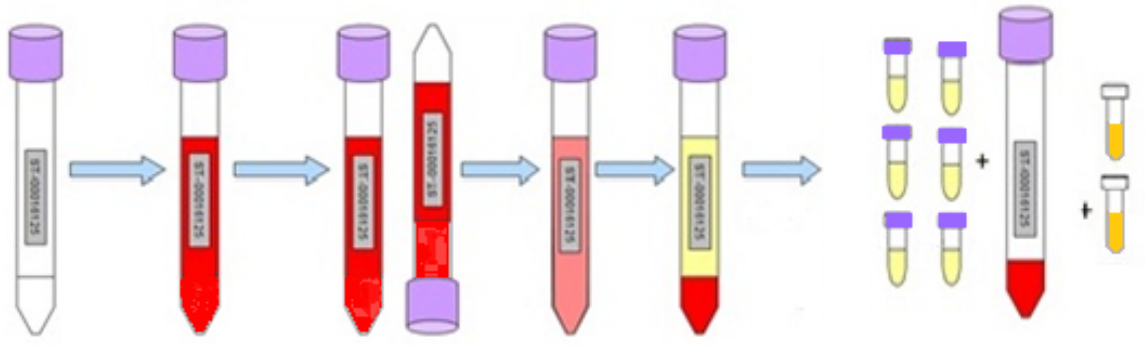
8. In the BASELINE visit ONLY, **after** plasma has been removed from the EDTA, purple top tube, aliquot the buffy coat layer into labeled cryovial with a disposable graduated pipette.
    a. With the tube upright, carefully collect the white buffy coat layer, getting as little of the pellet as possible into the pipette.
    b. Be sure to only place **buffy coat** in cryovial labeled with the suffix **D01.**
    c. Place a CLEAR cap on each cryovial filled with buffy coat.


Place labeled buffy coat cryovial in a separate cryovial box and place the box in **-80°C Freezer. Store all samples at -80°C until shipped to NTBI-BR on dry ice.**

9. Dispose of the purple EDTA vacutainer tube with cell pellet into biohazard container.

10. Fill in the applicable CRF F181 during processing with:
    a. Plasma Aliquots Collected
    b. Level of Hemolysis
    c. Buffy Coat Aliquots Collected (enrollment timepoint only)
    d. Time of Plasma in Freezer
    e. Time Buffy Coat in Freezer



1: Store tubes at *room* temperature	2: Collect blood into the purple top tube, allowing blood to flow 10 seconds and ensuring blood has stopped flowing.	3: Immediately after blood draw, invert tube gently 8-10 times to mix sample.	4: Within 60 minutes of whole blood collection, centrifuge sample for 15 min at 1500 x g.	5: Label cryovials with preprinted "plasma" and "buffy coat" labels. Using graduated pipette, aliquot 0.5 ml plasma into each "plasma" cryovial and 0.5 ml buffy coat into "buffy coat" cryovials. Cap plasma cryovials with purple caps; and buffy coat cryovials with clear caps. Store at -80°C until shipment.

5.6 RNA Collection

Steps for Whole Blood Collection for Extraction of RNA: PAXgene™ Tubes

1. **Store PAXgene™ Blood RNA Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

2. Using a blood collection set and a holder, collect blood into the **PAXgene™ Blood RNA Tube** using your institution's recommended procedure for collection of whole blood by venipuncture or an indwelling catheter.

3. **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times**.

4. Place "**RNA**" label on the PAXgene RNA tube prior to blood draw with the **R01** label. **<u>This single tube is to be shipped to the NTBI-BR frozen, without processing, at the collection site.</u>**

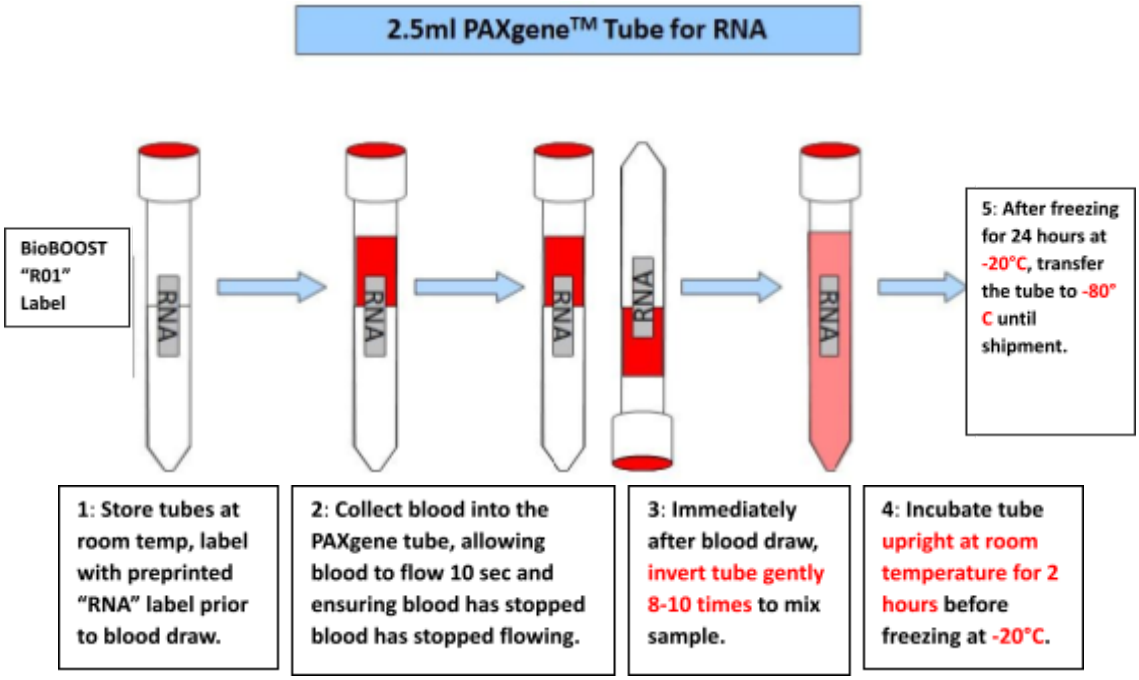
6. Incubate the PAXgene™ Blood RNA Tube **UPRIGHT at room temperature (18°C to 25°C) for 2 hours**.

7. After **2 hours** at room temperature, place the PAXgene tube upright in a **<u>WIRE</u>** rack and transfer the PAXgene tube to a **-20°C freezer.**

8. Keep the **PAXgene™ Blood RNA Tube at -20°C** for at least 24 hours

9. Record Time when PAXgene is placed in **-20°C** freezer on the applicable CRF F181 .

10. Transfer the tube to an **-80°C freezer** for storage until you ship on dry ice to the NTBI-BR.



11. If a -20°C freezer is not available, PAXgenes may be stored directly in a -80°C freezer after sitting for 2 hours at room temperature.
    a. If this occurs, select "No" to Q34 on Form 181.

6.0 CSF Collection and Processing Procedures

6.1 General Guidelines

The decision to place an External Ventricular Drainage (EVD) is a local clinical decision and is not affected by a patient's participation in BOOST III. Similarly, indications and procedures for CSF drainage (continuous vs. intermittent drainage) is a local clinical decision and not prescribed in the BOOST III protocol.

CSF collected for research purposes is fluid that would otherwise be discarded.

- Procedures for inserting the EVD and for collecting fluid from the system are also governed by local Neuro ICU protocols.

- Published guidelines from the American Association of Neuroscience Nurses are available (Am Assc Neurosci Nurses [2011]) Care for the patient undergoing intracranial pressure monitoring/external ventricular drainage or lumbar drainage. Glenview (IL) 37 p. [164 Refs]). Link to PDF

- A video demonstrating CSF collection is available here: https://vimeo.com/user120054989/CSFfromEVD
  DISCLOSURE: This tutorial is to assist trained personnel in CSF collection from an EVD.
  - Each site may differ in procedure. Check your local Neuro ICU protocol.
  - Also, this video shows betadine for cleaning the port in a sterile fashion; at some institutions, this may have been changed to chlorhexidine.

- The collection of CSF from the EVD system is performed by trained Neuro ICU nurses or physicians; however, trained research personnel may be granted permission at your institution (check local hospital protocols).

- At most centers, collection of 0.5 – 1 mL of CSF is routinely done daily, to monitor for infection. CSF for research purposes will in most cases be collected at the same time as the daily routine accession of the system.
  - If insufficient CSF is produced, priority will be given for fluid required for patient care.

- An effort should be made to collect the first CSF available at the time of insertion of the EVD. Up to 5 mL should be collected.

6.2 .CSF Collection

Steps for Whole CSF Collection

1. CSF is collected daily from the buretrol. If a bag change occurs in the morning, allow at least 2 hours before collection from buretrol.

2. **Fresh fluid** is collected as follows:
   a. Fluid is collected **using sterile technique** directly from the buretrol.
   b. Up to 5 mL is collected (although in most cases it will be less) and transferred to a single polypropylene conical centrifuge tube.
   c. Fluid is allowed to drain into buretrol by gravity (**never aspirated**).

3. Cell contamination of ventricular CSF is a significant confound. To minimize, CSF is centrifuged.

4. Transport fluid within 30 minutes of collection to the laboratory and centrifuge at 1500 RCF (x g) for 15 minutes. This can be done at the same time as blood processing.

5. Aliquot supernatant into 1.5 ml polypropylene cryovials (provided) using a micropipette with disposable tip.
   a. Up to 5 aliquots are prepared, each containing to 0.5 mL. If more fluid is collected, increase the volume of aliquots up to a maximum of 1.0 mL.
   b. Examples:
      i. If 5 mL are collected, distribute into 5x 1 mL aliquots
      ii. If 2.0 mL are collected, distribute into 4 x 0.5 mL aliquots.
      iii. If 1.2 mL are collected, distribute into 2 x 0.6 mL mL aliquots.
   c. Place a CLEAR cap on each cryovial filled with CSF.

6. Place cryovials in an 81-grid cryovial box and freeze samples immediately in a -80^{o}C freezer.

7. The following are noted on the applicable CRF F181:
   a. Appearance of fluid (clear, cloudy, bloody)
   b. Time of collection
   c. CSF aliquots collected
   d. Time of freezing

8. This CSF collection protocol can run for a maximum of five (5) days:
   a. Collect up to three (3) CSF samples on Day 1.
   b. Collect up to two (2) CSF samples on Days 2 through 5.
   c. If possible, collect the BioBOOST blood sample at the same time as collecting the CSF sample. This will provide a paired blood sample for some of the CSF samples

Samples will be shipped to the NTBI-BR at the University of Pittsburgh using the same procedures as done for blood samples.

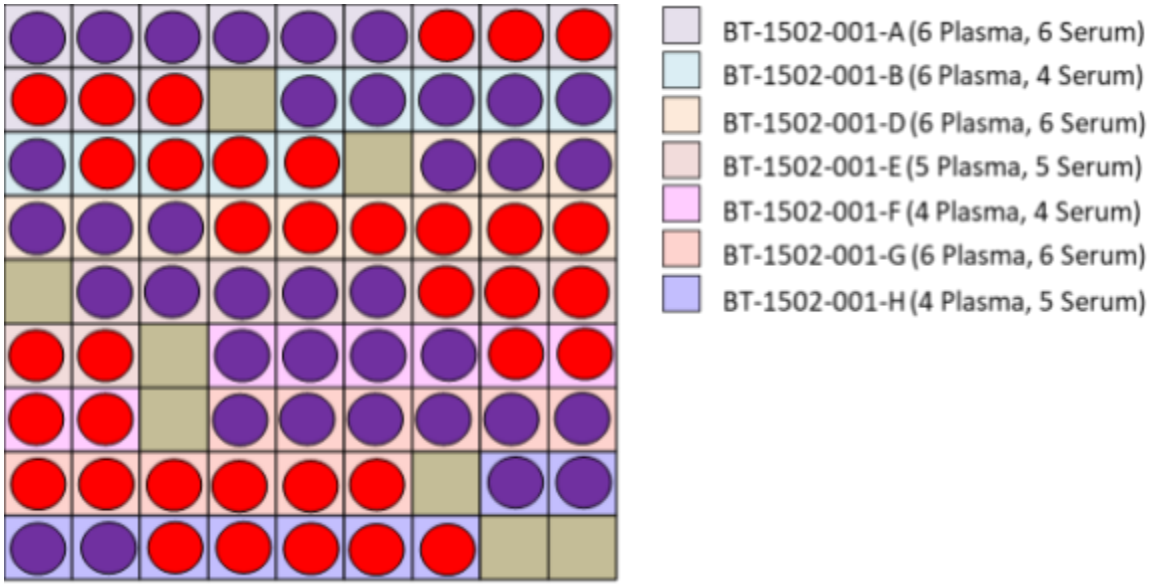
7.0 Storage of Specimens

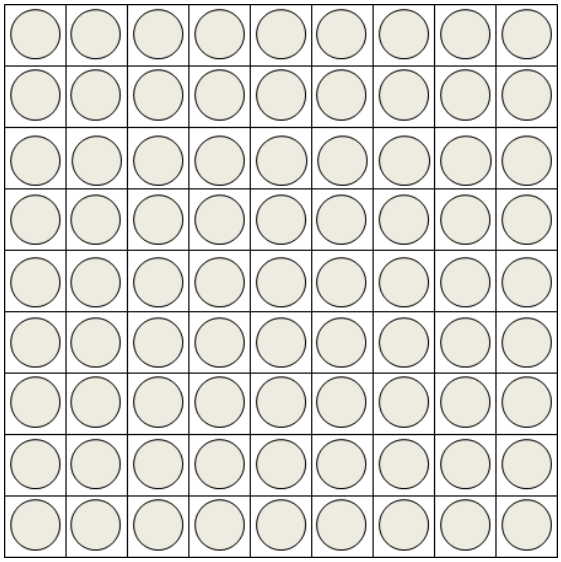
7.1 Storage of Serum, Plasma, Buffy Coat, and CSF Cryovials in Freezer

The supplied cryoboxes should be filled in chronological order of collection to minimize misplacement of samples within the box due to reshuffling of cryovials to keep subject samples next to one another.

- Plasma and serum from all visits may be placed in the box
- All aliquots from a single subject/timepoint are kept together
- Leave an empty space in the box between subject/timepoint

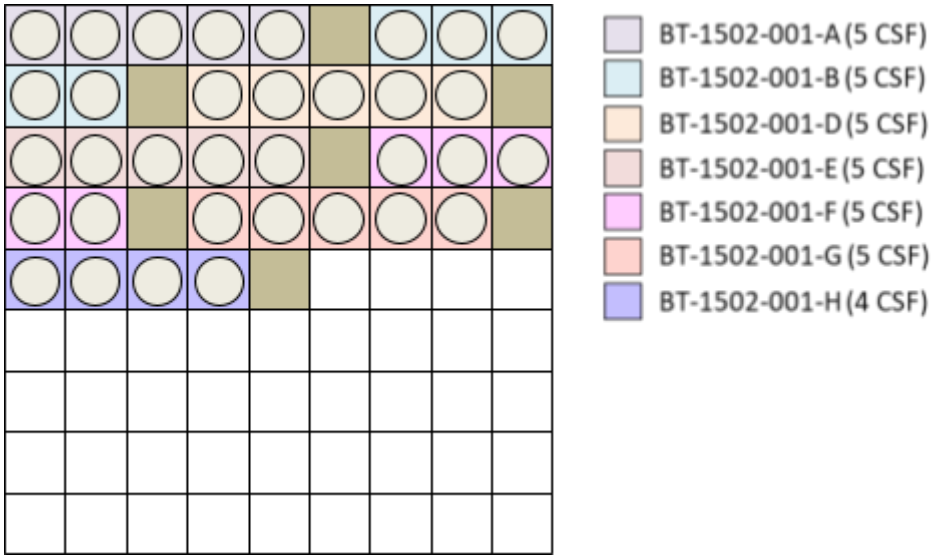
Buffy Coat should be placed in a separate cryobox. It is not necessary to leave spaces between subject/timepoint as done in the plasma/serum box above.





CSF should be placed in a separate cryobox from plasma/serum and buffy coat. CSF should be stored within the cryobox chronologically (as in the plasma/serum cryobox above).

- Plasma and serum from all visits may be placed in the box
- All aliquots from a single subject/timepoint are kept together
- Leave an empty space in the box between subject/timepoint



7.2 Storage of PAXgene Tubes in Freezer

PAXgene tubes must be stored upright until the specimen is fully frozen. It is best to keep PAXgenes in a freezer-safe rack in the -20°C freezer until transfer to -80°C freezer for long-term storage prior to shipping.

- PAXgenes must be kept in -20°C freezer for a minimum of 24 hours but may be stored at the temperature indefinitely.
- It may be most efficient to keep the rack at -20°C until full, then transfer the entire rack to -80°C freezer.
- PAXgene tubes need not be stored in any specific order, but it is imperative that PAXgenes are shipped concomitant with their respective plasma, serum, and CSF.

8.0 Packaging and Shipping Instructions

8.1 Biospecimens to be sent to the NTBI-BR:

The following samples will be collected on each subject:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)
- PAXgene (for RNA extraction)
- Cerebrospinal Fluid (CSF)

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academics or industry through the NTBI-BR. A copy of the consent form for each subject should be kept on file by the investigator.

ALL study personnel responsible for shipping must have IATA certification in Dangerous Goods Shipping. Check your local institution's Environmental Health & Safety if additional training in the Shipment of Hazardous Material is needed.

8.2 NTBI-BR Shipping Instructions

*****Important Note*****

For frozen shipments, include no more than SIX Pathopouch A3 envelopes per GDI-80 shipping container in order to have room for enough dry ice to keep samples frozen up to 36 hours.

The labeled, processed, aliquoted and frozen cryovials of plasma, buffy coat, serum, and frozen unprocessed PAXgene RNA tubes will be shipped to the NTBI-BR as outlined below.

Baseline and Follow-up Shipments to the NTBI-BR include the following:

- **Frozen 0.5 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 0.5 ml aliquots of buffy coat (for DNA, BASELINE visit only, FROZEN SHIPMENT)**
- **Frozen 0.5 ml aliquots of serum (FROZEN SHIPMENT)**
- **Frozen PAXgene Tube (FROZEN SHIPMENT)**
- **Frozen 0.5 - 1.0 ml aliquots of CSF (FROZEN SHIPMENT)**

Specimens being shipped to the NTBI-BR should be considered as Clinical/Diagnostic specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

All specimens must be entered into WebDCU PRIOR to specimen shipment. Sites are able to review the specimens that have been entered into WebDCU with the "Specimen Shipping Manifest" option within

the Study Material Tracking tile. Specimens will only appear on the Specimen Shipping Manif est list if the specimens were indicated as collected on a Form 181 eCRF within WebDCU. See instructions for Lab Kit Labeling provided in the "Bio-BOOST Site Lab Kit Receiving and Shipping" instructions within Toolbox > Project Documents in WebDCU to create Form 181 eCRFs.

Sites must provide a manifest with their shipment. Manifests must be created via the Specimen Shipping Manifest in WebDCU. Use the "Dump to Excel" option on the drop-down menu on the top-right of the Specimen Shipping Manifest screen in WebDCU to create an excel document containing your site's specimens. Only specimens included in a shipment may appear on the final Specimen Shipping Manifest provided to the NTBI-BR. Sites must verify that their manifest is accurate prior to contacting the NTBI-BR or World Courier for shipping.

8.3 World Courier Instructions

World Courier will arrange delivery of packaging and dry ice to your site. **Packaging and shipping labels should be ordered three days in advance of shipment.** These will be delivered directly to your site prior to the shipping day. Dry ice will be delivered at the time of pick up. Please note that World Courier drivers cannot assist with packing your shipments.

To arrange for the packaging and pick-up of samples, please contact:

World Courier Tel: (800) 221-6600

Provide the World Courier Representative with the following information:

1. Study Account Number: # 21408
2. Time that pick-up is required (Ship only on Monday-Wednesday!)
3. Specify the type of samples being sent:
   Blood, serum, plasma. Biological substance category B, Frozen at -80°C. on dry ice, to Pittsburgh.
4. State that you will need ALL shipping materials delivered to your site.
5. Specify that dry ice is required at time of shipping

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED ON MONDAY - WEDNESDAY ONLY!

All shipments require the following items:

Shipping Materials	Quantity	Description
House WayBill	1	Comes pre-printed with Shipper and Consignee information
Box labels		World Courier provides these (both UN 3373 and Dry Ice labels.)
Dry ice (10-kilo bag)	1-2 (see below)	World Courier will bring dry ice when picking up your shipment. Specify that you need pellets rather than blocks of dry ice.
Intelsius DGP Pathopouch 95 (A3 size)	Varies (see below)	The Pathopouch A3 is a large Secondary envelope. One Pathopouch A3 can maximally hold 4 81-grid cryoboxes, stacked 2 high and 2 deep. One Pathopouch A3 can maximally hold 24 PAXgenes in 6 dividers.
Absorbent material vial dividers	Varies (see below)	World Courier provides these. Be sure to ask for them. Include 1 absorbent material pad in the Secondary envelope with the cryoboxes. Additionally, one pad may be used to hold 6 PAXgenes.

Use the following guide to determine which size of shipping container is necessary:

Shipping Box	Cryoboxes	PAXgene Dividers	Comment
GDI-80	Up to 16	Up to 8	A GDI-80 insulated box can hold 6 Pathopouch A3 packages. This should be large enough for 16 cryoboxes (4 boxes in each of 4 Pathopouches) + 48 PAXGene tubes (24 tubes in each of 2 Pathopouches) + dry ice (2 bags).
GDI-45	Up to 12	Up to 8	A GDI-45 insulated box can hold 3 Pathopouch A3 packages. This should be large enough for 12 cryoboxes (4 boxes in each of 3 Pathopouches) + 48 PAXGene tubes (24 tubes in each of 2 Pathopouches) + dry ice (order 2 bags, may need more than 1 to fill box).
GDI-30	Up to 4	Up to 4	A GDI-30 insulated box can hold 2 Pathopouch A3 packages. This should be large enough for 12 cryoboxes (4 boxes in 1 Pathopouch) + 24 PAXGene tubes (24 tubes in 1 Pathopouch) + dry ice (1 bag).
GDI-15	1	1	A GDI-15 insulated box can hold 1 Pathopouch A3 package. This should be large enough for only a single cryobox + 6 PAXGene tubes (all in 1 Pathopouch) + dry ice (1 bag, about half of the bag should fit).

World Courier model GDI-80 insulated shipper

The GDI-80 insulated shipping box is large enough for 6 filled A3 Pathopouches.

6 A3 Pathopouches should fit 16 cryoboxes and 48 PAXgenes, along with adequate dry ice.

Outer Dimensions: 23 x 21 x 25 inches (57.0 x 52.0 x 63.5 cm)
Inner Dimensions: 17.4 x 15.2 x 19 inches (44.4 x 38.8 x 48.5 cm)

Intelsius DGP Pathopouch 95, Size A3

Each A3 Pathopouch can fit up to four 5 x 5 x 2 inch cryoboxes, stacked 2 x 2.

Each A3 Pathopouch can fit up to four 6-place absorbent vial dividers, stacked 2 x 2.

Outer Dimensions: 16.5 x 11.5 inches (41.9 x 29.2 cm)
Inner Dimensions: 14.1 x 10.3 inches (35.9 x 26.1 cm)

The International Air Transport Association packing instructions for shipping Biological materials, IATA 650, can be found at https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

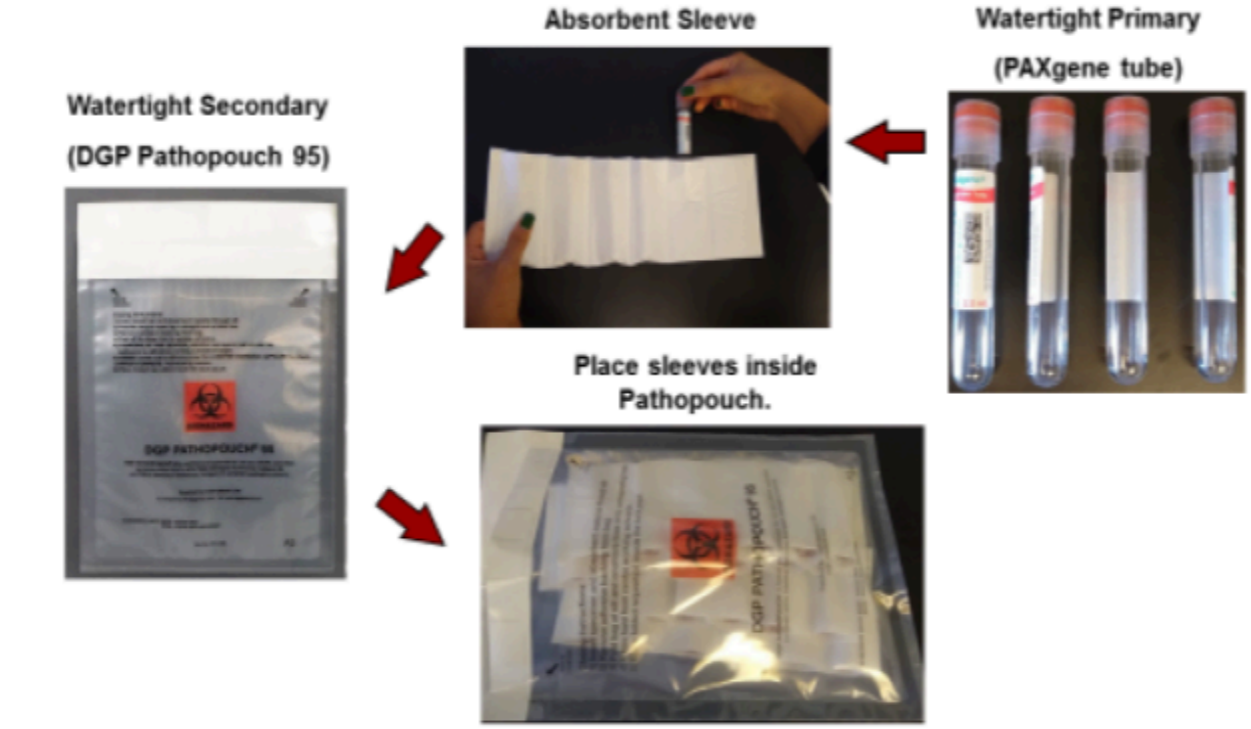
Triple packaging consists of a primary receptacle, secondary packaging and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IMPORTANT!

IT IS ESSENTIAL TO KEEP YOUR SAMPLES FROZEN AT ALL TIMES DURING THE PACKING PROCESS

Shipment Packing Instructions

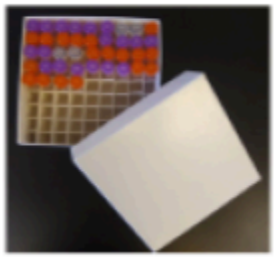
for Frozen Shipments (PAXgenes)



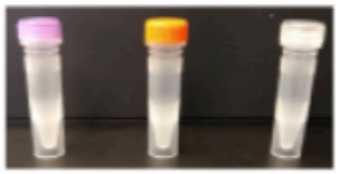
Shipment Packing Instructions for Frozen Shipments (Plasma/Serum Cryovials)

Watertight Secondary (DGP Pathopouch 95)

Cryovial box



Watertight Primary (cryovial)



Stack boxes 2 x 2 inside Pathopouch. Ensure that boxes pushed into pouch such that the adhesive-lined edges can meet.

Place an absorbent sheet above AND below boxes.

***** Sealing Pathopouches *****

- ☐ It is imperative that the Pathopouches are fully sealed. Do not remove the protective tape covering the adhesive until the pouch has been filled.
- ☐
- ☐ Once pouch is filled, remove tape and fold at opening. Line up black lines on both sides of the opening.
- ☐
- ☐ When packing cryoboxes, ensure that corners are adequately sealed.

IMPORTANT!

IT IS ESSENTIAL TO KEEP YOUR SAMPLES FROZEN AT ALL TIMES DURING THE PACKING PROCESS

Shipment Packing Instructions for filling the shipping container with sealed secondary envelopes and dry-ice

Place a layer of dry ice on the bottom of the Styrofoam-lined shipping carton.

Place the sealed Pathopouches **UPRIGHT** in the Styrofoam-lined shipping carton. You should be able to fit 6 within a GDI-80 box (not pictured).

FILL the remaining space in the shipping carton with dry ice, ensuring dry ice surrounds the envelopes and reaches the TOP of the carton

The following video is a guide to filling your GDI-80 shipping box:
https://vimeo.com/211217233
You may ignore the portions pertaining to the cardboard stabilizer (we will be shipping too large a volume to make use of these) and temperature monitor.

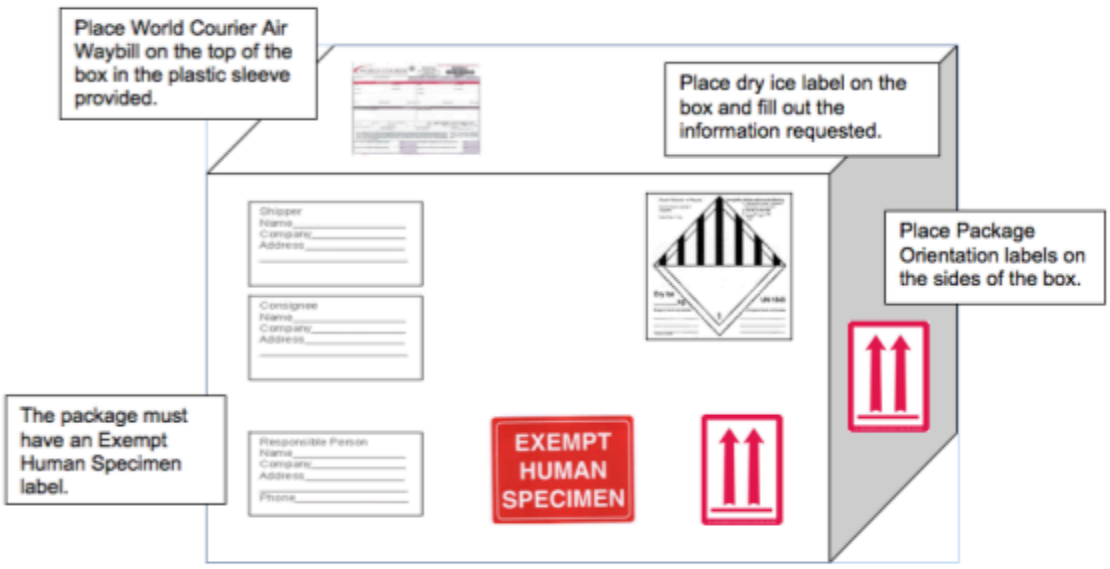
IMPORTANT!
AN ITEMIZED LIST OF CONTENTS MUST BE ENCLOSED BETWEEN THE SECONDARY PACKAGING AND THE OUTER PACKAGING.

*** Packing and Labeling Guidelines ***

IATA 650 guidelines: https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

- ☐ The primary receptacle (PAX RNA tube or frozen cryovials) must be leakproof and must not contain more than 1L total.
- ☐ The secondary packaging (DGP Pathopouch 95) must be leakproof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- ☐ Absorbent material must be placed between the primary receptacle (cryovial box containing the frozen cryovials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls or cellulose wadding.
- ☐ A shipping manifest of specimens being shipped must be included between the secondary and outer packaging (i.e. between the Styrofoam container and cardboard box).
- ☐ The outer shipping container must display the following labels:
  - ✔ Sender's name and address
  - ✔ Recipient's name and address
  - ✔ Responsible Person
  - ✔ The words "Biological Substance, Category B"
  - ✔ UN3373
  - ✔ Class 9 label including UN 1845, and net weight of dry ice contained

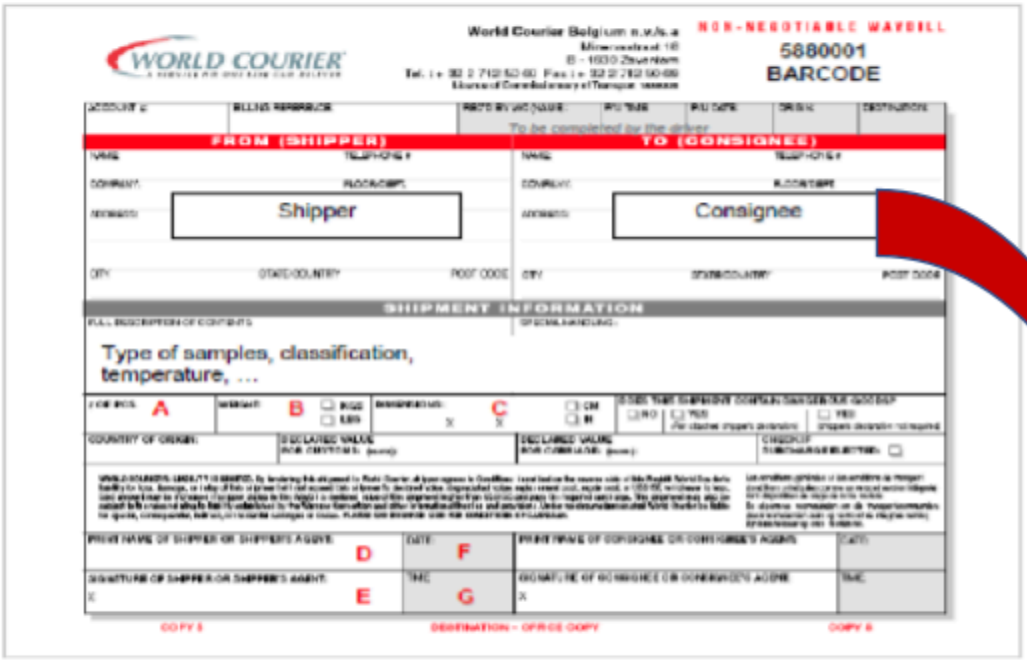
Labeling & Marking Instructions



Required documents

House Waybill (HWB)

- Please affix a waybill (or HWB) to the exterior of each shipment tendered to World Courier.
- World Courier will provide these forms with shipper and consignee information pre-printed for your convenience at the time of pick-up.
- This form is an internal tracking form used to identify your shipment from pick-up to delivery. When inquiring about your shipment, please reference the waybill number in the right hand corner.



Please complete the following information :

- A = number of packages
- B = total weight (kg)
- C = dimensions of the box (thermal box)
- D = full name of the shipper (in capitals)
- E = signature of the shipper
- F = collection date
- G = collection time

Ensure that the Consignee address is:

University of Pittsburgh, Neurotrauma Clinical Trials
ATTN: Miri Rabinowitz
3550 Terrace Street
Scaife Hall, Room S918
PITTSBURGH, PA 15261

Specimen Shipping Workflow Summary

1. Notify the NTBI-BR of your intent to send a shipment by sending an email to the following addresses:
   - mancinellimd@upmc.edu
   - rabinowitzmk@upmc.edu

2. Generate Specimen Shipping Manifest as directed in Section 8.2 and verify the manifest matches the shipment contents.

3. Contact World Courier to confirm service is available and schedule package supplies to be delivered and schedule the container to be picked up.

4. When the shipment is sent, send an email to the address above and include the **manifest generated by WebDCU.** See Appendix E for a sample.

5. Send an email verifying the shipment was picked up by World Courier to the NTBI-BR

6. If you have any questions or concerns, contact Mike Mancinelli, NTBI-BR Manager.

SHIP ALL FROZEN SAMPLES MONDAY-WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES

9.0 Sample Quality Checks and Feedback to Projects

In addition to tracking and reconciliation of samples, the condition and number of samples received are tracked by the NTBI-BR for each sample type. (See Shipment Tracking form – Appendix D) Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

10.0 Data Queries and Reconciliation

The paper version of the applicable CRF (see Appendix C) must be completed concurrently with sample collection/processing, as they capture information related to the details of the sample collection/processing. These forms include information that will be used to serve as source documentation for WebDCU eCRFs, reconcile sample collection and receipt, and record information essential to future analyses. The corresponding eCRF in WebDCU must be completed within 5 days of sample collection/processing.

WebDCU will be collaborating with the NTBI-BR to reconcile information captured in the WebDCU database compared to samples received and logged at the NTBI-BR. Information that appears incorrect in the WebDCU database will be queried through the standard system.

Data queries or discrepancies with samples shipped versus received at the NTBI-BR may result from:

- Missing samples at the NTBI-BR
- Incorrect samples collected and shipped to the NTBI-BR
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information logged at the NTBI-BR compared to information entered into the WebDCU database

Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication.

11.0 Specimen Destruction

Requests for specimen destruction must be sent in writing to the study PIs, Drs. Diaz-Arrastia or Korley. If the specimen(s) in question have already been shipped to the NTBI-BR, the NTBI-BR Manager must also be included in the request. Specimens shall never be destroyed without prior authorization by the study PI or a study team member designated by the study PI.

Procedures for destroying a specimen within the WebDCU database are provided in the “Bio-BOOST Site Lab Kit Receiving and Shipping” instructions within Toolbox > Project Documents in WebDCU.

12.0 Appendices

Appendix A: **Rate of Centrifugation Worksheet**

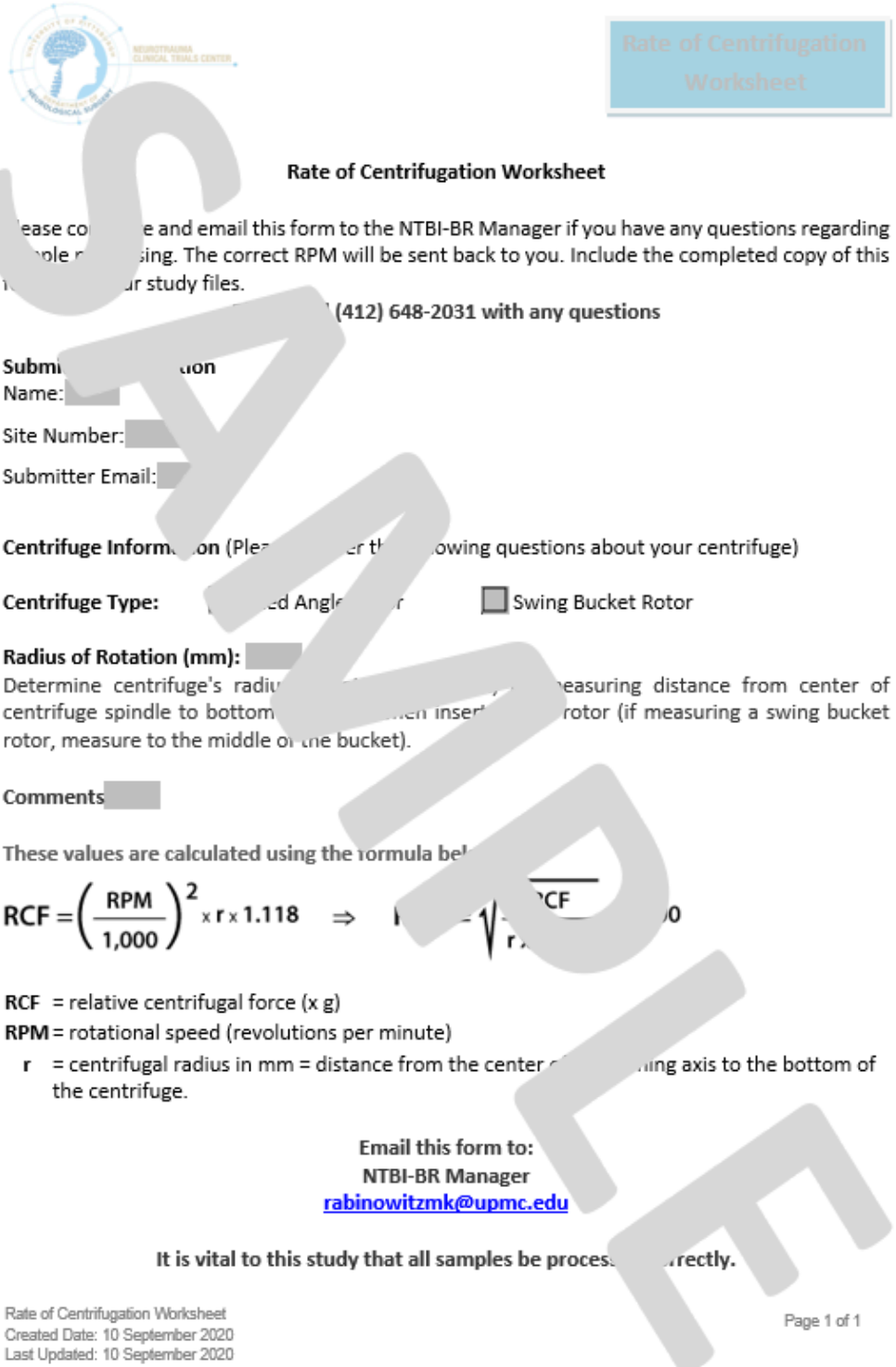
Appendix B: **BioBOOST Sample Processing Quick Reference**

Appendix C: **Form 181: Biospecimen Collection**

Appendix D: **Shipment Tracking Form**

Appendix E: **Shipping Manifest**

12.1 Appendix A: Rate of Centrifugation Worksheet



Rate of Centrifugation Worksheet

Rate of Centrifugation Worksheet

ease co e and email this form to the NTBI-BR Manager if you have any questions regarding ple sing. The correct RPM will be sent back to you. Include the completed copy of this ur study files.

(412) 648-2031 with any questions

Submi ion
Name:

Site Number:

Submitter Email:

Centrifuge Inform on (Ple er th owing questions about your centrifuge)

Centrifuge Type: ed Angl r ☐ Swing Bucket Rotor

Radius of Rotation (mm):
Determine centrifuge's radiu easuring distance from center of centrifuge spindle to bottom inser rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Comments

These values are calculated using the formula bel

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

RCF = relative centrifugal force (x g)
RPM = rotational speed (revolutions per minute)
r = centrifugal radius in mm = distance from the center ing axis to the bottom of the centrifuge.

Email this form to:
NTBI-BR Manager
rabinowitzmk@upmc.edu

It is vital to this study that all samples be proces rrectly.

Rate of Centrifugation Worksheet
Created Date: 10 September 2020
Last Updated: 10 September 2020

Page 1 of 1

12.2 Appendix B: BioBOOST Specimen Processing Quick Reference

BioBOOST Specimen Processing Quick Reference

Serum - 1 6 mL SCA Red Top Tube

- Allow to clot 30-60 mins.
- Spin for 15 Mins. @ 1.5 RCF
- Aliquot Serum into prelabeled 1.5 mL cryovials **(S01-S06)**
- Aliquot in portions of at least 500 uL (0.5 mL)
- Store in -80°C

PLASMA - 1 6 mL K_3EDTA Purple Top Tube

- Spin for 15 Mins. @ 1.5 RCF Immediately
- Aliquot Plasma into prelabeled 1.5 mL cryovials **(01-P06)**
- Aliquot in portions of at least 500 uL (0.5 mL)
- **DNA (**A ONLY): Aliquot Buffy Coat into prelabeled 1.5 mL cryovial **(D01)**
- Store in -80°C

RNA - 1 2.5 mL PaxGene® Tube

- Label PaxGene Tube **(R01)**
- Sit for 2-72 Hours at Room Temp.
- Store -20°C for 24+ Hours
- Move to -80°C

CSF – 1 15 mL Conical Blue Top Tube

- Collect up to 5 mL
- Should be processed within 30 minutes of collection
- Spin for 15 Mins @ 1.5 RCF for 15 minutes
- Aliquot CSF into prelabeled 1.5 mL cryovials **(C01-C05)**
- Aliquot in portions of at least 500 uL
- Store in -80°C

12.3

12.4 Appendix C: Form 181: Biospecimen Collection

The current version of Form 181 is available within Toolbox > Project Documents in WebDCU.

BioBOOST	Subject: ___-___-___-___	

Form 181: Biospecimen Collection Version 2 (11-Oct-2021) Page 1 of 3

Q01		Biospecimen collection timepoint	
Q07		Lab kit ID	
Q04		WebDCU Lab kit code	
Q02		Specimen collected	○ No ○ Yes
Q03	*If Q02 is 'Yes'*	Date of specimen collection	__ __-__ __ __-__ __ __ __ dd-mmm-yyyy
Instruction: 1. *Transport blood tubes at room temperature.* 2. *Start centrifuge within 90 minutes from blood draw.*			
Q05	*If Q02 is 'Yes'*	Blood sample collected	○ No ○ Yes
Q06	*If Q05 is 'Yes'*	Time of blood draw	__ __:__ __ 24 hour clock; hh:mm
Q08		Any issues with collecting, processing, or storing samples	
Q60	*If Q01 is 'X'*	Type of blood draw triggering event	○ Hypoperfusion ○ Hypoxia
Serum aliquots			
Q10	*If Q05 is 'Yes'*	Time serum samples placed in freezer	__ __:__ __ 24 hour clock; hh:mm
Q11		Aliquot S01 collected	○ No ○ Yes
Q12		Aliquot S02 collected	○ No ○ Yes
Q13		Aliquot S03 collected	○ No ○ Yes
Q14		Aliquot S04 collected	○ No ○ Yes
Q15		Aliquot S05 collected	○ No ○ Yes
Q16		Aliquot S06 collected	○ No ○ Yes
Q17		Reason any serum aliquot not collected	
Q18		Amount of hemolysis in serum samples	○ Less than 100 mg/dL ○ 100 mg/dL or more

If this is a source document, sign and date:	_____ *Print name*	_____ *Signature*	__ __-__ __ __-__ __ __ __ *dd-mmm-yyyy*

Medical University of South Carolina, Data Coordination Unit

BioBOOST	Subject: _____	

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Plasma aliquots				
Q20		Time plasma samples placed in freezer	__ __ : __ __ 24 hour clock; hh:mm	
Q21		Aliquot P01 collected	○ No	○ Yes
Q22		Aliquot P02 collected	○ No	○ Yes
Q23		Aliquot P03 collected	○ No	○ Yes
Q24	*If Q05 is 'Yes'*	Aliquot P04 collected	○ No	○ Yes
Q25		Aliquot P05 collected	○ No	○ Yes
Q26		Aliquot P06 collected	○ No	○ Yes
Q27		Reason any plasma aliquot not collected		
Q28		Amount of hemolysis in plasma samples	○ Less than 100 mg/dL	○ 100 mg/dL or more
DNA (Buffy Coat)				
Q30	*If Q05 is 'Yes'*	Aliquot D01 collected	○ No	○ Yes
Q31	*If Q30 is 'Yes'*	Time DNA sample placed in freezer	__ __ : __ __ 24 hour clock; hh:mm	
RNA (PAXgene)				
Q32	*If Q05 is 'Yes'*	Aliquot R01 collected	○ No	○ Yes
Q35	*If Q32 is 'Yes'*	PAXgene sample stored at room temperature for at least 2 hours before being placed in the –20 freezer	○ No	○ Yes
Q33		Time PAXgene sample placed in –20 freezer	__ __ : __ __ 24 hour clock; hh:mm	
Q34		PAXgene sample stored in freezer at –20 °C for at least 24 hours	○ No	○ Yes

If this is a source document, sign and date:	_____ Print name	_____ Signature	__-___-____ dd-mmm-yyyy

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BioBOOST	Subject: ___-___-___-___	

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CSF aliquots

Collect CSF sample as close to the blood collection as possible.

Q40	*If Q01 is Day 1-Day 5 and Q02 is 'Yes'*	CSF collected	○ No ○ Yes
Q41	*If Q40 is 'Yes'*	Time CSF collected	__ __ : __ __ 24 hour clock; hh:mm
Q42		Time CSF centrifuge started	__ __ : __ __ 24 hour clock; hh:mm
Q43		Time CSF sample placed in freezer	__ __ : __ __ 24 hour clock; hh:mm
Q44		CSF sample appearance	○ Clear ○ Bloody ○ Cloudy
Q51		Aliquot C01 collected	○ No ○ Yes
Q52		Aliquot C02 collected	○ No ○ Yes
Q53		Aliquot C03 collected	○ No ○ Yes
Q54		Aliquot C04 collected	○ No ○ Yes
Q55		Aliquot C05 collected	○ No ○ Yes
Q56		Reason any CSF aliquot not collected	

General comments

If this is a source document, sign and date:	_____ *Print name*	_____ *Signature*	__ __-___-__ __ *dd-mmm-yyyy*

Medical University of South Carolina, Data Coordination Unit

12.5 Appendix D: Shipment Tracking Form

Shipment Tracking Form

Shipp Center Name or umber: _____

NCT ication e: _____

Electronic M sent: ☐ Yes ☐ No

(Manifest must be ser .xls, or .txt form

Courier: _____

Shipment Tracking Nu : _____

Shipment Date: _____

Paper Manifest Included: ☐ ☐ No

(Printout of electronic manifest in box with specimens)

Number of Specimens: _____

Receipt Details: To Be Completed by Staff

Receipt Date: _____

Adequate Dry Ice: ☐ Yes ☐ No

(If no, ensure that boxes are checked for thaws)

Number of Specimens: _____

Shipment Tracking Form

Instructio

pping C er: Na e and Site ID of collection site shipping samples to BR

NCT ification Date: ate of first notification to BR staff (Mike Mancinelli or Miri Rabinowit **NOTIFIED PRIOR TO SHIPMENT.**

Electronic Mani Ser nitial notification email, an electronic manifest of all specimens include ment must be attached. Manifest must be in .csv, .xls, or .txt format.

Courier: The name of co ed f ipping. This should be World Courier except in extremely ses.

Shipment Tracking Numbe t will be used for shipment.

Shipment Date: Anticipated date of sh

Paper Manifest Included: A printout of the c ic ma st should be included in the shipping box.

Number of Specimens: Total number of specimens i ded in s ment.

12.6 Appendix E: Shipping Manifest

All information is generated based on data currently in the database. Data may not be verified or validated.
The report is generated to assist in trial operations only, and is not valid to support any statistical analysis of study data.
Unless noted, the Data Coordination Unit (DCU) assumes no responsibility for the use of this report. This report may contain protected health information covered by the Health Insurance Portability and Accountability Act (HIPAA).
You are prohibited from disclosing this information without the specific written consent of the person to whom it pertains. Anyone using this data specifically assumes responsibility for maintaining the confidentiality of the protected data.

Specimen label (1)	Site (2)	BioBOOST Subject ID (3)	Timepoint (4)	Lab kit code (2)	Specimen type (5)	Specimen collection date time (6)	Specimen shipping tracking number (7)	Specimen shipment date time (8)
BT-1170-001-A-S01	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-S02	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-S03	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-S04	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-S05	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-S06	1170	1	A - Baseline	40001	Serum	2/1/2021 15:16		
BT-1170-001-A-P01	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-P02	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-P03	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-P04	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-P05	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-P06	1170	1	A - Baseline	40001	Plasma	2/1/2021 15:16		
BT-1170-001-A-D01	1170	1	A - Baseline	40001	DNA (Buffy Coat)	2/1/2021 15:16		
BT-1170-001-A-R01	1170	1	A - Baseline	40001	RNA (PAXgene)	2/1/2021 15:16		
BT-1170-001-B-S01	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-S02	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-S03	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-S04	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-S05	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-S06	1170	1	B - 16 Hours post Injury	40002	Serum	2/2/2021 2:33		
BT-1170-001-B-P01	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-P02	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-P03	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-P04	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-P05	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-P06	1170	1	B - 16 Hours post Injury	40002	Plasma	2/2/2021 2:33		
BT-1170-001-B-R01	1170	1	B - 16 Hours post Injury	40002	RNA (PAXgene)	2/2/2021 2:33		