Manual of Procedures

TRACK-TBI Biorepository

5 May 2016

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1. Abbreviations

ACD	Acid Citrate Dextrose
BD	Becton, Dickinson
BR	BioRepository
BSIS	Biological Specimen Inventory System
TRACK-TBI BR	Transforming Research and Clinical Knowledge in TBI BioRepository
DoD	Department of Defense
EDTA	Ethylene Diamine Tetra-acetic Acid
FAA	Federal Aviation Administration
FDA	Food and Drug Administration
IATA	International Air Transport Association
ISBER	International Society For Biological and Environmental Repositories
MTA	Material Transfer Agreement
PPE	Personal Protective Equipment
RCF	Relative Centrifugal Force
RIMS	Repository Inventory Management System
RPM	Revolutions Per Minute
SST	Serum Separator Tube
TBI	Traumatic Brain Injury
USU	Uniformed Services University

2. Purpose

The collection of biofluids is central to the goals of the TRACK-TBI initiative. The development of valid and reliable biomarkers of the effects of TBI is needed to:

- Aid in the recognition and assessment of TBI-associated neuronal injury
- Distinguish between different types of TBI-associated neuronal injury
- Evaluate and monitor effects of therapeutic agents in clinical trials of TBI

The purpose of this Manual is to provide TRACK-TBI staff (PIs, AIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for TRACK-TBI studies. It includes instructions for biospecimen submission to the TRACK-TBI BioRepository located in Pittsburgh. The following samples will be collected:

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

This manual includes instructions for collection of blood, fractionation of blood from vacutainer tubes, aliquotting, labeling, storage prior to shipping and shipping to the TRACK-TBI Biorepository.
Note: **PAXgene tubes will be frozen at the collection site without processing.**

These procedures are relevant to all study personnel responsible for processing blood specimens entering the TRACK-TBI Repository for participating TRACK-TBI protocols.

3. TRACK-TBI BioRepository Laboratory Contacts

Contact information for the TRACK-TBI BioRepository at the University of Pittsburgh:
Attn: Ava Puccio, RN, PhD
Neurotrauma Clinical Trials Center
University of Pittsburgh Department
of Neurological Surgery
200 Lothrop Street, Suite B-400
Pittsburgh, PA 15213
Phone: 412-648-9246

After Hours Phone: (412) 298-7033
Email: puccioam@upmc.edu

Secondary Contact: Miri Rabinowitz, PhD
TRACK-TBI Biorepository Lab Manager Office:
412-648-2031 Mobile: 412-491-6199
Email: rabinowitzmk@upmc.edu

4. TRACK-TBI BioRepository Information

4.1 Hours of Operation

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

Frozen samples must be shipped Monday – Tuesday only.

Frequency of shipments will depend on enrollment rate at each study site. A standard shipping box can maximally hold sixteen 5”x5”x2” cryovial boxes, each holding up to 81 cryovials per box and 1 biospecimen bag containing up to 12 frozen PAXgene tubes. For common scenarios with packing and shipment, please refer to Section 8 of this protocol.

Frozen samples should be shipped after at least quarterly. Please ensure adequate storage at -80°C prior to shipment.

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

4.2 Holiday 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
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 at this time.

5. TRACK-TBI BioRepository Research Laboratory Collection

5.1 Materials and Equipment Required at the Collection Site for Local Processing Prior to Shipping

The following materials and equipment are necessary for the processing of specimens at the collection site and to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses, gloves
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles
- Microcentrifuge tube rack
- Gloves
- Sharps bin and lid

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- Centrifuge capable of ≥ 1500 rcf (1500 x g)
- -80°C Freezer

5.2 Biospecimens to be sent to the TRACK-TBI BR Laboratory:

Biospecimens collected include whole blood. The following samples will be collected on each subject:

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

Whole blood collected for the TRACK-TBI BR is collected into three different vacutainer tubes. It is then processed locally into plasma, serum and buffy coat fractions, aliquotted, frozen at the study site and then shipped to the TRACK-TBI BR. PAXgene tube is frozen locally without further processing.

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

Frozen samples are to be submitted at least quarterly according to the schedule in Section 3.2 above. In general, the following volumes must be submitted to the TRACK-TBI Repository for each sample type:

- Plasma and Buffy Coat (purple-EDTA tube): 6 milliliters (ml)
- Serum (red top tube): 6 milliliters (ml)
- PAXgene™ tube (for RNA): 2.5ml

Guidelines for the processing, storage location and timing of sample collection are in the order in which they appear in the tables below.

TRACK-TBI Biospecimen Collections (Baseline and Follow-up Visits):

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquotting	Tubes to TRACK-TBI BR
Whole blood for isolation of plasma & buffy coat (for DNA* extraction)	**6 ml Purple Top EDTA Tube**	**1**	**N/A**	**N/A**
	PLASMA: 2 ml cryotubes with purple caps	6	0.5 ml plasma aliquots per 2 ml cryotube	5-6
	BUFFY COAT: 2 ml cryotubes with clear caps	2	0.5 ml buffy coat aliquots per 2 ml cryotube	2* (baseline visit only)
Whole blood for isolation of serum	**6 ml Red Top**	**1**	**N/A**	**N/A**
	SERUM: 2 ml cryotubes with orange caps	6	0.5 serum aliquots per 2 ml cryotube	5-6
Whole blood for RNA extraction	**2.5 ml PAXgene™ Tube**	**1**	**N/A**	**1**
Blood TOTAL	15 ml	14- cryovials with color-coded caps 1 purple top- 6 ml 1 red top- 6 ml 1 PAXgene™		14 cryovials 1 PAXgene™

*"Buffy coat" for DNA is **collected only** at the Day 1 of the Baseline Visit; all subsequent visits will **not** include the collection of the buffy coat layer. However, the purple top tube is still needed for plasma collection.

If a sample is not obtained at a particular visit, this should be recorded on the **Sample Record Summary and Shipment Notification Form (see Appendix B).** Submit a copy to the TRACK-TBI BioRepository with a reason provided for the omission.

6. Specimen Collection Kits, Shipping Kits and Supplies

Research specimen collection kits (except equipment supplies listed above) will be provided to you by the TRACK-TBI BioRepository with materials needed for blood collection and containers for plasma/serum/buffy coat aliquots. Cryovial tube labels will be provided to you by the TRACK-TBI BioRepository. Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment.

6.1 TRACK-TBI BioRepository – Specimen Collection Kit Contents

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from TRACK-TBI BioRepository Laboratory Manager to do so.

Kit Supplies

Quantity	Baseline Kit Components
14	Polypropylene microcryovial tubes
6	Purple caps (for plasma)
6	Orange caps (for serum)
2	Clear caps (for buffy coat/DNA) *
15	Pre-printed RNA, plasma, serum, buffy coat cryovial tube labels**
1	PAXgene™ blood collection tube
1	EDTA (purple top) blood collection tube
1	Serum determination tube (red top)
3	Fisherbrand™ disposable graduated transfer pipettes

* The baseline visit includes aliquotting the buffy coat in cryovials using the clear caps; the buffy coat is NOT collected in visits after the baseline visit.

6.2 TRACK-TBI BioRepository – Initial Supply to Study Sites

Each site will initially be supplied with 10 blood draw kits and 1 shipping kit. Blood draw kits contain all blood collection supplies for Day 1, 3, and 5. This means project sites will have sufficient baseline visit kits for up to 10 subjects and a shipping kit for up to 10 subjects. Subsequent specimen kits, including those for 2-weeks and 6-months, should be ordered from the TRACK-TBI BioRepository Laboratory Manager when in anticipation of need. Shipping kits will be sent to the site from the TRACK-TBI BR upon request, and automatically every time a study site makes a specimen shipment to the TRACK-TBI BR.

6.3 TRACK-TBI BioRepository – Resupply to Study Sites

Each individual site will be responsible for ordering additional kits from the TRACK-TBI BR after the initial supply has been sent. Be sure to check your supplies and order additional materials before you run out so you are prepared for both scheduled and unanticipated visits. Please allow **10 days** for kit orders to be processed and delivered.

7. 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 specific collection, processing and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies 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.

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES. See Appendix C for lab worksheet for processing of all lab samples.

7.1 Labeling Samples

In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Each kit is supplied with labels for the specimens shipped to the TRACK-TBI BioRepository (15 for the baseline blood draw and 13 for all subsequent blood draws as DNA is collected only on the initial visit).
- Each vial will be labeled with an alphanumeric string. For example:
  TR-01-1001-A-01P (represents the first plasma cryovial label from the first (baseline) blood draw on the first subject at TRACK-TBI site 1)
- The code used in the labeling is as follows:

Study	Site	Subject ID	Blood Draw	Specimen Type and Vial
TR	01 to 11	1001 to 1999	A thru E A: Day 1; B: Day 3 C: Day 5; D: 2-wk E: 6-month	Plasma: 01P to 06P Buffy Coat: 07D* to 08D** Serum: 09S to 14S PAXgene: 15R***

*Since DNA will only be collected at the baseline blood draw, only samples with an "A" designation will have labels 07D or 08D.
** D for DNA
***R for RNA
Note: There are 6 aliquot tubes for plasma, 6 aliquot tubes for serum, and 2 aliquot tubes for buffy coat. The provided cryovial caps are color-coded. Use the **Purple caps** for plasma; **Orange caps** for serum; and **Clear caps** for buffy coat.

- Place labels on **ALL** collection and aliquot tubes **BEFORE** any sample collection or sample processing/freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place label **horizontal** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.

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

- Be sure to only place **plasma** in cryovials labeled with the suffix **01P to 06P** (depending on the amount of blood drawn you may fill less than 6 cryovials: use the lowest numbered labels, i.e. 01P, 02P and 03P rather than 03P, 04P and 05P if only 3 cryovials used). Cap with the **PURPLE** caps.

- Be sure to only place **buffy coat** in cryovials labeled with the suffix **07D or 08D** (again you may only use 1 cryovial depending on your blood draw yield). Cap with the **CLEAR** caps.

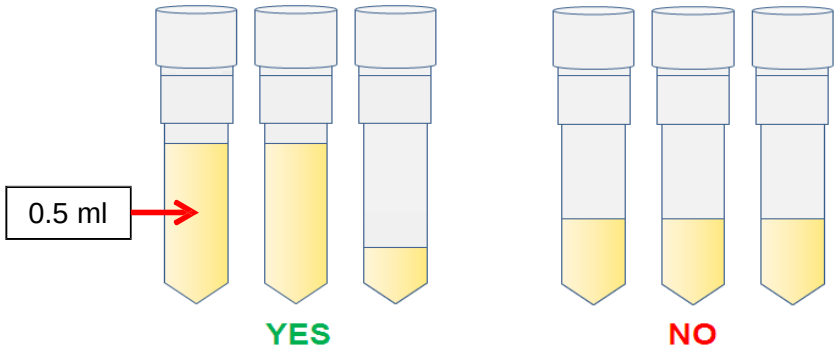
- Be sure to only place **serum** in cryovials labeled with the suffix **09S to 14S** (as before, you may fill less than 6 cryovials with serum- use the lowest numbered labels first as above). Cap with the **ORANGE** caps.

- Finally, label the **PAXgene** tube with the **15R** suffix label.

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

7.2 Filling Aliquot Tubes (Plasma and Serum)

In order to ensure that the TRACK-TBI BioRepository receives a sufficient amount of the sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should ideally be filled to 0.5 milliliters (see picture below) with the respective biologic material after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample. If there is biologic material remaining that will not fill a subsequent aliquot tube, that remaining amount should still be included and shipped to the TRACK-TBI BioRepository. Essentially, all material should be shipped to the TRACK-TBI BioRepository, ensuring maximum amount in as many aliquot tubes as will allow after processing the sample. You do not have to fill all cryovial tubes provided; you should attempt to fill as many tubes as possible with 0.5 ml of sample. For example, if 2.7 ml of sample is obtained, you should fill 5 cryovial tubes each with 0.5 ml, and one additional cryovial tube with the remaining 0.2 ml.



7.3 Whole Blood Collection for Isolation of Plasma and Buffy coat (buffy coat is shipped in baseline blood draw only for DNA extraction): 6 ml EDTA Purple Top Tube (for processing of plasma and buffy coat aliquots)

1. Place the
   pre-printed "**PLASMA**" labels on the 2 ml cryovial tubes (6)
   and pre-printed "**BUFFY COAT**" labels on 2 ml cryovial tubes (2).

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

3. Using a blood collection set and a holder, collect blood into the **6 ml EDTA-Purple tube** using your institution's recommended procedure for standard venipuncture technique.

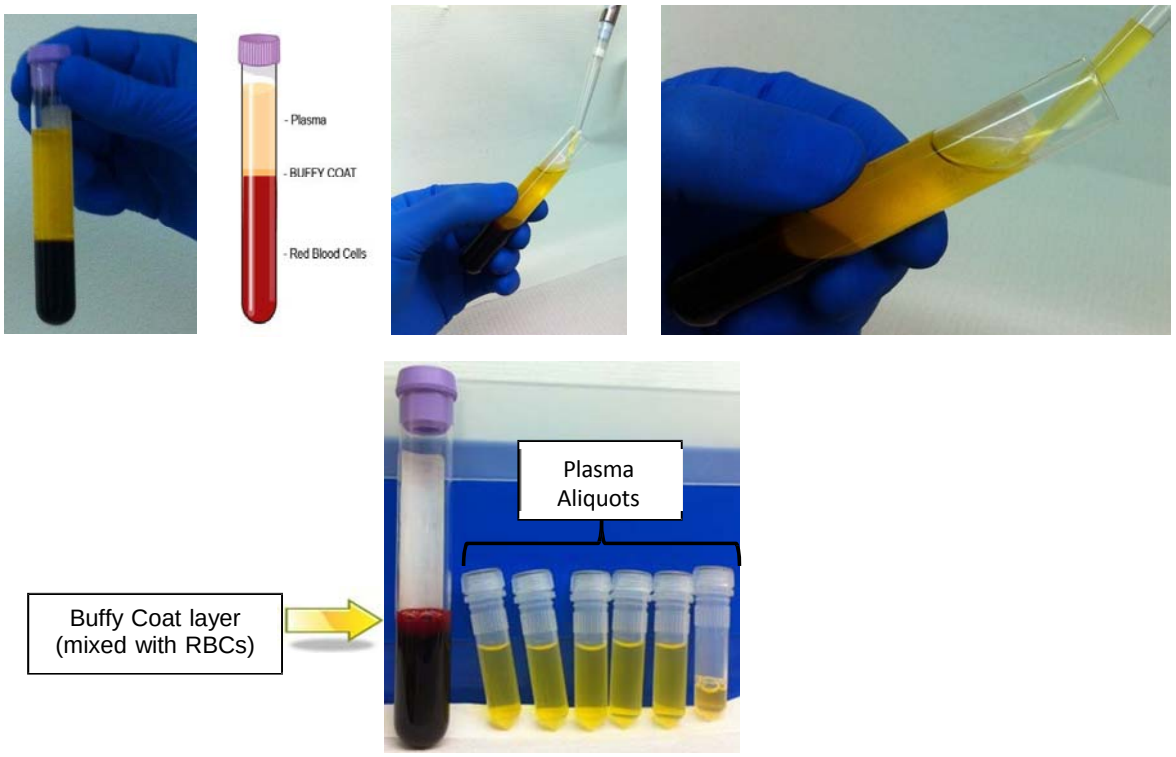
   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

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

6. Within 30 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.

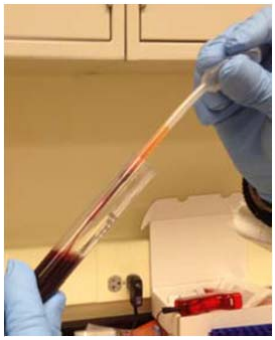
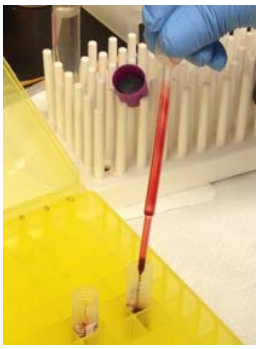
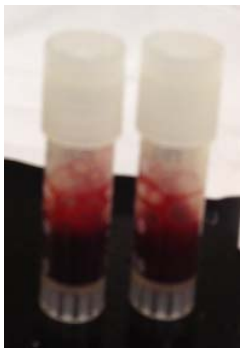
7. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the vacutainer tube, by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material (see below). Using a Fisherbrand™ disposable graduated transfer pipette, transfer plasma into the pre-labeled cryovials. 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). . Be sure to only place **plasma** in cryovials labeled with the suffix **01P to 06P.** Take caution not to disturb the pellet at the bottom of the tube. Place a PURPLE cap on each cryovial filled with plasma.



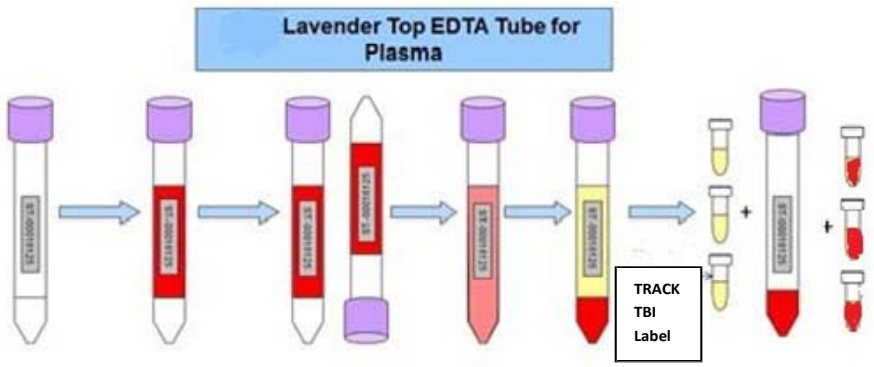
8. Place the labeled cryovials in the 81 grid cryovial box and freeze samples immediately following processing by transferring to **-80°C Freezer**. Record vial locations within the cryovial box and freezer on batch record and in BSIS. Store all samples at **-80°C until shipped** to TRACK-TBI BR on dry ice.

9. <u>In the BASELINE visit blood collection ONLY</u>, **after** plasma has been removed from the EDTA, purple top tube, aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs- see figure above) into labeled cryovials with a disposable graduated micropipette. Each aliquot should be 0.5 ml of buffy coat per cryovial tube in two cryovial tubes (as supplied in each specimen kit). Be sure to only place **buffy coat** in cryovials labeled with the suffix **07D or 08D** (again you may only use 1 cryovial depending on your blood draw yield). Place a CLEAR cap on each cryovial filled with buffy coat.

10. Dispose of the vacutainer with cell pellet.

11. Place labeled buffy coat cryovials in 81 grid cryovial box and place the box in **-80°C (or colder) Freezer.** Record vial locations within cryovial box and freezer on batch record.



1: Store tubes at *room* temp, label cryovial tubes with preprinted "plasma" and "buffy coat" labels prior to blood draw.

2: Collect blood into the purple top tube, allowing blood to flow 10 sec and ensuring blood has stopped flowing.

3: Immediately after blood draw, invert tube gently 8-10 times to mix sample.

4: Centrifuge sample for 15 min at 1500 x g.

5: Using graduated pipette, aliquot 0.5 ml plasma into "plasma" cryovials 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.

7.4 SERUM COLLECTION:

Whole Blood Collection for Isolation of Serum: 6 ml Red Top Tube (for processing of serum aliquots). One red top tube is collected at every study visit (baseline and follow-up).

1. Place pre-printed "**SERUM**" labels on the 2 ml cryovial tubes.

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

3. Using a blood collection set and a holder, collect blood into **6 ml red top tube** using your institution's recommended procedure for standard venipuncture technique

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

5. **Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

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

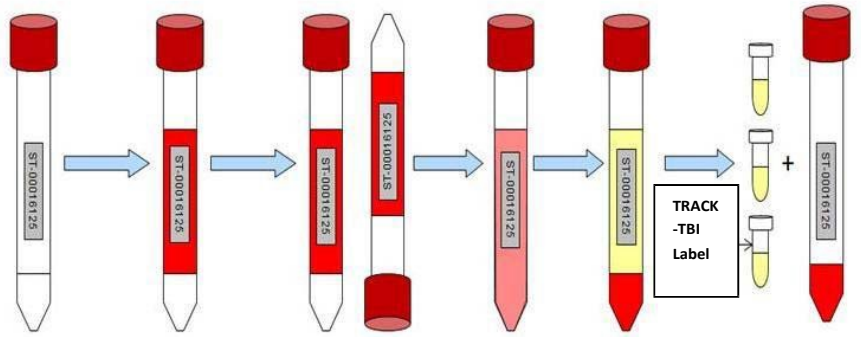
7. After 30 minutes of clotting, centrifuge the balanced vacutainer tube for 15 minutes at 1500 rcf (x g). **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix A to calculate RPM in your particular rotor, or refer to: http://www.sciencegateway.org/tools/rotor.htm**.

8. Remove the serum, being careful not to disturb the clot at the bottom of the tube, by tilting the tube and placing the Fisher disposable graduated pipette tip along the lower side of the glass wall without touching the pellet. Using a Fisherbrand™ disposable graduated transfer pipette, transfer 0.5 ml aliquots into each pre-labeled cryovial (5-6). The red top tube should yield, on average, 3 ml of blood serum for a total of 5-6 aliquot cryovial tubes per subject. Be sure to only place **serum** in cryovials labeled with the suffix **09S to 14S** (as before, you may fill less than 6 cryovials with serum- use the lowest numbered labels first). Place an ORANGE cap on each cryovial filled with serum.

9. Place cryovials in 81 grid cryovial box and freeze samples immediately in **-80°C Freezer**. Record vial locations within the cryovial box and freezer on batch record and.

10. Dispose of vacutainer with clotted blood in the bottom of the tube.

6ml Red Top Tubes for Serum



1: Store tubes at room temp, label tube with subject ID prior to blood draw.

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 mins. Then centrifuge tubes at 1500 x g for 15 minutes.

5: Label cryovials with TRACK-TBI BR "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.

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

See training videos for blood collection (http://www.preanalytix.com/videos/rna-tube-collection-video/) and freezing (http://www.preanalytix.com/videos/rna-tube-freezing-video/).

1. Place "**RNA**" label on the PAXgene RNA tube prior to blood draw with the **15R** suffix label (per Section 6.1); no processing is required for this tube**; the single tube is to be shipped to the TRACK-TBI BioRepository frozen without processing at the collection site.**

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

3. Using a blood collection set and a holder, collect blood into the **PAXgene™ Blood RNA Tube** using your institution's recommended procedure for standard venipuncture technique.

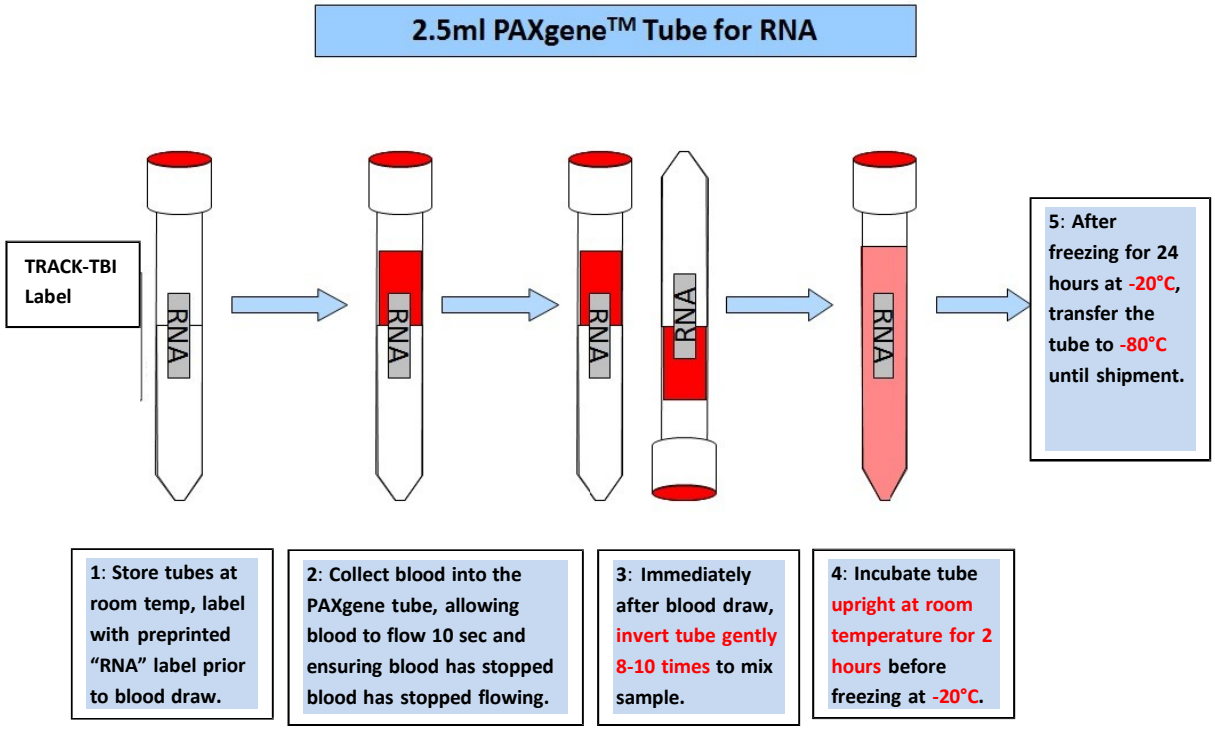
   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record time of blood draw on Laboratory Procedures data form.

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

6. Incubate the PAXgene™ Blood RNA Tube **UPRIGHT at room temperature (18°C to 25°C) for 2 hours**. Record time and date of draw on Laboratory Procedures data form.

7. After **2 hours** at room temperature, place the PAXgene tube upright in a **WIRE** rack and transfer the PAXgene tube to a **-20°C freezer.** Keep the **PAXgene™ Blood RNA Tube at -20°C** for 24 hours, then transfer the tube to an **-80°C freezer** for storage until you ship on dry ice to the TRACK-TBI BioRepository. Complete remainder of the Laboratory Procedures data form.


2.5ml PAXgene™ Tube for RNA
TRACK-TBI Label
RNA
RNA
RNA
RNA
RNA
5: After freezing for 24 hours at -20°C, transfer the tube to -80°C until shipment.
1: Store tubes at room temp, label with preprinted "RNA" label prior to blood draw.
2: Collect blood into the PAXgene tube, allowing blood to flow 10 sec and ensuring blood has stopped blood has stopped flowing.
3: Immediately after blood draw, invert tube gently 8-10 times to mix sample.
4: Incubate tube upright at room temperature for 2 hours before freezing at -20°C.

8. Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping and can obtain training and certification through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/). Check your local institution's Environmental Health & Safety if additional training in the Shipment of Hazardous Material is needed.

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

For frozen shipments, include no more than **five** STP-740 Extra-large Secondary envelopes per E200 shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 36 hours.

The labeled, processed, aliquotted and frozen cryovials of plasma, buffy coat, serum, and frozen unprocessed PAXgene RNA tubes will be shipped to the TRACK-TBI BioRepository as outlined below.

8.1 TRACK-TBI BR Shipping Instructions

Baseline and Follow-up Shipments to the TRACK-TBI 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)**

Specimens being shipped to the TRACK-TBI BioRepository 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.*

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.

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED ON MONDAY OR TUESDAY ONLY!

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 or Tuesday!)
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

To ship 16 cryoboxes and 12 PaxGene tubes
Request from World Courier the following items in the quantities indicated:

Shipping materials	Quantity	Description
Thermosafe E200	**1**	An E200 insulated box can hold 5 STP-740 packages. This should be large enough for 16 cryoboxes + 12 PAXGene tubes + dry ice
STP-740	**5**	The STP-740 is an Extra large Secondary envelope. Each STP-740 can hold 4 81- grid cryoboxes. or an STP-710 envelope filled with wrapped PAXGene tubes.
STP-710	**1**	The STP-710 is a smaller Secondary envelope Each STP-710 holds 12 10ml PAXGene tubes
absorbent material vial dividers	**6**	**World Courier provides these. Be sure to ask for them.** Place the PAXGene tubes in the vial dividers. Include 1 absorbent material pad in the Secondary envelope with the cryoboxes.
House WayBill	**1**	Comes pre-printed with Shipper and Consignee information
Box labels		World Courier provides these
Dry ice		World Courier will bring when picking up your shipment

ThermoSafe model E200UPS is the insulated shipper.

The E200 insulated shipping box is large enough for 6 filled STP-740 packages.

This should be large enough to hold
16 cryoboxes + 12 PAXgene tubes + dry ice.

E200UPS –
Polyurethane foam insulated container with plug.

Outer Dimensions: 20 x 20 x 19 inches (50.8 x 50.8 x 48.3 cm)
Inner Dimensions: 16 x 16 x 13 inches (40.6 x 40.6 x 33.0 cm)

STP-710 Disposable 2-Part Secondary Pressure Vessel, Medium

STP-710

STP-710 Disposable 2-Part Secondary Pressure Vessel, Medium

STP-710 Disposable 2-Part Secondary Pressure Vessel, Medium. System Components: Inner leak proof polybag - 9 1/4 X 12 inches and Tyvek outer envelope - 7 1/2 X 9 1/2 inches. Maximum Capacity - Ten 10ml vials or 2 inch grid box. 50 per Case

Use the STP-710 packaging for the wrapped PAXGene tubes.

STP-740 Disposable 2- Part Secondary Pressure Vessel, Extra Large

STP-740

STP-740 Disposable 2- Part Secondary Pressure Vessel, Extra Large

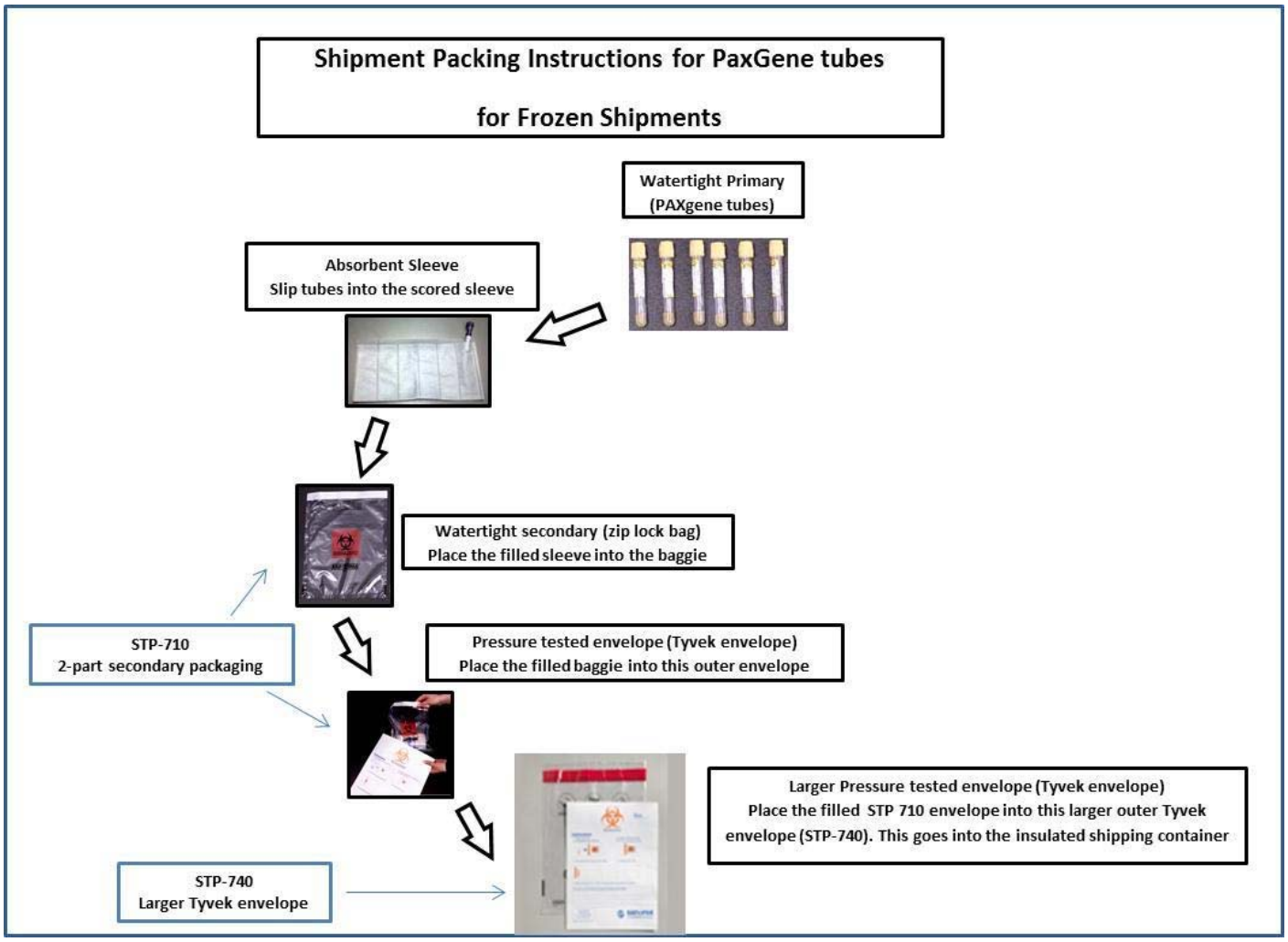
STP-740 Includes Inner Leak Proof Polybag: 14 X 19 Inches and Tyvek Outer Envelope: 12 X 16 Inches. Excellent for transport of forensic evidence, body parts and diagnostic kits. 50 per Case

For the STP-740 packaging
1. Place the filled STP-710 envelope into a STP-740 envelope
2. Place 4 filled 81-grid cryoboxes into a STP-740

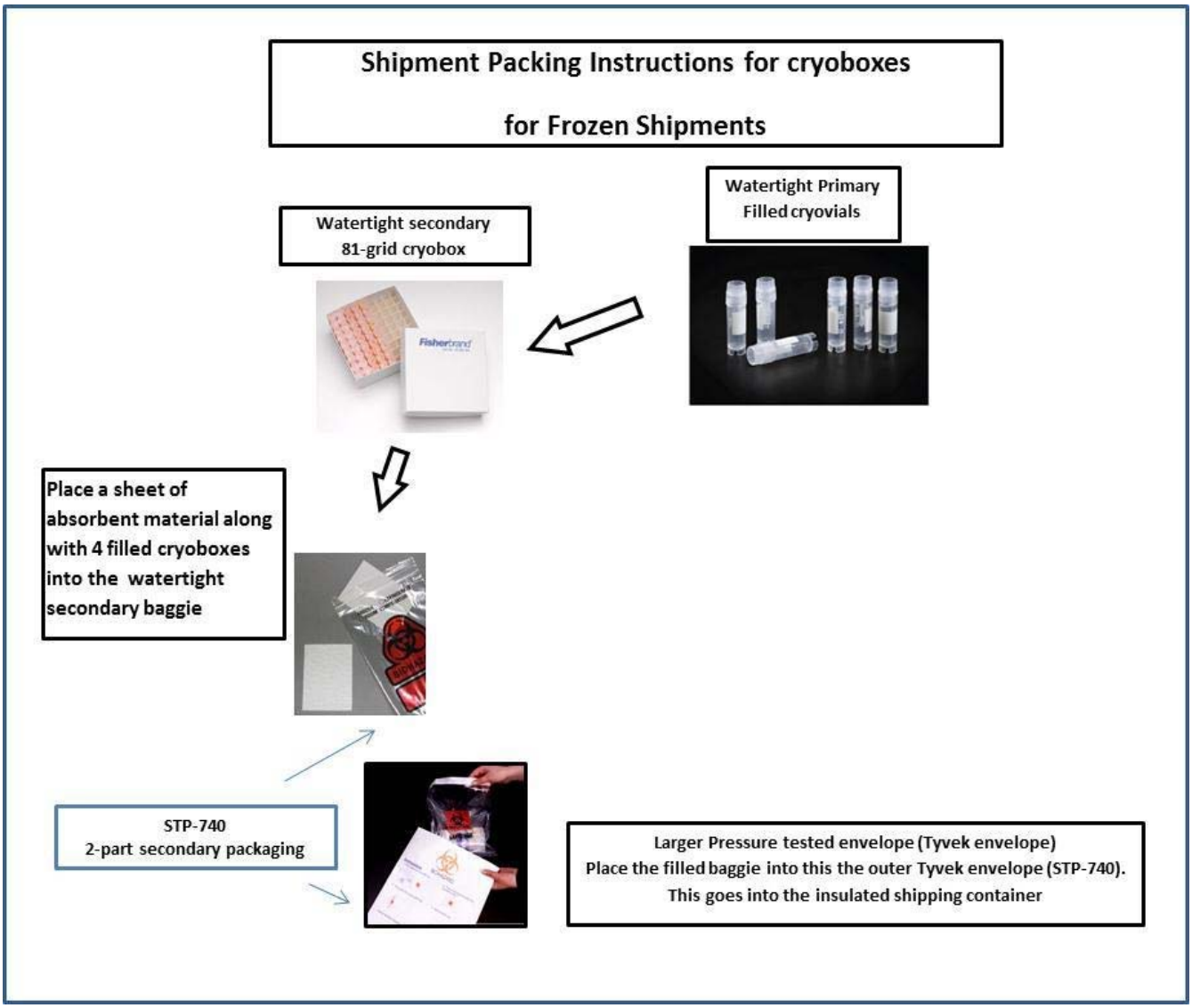
The International Air Transport Association packing instructions for shipping Biological materials, IATA 650, can be found at https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

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



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 large, sealed Tyvek envelopes **UPRIGHT** in the Styrofoam-lined shipping carton.

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

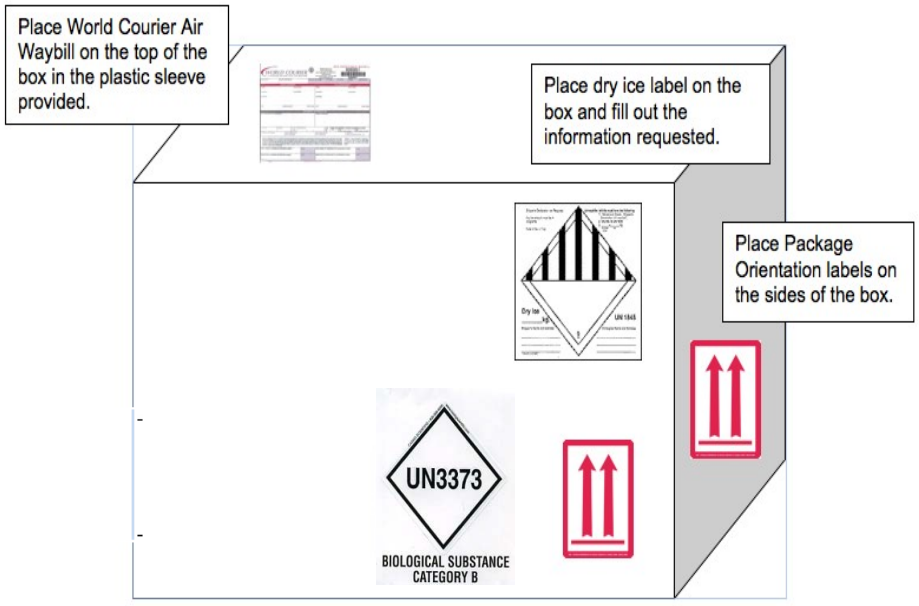
IMPORTANT!
AN ITEMIZED LIST OF CONTENTS MUST BE ENCLOSED BETWEEN THE SECONDARY PACKAGING AND THE OUTER PACKAGING.

***** 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 (biohazard bag) 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.
- 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

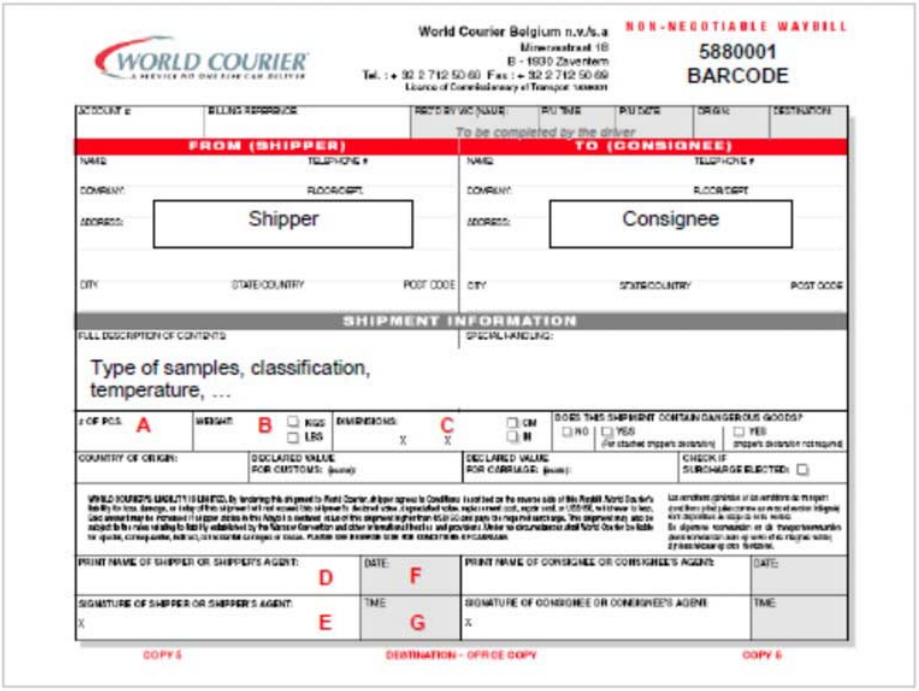


The package must have a UN3373 Biological Substance, Category B label

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 S-916
PITTSBURGH, PA 15261

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

2. **Notify the TRACK-TBI BioRepository of your intent to send a shipment by emailing the TRACK-TBI BR at:**

   rabinowitzmk@upmc.edu

3. When the shipment is sent send an email to the address above and include the

   Excel electronic manifest (see APPENDIX E for an example of the TRACK-TBI manifest)

   The Excel electronic manifest is uploaded into the database and should match the specimens being sent. This helps in accessioning the specimens into the database at the TRACK-TBI BR.

4. If you have any questions or concerns, contact Miri Rabinowitz, TRACK-TBI Biorepository manager.
   rabinowitzmk@upmc.edu or (412)-648-2031

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

9. Sample Quality Checks and Feedback to Projects

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by the TRACK-TBI BioRepository for each sample type. 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. Data Queries and Reconciliation

The Laboratory worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

QuesGen will be collaborating with the TRACK-TBI BR to reconcile information captured in the QuesGen database compared to samples received and logged at the TRACK-TBI BR. Information that appears incorrect in the QuesGen database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication.

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

- Missing samples at the BR
- Incorrect samples collected and shipped to the BR
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged at the TRACK-TBI BR compared to information entered into the QuesGen database.

11. Appendices

Appendix A: Rate of Centrifugation Worksheet

Appendix B: Sample Record Summary and Shipment Notification Form

Appendix C: TRACK-TBI BioRepository Lab Worksheet

Appendix D: TRACK-TBI BioRepository Sample Submission Non-Conformance Report

Appendix E: TRACK-TBI BioRepository Electronic Manifest Form

Appendix F: TRACK-TBI CSF Collection protocol

Appendix G: TRACK-TBI add-On study for Abbott Laboratories

APPENDIX

App A: Rate of Centrifugation Worksheet

Please complete and email this form to the TRACK-TBI BR Lab Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your TRACK-TBI Biologics MOP. Alternatively, use the formula below or refer to the following website to perform the calculations yourself (www.hettweb.com/mobile-app). **Please call with any questions at (412) 648-2031.**

Submitter Information

Name:

Site Number:

Submitter Email:

Centrifuge Information (Please answer the following questions about your centrifuge)

Centrifuge Type:

☐ Fixed Angle Rotor
☐ Swing Bucket Rotor

Radius of Rotation (mm):

Determine centrifuge's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Comments

These values are calculated using the formula below:

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

RCF = relative centrifugal force (x g)

RPM = rotational speed (revolutions per minute)

r = centrifugal radius in mm = distance from the center of the turning axis to the bottom of the centrifuge.

Email this form to:
TRACK-TBI BR Lab Manager
rabinowitzmk@upmc.edu

It is very important to this study that all samples be processed correctly.

App B: TRACK-TBI BioRepository Sample Record Summary and Shipment Notification

Site Name/Number:_____ **Coordinator:**_____

Tel:_____ **Email:**_____

Please list only ONE subject per Sample Record Summary and Shipment Notification Form

TRACK-TBI Study ID (TR-XX-XXXX-X-XXX): _____ **Visit ID:** _____

Date Sample(s) Shipped: _____ **World Courier Tracking Number:** _____

Instructions: Ship frozen shipments Monday or Tuesday ONLY! This form must be completed for shipment of all research samples. Notify TRACK-TBI BR (email preferred) in advance of shipment using contact information below. Place a copy of this form in the shipment box email a copy to the TRACK-TBI BR and file a copy of the completed form in the study binder. **Ensure all frozen shipments are filled with DRY ICE. In the table below, please indicate the tube ID (from pre-printed labels)**

Completed by Submitter/Site			Completed by TRACK-TBI BioRepository
Date of Draw	Specimen Type	Tube ID Number (BSIS#)	Notation of Problems
	Plasma		
	Buffy Coat		
	Serum		
	RNA		

Contact Information:
Miri Rabinowitz, Manager TRACK-TBI BioRepository
Email: rabinowitzmk@upmc.edu
Phone: 412-648-2031

App C: TRACK-TBI BioRepository LAB WORKSHEET

Processing of Blood Samples at Time of Baseline Collection	
1. EDTA-treated blood (One 6 ml purple-top tube). Centrifuge at 1500 g x 15 minutes within 30 minutes of collection. Process as follows:	
Plasma vials with purple caps Numbered 1-6	*For TRACK-TBI plasma*: 0.5 ml of plasma aliquotted to each TRACK-TBI BR labeled cryovial. Store in cryovial box at -80°C until shipment to TRACK-TBI BR.
Buffy Coat vials with clear caps Numbered 7-8	*For TRACK-TBI DNA extraction:* 0.5 ml of EDTA-treated buffy coat / red blood cells transferred to TRACK-TBI labeled "DNA" cryovials. Store in cryovial box at -80°C until shipment to TRACK-TBI BR.
2. Serum tube (One 6 ml red-top tubes). Tube is placed at room temperature at time of collection, and allowed to clot for 30 minutes. Then the red top tube is centrifuged at 1500 x g for 15 minutes. Serum is aspirated and 0.5 ml aliquots transferred to serum cryovials for the TRACK-TBI BR. Clotted RBCs are discarded.	
Serum vials with orange caps Numbered 9-14	*For TRACK-TBI serum*: 0.5 ml of serum aliquotted to each TRACK-TBI BR labeled cryovial. Store in cryovial box at -80°C until shipment to TRACK-TBI BR.
3. PAXgene™ tube (One 2.5 ml tube). *For TRACK-TBI BR RNA collection:* Sit tube upright at room temperature for 2 hours after collection. Then freeze at -20°C for 24 hours. Then transfer to -80°C until shipment to TRACK-TBI BR.	

Time of collection:_____Time of centrifugation:_____Time of freezing (20°C):_____

Processing of Blood Samples at All Follow-Up Collections	
1. EDTA-treated blood (One 6 ml purple-top tube). Centrifuge at 1500 g x 15 minutes within 30 minutes of collection. Process as follows:	
Plasma vials numbered 1-6 (*no vials numbered 7 or 8- no DNA collected*)	*For TRACK-TBI plasma*: 0.5 ml of plasma aliquotted to each TRACK-TBI BR labeled cryovial. Store in cryovial box at -80°C until shipment to TRACK-TBI BR.
2. Serum tube (One 6 ml red-top tubes). Tube is placed at room temperature at time of collection, and allowed to clot for 30 minutes. Then the red top tube is centrifuged at 1500 x g for 15 minutes. Serum is aspirated and 0.5 ml aliquots transferred to serum cryovials for the TRACK-TBI BR. Clotted RBCs are discarded.	
Serum vials Numbered 9-14	*For TRACK-TBI serum*: 0.5 ml of serum aliquoted to each TRACK-TBI BR labeled cryovial. Store in cryovial box at -80°C until shipment to TRACK-TBI BR.
3. PAXgene™ tube (One 2.5 ml tube). *For TRACK-TBI BR RNA collection:* Sit tube upright at room temperature for 2 hours after collection. Then freeze at -20°C for 24 hours. Then transfer to -80°C until shipment to TRACK-TBI BR.	

Time of collection:_____Time of centrifugation:_____Time of freezing (20°C):_____

App D: TRACK-TBI BioRepository Biomarker Sample Submission Non-Conformance Report

This form is to be completed by the TRACK-TBI BioRepository personnel when a sample has been received and issues are noted. Completed form is to be emailed or faxed to submission site coordinators and Coordination Centers.

Site Name/Number:_____

TRACK-TBI Study ID:_____ **Visit ID:** _____

Received by:_____ **Date:**_____

Your shipment was received with the observed problem(s) checked below. Please take note so that your future shipments are received without incident.

	Samples shipped on Thursday, Friday or Saturday
	Samples arrived on Saturday or Sunday
	Advanced notice of shipment not provided
	Shipment notification does not match shipment notification form received with samples
	No shipment notification form included in package
	Shipment Notification form incomplete
	Package contents do not match shipment notification form
	Package received has little/no dry ice
	Signs of sample thawing present
	Samples submitted in non-standard tubes
	Sample tubes damaged/cracked
	Samples not labeled appropriately/labels peeling off
	PAXgene™ tube received with low volume
	Cryovial tubes received with low volume
	Unexpected sample(s) received (specified in comments below)
	Other (specified in comments below)

Comments:

_____

_____

APPENDIX E: *SAMPLE*- TRACK-TBI BioRepository Electronic Manifest Form

Sample ID (Subject)	Visit	Material Type	Material Modifier	Date/Time Drawn	Date/Time Processed	Date/Time Frozen	Date/Time Shipped	Site
TR-01-1001-A-01P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-02P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-03P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-04P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-05P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-06P	Day 1	PLASMA	EDTA					Baylor
TR-01-1001-A-07D	Day 1	Buffy Coat	EDTA					Baylor
TR-01-1001-A-08D	Day 1	Buffy Coat	EDTA					Baylor
TR-01-1001-A-09S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-10S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-11S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-12S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-13S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-14S	Day 1	SERUM	SST					Baylor
TR-01-1001-A-15R	Day 1	WHOLE BLOOD	PAX Gene					Baylor
TR-01-1001-B-01P	Day 3	PLASMA	EDTA					Baylor
TR-01-1001-B-02P	Day 3	PLASMA	EDTA					Baylor
TR-01-1001-B-03P	Day 3	PLASMA	EDTA					Baylor
TR-01-1001-B-04P	Day 3	PLASMA	EDTA					Baylor
TR-01-1001-B-05P	Day 3	PLASMA	EDTA					Baylor
TR-01-1001-B-06P	Day 3	PLASMA	EDTA					Baylor

Appendix F: Cerebrospinal Fluid Collection Procedures

1. The decision to place an External Ventricular Drainage (EVD) is a local clinical decision and is not affected by a patient's participation in TRACK-TBI. Similarly, indications and procedures for CSF drainage (continuous vs. intermittent drainage) is a local clinical decision and not prescribed in the TRACK-TBI protocol.
   CSF collected for research purposes is fluid that would otherwise be discarded.

2. Procedures for inserting the EVD and for collecting fluid from the system are also governed by local NeuroICU 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]). ww.aann.org/uploads/AANN11_ICP**EVD**new.pdf

   A video demonstrating CSF collection is available.
   DISCLOSURE: This tutorial is to assist trained personnel in CSF collection from an EVD. Each site may differ in procedure. Check your local NeuroICU protocol. Also, this video shows betadine for cleaning the port in a sterile fashion; this has been changed to chlorhexidine.

3. The collection of CSF from the EVD system is performed by trained NeuroICU 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.

4. An effort should be made to collect the first CSF available at the time of insertion of the EVD. This should be handled in the same way as fluid collected at later times (# 5 – 7 below). Up to 10 mL should be collected.

5. **Procedures for CSF collection.** CSF is collected twice daily, once in the morning and a second time at least 2 hours after the first collection.

   a. Fluid collected **overnight** in the buretrol (over a period of several hours, varying according to unit protocol and drainage rate) is drained into the collection bag. The sample can be collected from the port in the buretrol or the bag **(using sterile technique)**, or coordinate with the ICU nurse to connect a new bag, collecting fluid from the old bag. Up to 10 mL is collected for research processing in a single polypropylene conical centrifuge tube (provided).

   b. **Fresh fluid** is collected as follows: After emptying the buretrol into the collection bag, wait at least 1 hour to obtain 3-10mL (this is patient dependent. Some patients produce more than others). Fluid collected over this > 1 hour period in the buretrol is collected **using sterile technique** directly from the buretrol. Up to 10 mL is collected (although in most cases it will be less) and transferred to single polypropylene conical centrifuge tube. Fluid is allowed to drain into buretrol by gravity (**never aspirated**).

c. The following are noted in the Case Report Form (see below)

i. Appearance of fluid (clear, cloudy, bloody)

ii. Date and Time of collection

iii. Volume collected (from overnight collection as well as from the 1 hour collection)

iv. Whether CSF drainage was being done continuously or intermittently

v. Time of centrifugation and freezing (see #8 below)

6. **Processing of CSF.** Cell contamination of ventricular CSF is a significant confound. To minimize, CSF is centrifuged.

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

b. Supernatant from both overnight collection and 1-hour collection are kept separate.

c. Transfer supernatant to polypropylene cryovials (provided) using a transfer pipette (provided). Up to 5 aliquots are prepared, each containing up to 1.0 mL. If less fluid is collected, decrease volume of aliquots down to a minimum of 0.5 mL.

i. For example, 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.

d. Transfer to -80^{o}C freezer. Temporary transfer to -20^{o}C freezer is allowed if that is only what is available.

7. This CSF collection protocol can run for a maximum of 7 consecutive days (maximum of 13 CSF collections)

a. Collect 1 CSF sample at EVD placement.
b. Collect CSF twice each day for the next 6 days.
c. On days 1, 3, and 5 collect the TRACK-TBI blood sample at the same time as collecting one of the CSF samples. This will provide a paired blood sample for some of the CSF samples

9. Samples are shipped to the TRACK-TBI Biorepository at the Univ. of Pittsburgh using same procedures as done for blood samples.

CSF Collection Case Report Forms

FOR EACH TRACK-TBI SUBJECT ENROLLED IN THIS ADD-ON CSF COLLECTION PROTOCOL, **MAKE A PHOTOCOPY OF THESE CRF SHEETS**.

BE SURE TO INCLUDE THE CSF PATIENT ID AND CORRESPONDING **TRACK-TBI PATIENT ID** ON **THE FIRST** PAGE. INCLUDE THE CSF PATIENT ID ON ALL OTHER PAGES

RETURN COPIES OF THESE COMPLETED FORMS TO THE TRACK-TBI BIOREPOSITORORY WHEN YOU SEND THE CRF SAMPLES TO THE BIOREPOSITORY.

IF YOU HAVE ANY QUESTIONS OR CONCERNS PLEASE CONTACT EITHER AVA PUCCIO, RN, PHD at PuccAM@UPMC.EDU OR MIRI RABINOWITZ, PHD at RABINOWITZMK@UPMC.EDU

CSF PATIENT ID: CS __ __ __ __ __ __

TRACK –TBI PATIENT ID: TR__ __ __ __ __ __

Sample at time of EVD Insertion (A):

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected:___mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 1 Overnight Fluid (B)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 1 Fresh Fluid (C)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 2 Overnight Fluid (D)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 2 Fresh Fluid (E)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 3 Overnight Fluid (F)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 3 Fresh Fluid (G)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 4 Overnight Fluid (H)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 4 Fresh Fluid (I)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 5 Overnight Fluid (J)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 5 Fresh Fluid (K)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 6 Overnight Fluid (L)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

Day 6 Fresh Fluid (M)

Available ☐ Not Available ☐

Date of Collection: _____ Time of Collection: _____

Volume Collected: _____mLs

Drainage protocol: Continuous ☐ Intermittent ☐

Appearance: Clear ☐ Bloody ☐ Cloudy ☐

Time of Centrifugation: _____ Time of Freezing: _____

CSF Kits:

The CSF protocol runs for 7 days (max) per subject.

1. Collect 1 CSF sample at EVD placement.
2. Collect CSF twice each day for the next 6 days.
3. At each time point collect 10mL CSF into one 15mL conical tube
4. Each CSF sample will be divided into 5 aliquots (max)

A kit for **each** separate CSF draw WILL include:

	CSF kit
1	15 ml conical tube
5	cryovials
5	clear caps
1	transfer pipette
1	biohazard pouch

(A subject who completes the entire protocol would use 13 kits)

Cryoboxes for sample storage at -80Cwill be included in the shipments.

Sheets of pre-printed labels (for conical tubes and cryovials) will be provided.

On the next page is an example of a sheet of preprinted labels for a single participant completing the full CSF protocol (13 CSF collections).
The following page explains the labels A-M.

SAMPLE SHEET OF LABELS

CS-03-1001-15ml conical (A: Day 0: EVD placement)		**CS-03-1001-15ml conical (B: Day 1: AM- Overnight)**	**CS-03-1001-15ml conical (C: Day 1: 2nd draw Fresh)**	**CS-03-1001-15ml conical (D: Day 2: AM- Overnight)**
CS-03-1001-15ml conical (E: Day 2: 2nd draw Fresh)	**CS-03-1001-15ml conical (F: Day 3: AM- Overnight)**	**CS-03-1001-15ml conical (G: Day 3: 2nd draw Fresh)**	**CS-03-1001-15ml conical (H: Day 4: AM- Overnight)**	**CS-03-1001-15ml conical (I: Day 4: 2nd draw Fresh)**
CS-03-1001-15ml conical (J: Day 5: AM- Overnight)	**CS-03-1001-15ml conical (K: Day 5: 2nd draw Fresh)**	**CS-03-1001-15ml conical (L: Day 6: AM- Overnight)**	**CS-03-1001-15ml conical (M: Day 6: 2nd draw Fresh)**	
CS-03-1001-A-01C	**CS-03-1001-A-02C**	**CS-03-1001-A-03C**	**CS-03-1001-A-04C**	**CS-03-1001-A-05C**
CS-03-1001-B-01C	**CS-03-1001-B-02C**	**CS-03-1001-B-03C**	**CS-03-1001-B-04C**	**CS-03-1001-B-05C**
CS-03-1001-C-01C	**CS-03-1001-C-02C**	**CS-03-1001-C-03C**	**CS-03-1001-C-04C**	**CS-03-1001-C-05C**
CS-03-1001-D-01C	**CS-03-1001-D-02C**	**CS-03-1001-D-03C**	**CS-03-1001-D-04C**	**CS-03-1001-D-05C**
CS-03-1001-E-01C	**CS-03-1001-E-02C**	**CS-03-1001-E-03C**	**CS-03-1001-E-04C**	**CS-03-1001-E-05C**
CS-03-1001-F-01C	**CS-03-1001-F-02C**	**CS-03-1001-F-03C**	**CS-03-1001-F-04C**	**CS-03-1001-F-05C**
CS-03-1001-G-01C	**CS-03-1001-G-02C**	**CS-03-1001-G-03C**	**CS-03-1001-G-04C**	**CS-03-1001-G-05C**
CS-03-1001-H-01C	**CS-03-1001-H-02C**	**CS-03-1001-H-03C**	**CS-03-1001-H-04C**	**CS-03-1001-H-05C**
CS-03-1001-I-01C	**CS-03-1001-I-02C**	**CS-03-1001-I-03C**	**CS-03-1001-I-04C**	**CS-03-1001-I-05C**
CS-03-1001-J-01C	**CS-03-1001-J-02C**	**CS-03-1001-J-03C**	**CS-03-1001-J-04C**	**CS-03-1001-J-05C**
CS-03-1001-K-01C	**CS-03-1001-K-02C**	**CS-03-1001-K-03C**	**CS-03-1001-K-04C**	**CS-03-1001-K-05C**
CS-03-1001-L-01C	**CS-03-1001-L-02C**	**CS-03-1001-L-03C**	**CS-03-1001-L-04C**	**CS-03-1001-L-05C**
CS-03-1001-M-01C	**CS-03-1001-M-02C**	**CS-03-1001-M-03C**	**CS-03-1001-M-04C**	**CS-03-1001-M-05C**

TIME	VIAL TYPE	LABELS				
EVD PLACEMENT (A)	One 15mL Conical tube	CS-03-1001-15ml conical (A: Day 0: EVD placement				
	Five 1.5 mL cryovials	CS-03-1001-A-01C	CS-03-1001-A-02C	CS-03-1001-A-03C	CS-03-1001-A-04C	CS-03-1001-A-05C
DAY 1 (B)	One 15mL Conical tube	CS-03-1001-15ml conical (B: Day 1: AM - Overnight)				
DAY 1 (B)	Five 1.5 mL cryovials	CS-03-1001-B-01C	CS-03-1001-B-02C	CS-03-1001-B-03C	CS-03-1001-B-04C	CS-03-1001-B-05C
DAY 1 (C)	One 15mL Conical tube	CS-03-1001-15ml conical (C: Day 1: 2nd draw Fresh))				
DAY 1 (C)	Five 1.5 mL cryovials	CS-03-1001-C-01C	CS-03-1001-C-02C	CS-03-1001-C-03C	CS-03-1001-C-04C	CS-03-1001-C-05C
DAY 2 (D)	One 15mL Conical tube	CS-03-1001-15ml conical (D: Day 2: AM - Overnight)				
DAY 2 (D)	Five 1.5 mL cryovials	CS-03-1001-D-01C	CS-03-1001-D-02C	CS-03-1001-D-03C	CS-03-1001-D-04C	CS-03-1001-D-05C
DAY 2 (E)	One 15mL Conical tube	CS-03-1001-15ml conical (E: Day 2: 2nd draw Fresh)				
DAY 2 (E)	Five 1.5 mL cryovials	CS-03-1001-E-01C	CS-03-1001-E-02C	CS-03-1001-E-03C	CS-03-1001-E-04C	CS-03-1001-E-05C
DAY 3 (F)	One 15mL Conical tube	CS-03-1001-15ml conical (F: Day 3: AM - Overnight)				
DAY 3 (F)	Five 1.5 mL cryovials	CS-03-1001-F-01C	CS-03-1001-F-02C	CS-03-1001-F-03C	CS-03-1001-F-04C	CS-03-1001-F-05C
DAY 3 (G)	One 15mL Conical tube	CS-03-1001-15ml conical (G: Day 3: 2nd draw Fresh)				
DAY 3 (G)	Five 1.5 mL cryovials	CS-03-1001-G-01C	CS-03-1001-G-02C	CS-03-1001-G-03C	CS-03-1001-G-04C	CS-03-1001-G-05C
DAY 4 (H)	One 15mL Conical tube	CS-03-1001-15ml conical (H: Day 4: AM- Overnight)				
DAY 4 (H)	Five 1.5 mL cryovials	CS-03-1001-H-01C	CS-03-1001-H-02C	CS-03-1001-H-03C	CS-03-1001-H-04C	CS-03-1001-H-05C
DAY 4 (I)	One 15mL Conical tube	CS-03-1001-15ml conical (I: Day 4: 2nd draw Fresh)				
DAY 4 (I)	Five 1.5 mL cryovials	CS-03-1001-I-01C	CS-03-1001-I-02C	CS-03-1001-I-03C	CS-03-1001-I-04C	CS-03-1001-I-05C
DAY 5 (J)	One 15mL Conical tube	CS-03-1001-15ml conical (J: Day 5: AM - Overnight)				
DAY 5 (J)	Five 1.5 mL cryovials	CS-03-1001-J-01C	CS-03-1001-J-02C	CS-03-1001-J-03C	CS-03-1001-J-04C	CS-03-1001-J-05C
DAY 5 (K)	One 15mL Conical tube	CS-03-1001-15ml conical (K: Day 5: 2nd draw Fresh)				
DAY 5 (K)	Five 1.5 mL cryovials	CS-03-1001-K-01C	CS-03-1001-K-02C	CS-03-1001-K-03C	CS-03-1001-K-04C	CS-03-1001-K-05C
DAY 6 (L)	One 15mL Conical tube	CS-03-1001-15ml conical (L: Day 6: AM - Overnight)				
DAY 6 (L)	Five 1.5 mL cryovials	CS-03-1001-L-01C	CS-03-1001-L-02C	CS-03-1001-L-03C	CS-03-1001-L-04C	CS-03-1001-L-05C
DAY 6 (M)	One 15mL Conical tube	CS-03-1001-15ml conical (M: Day 6: 2nd draw Fresh)				
DAY 6 (M)	Five 1.5 mL cryovials	CS-03-1001-M-01C	CS-03-1001-M-02C	CS-03-1001-M-03C	CS-03-1001-M-04C	CS-03-1001-M-05C

App G: TRACK-TBI Add-On study for Abbott Laboratories

Manual of Procedures

Abbott study

5 May 2016

- **You are receiving this protocol because your site has agreed to participate in this pilot project for Abbott Labs as an add-on to the TRACK-TBI protocol. The 7 participating sites include:**

  01 (Baylor)
  03 (UCSF)
  04 (Cincinnati)
  07 (Pittsburgh)
  08 (Austin)
  10 (Washington)
  11 (VCU)

- **DO NOT BEGIN USING THIS PROTOCOL UNTIL YOUR INSTITUTION HAS RECEIVED THE NECESSARY IRB APPROVAL.**

- **For this study 4 vacutainer tubes of whole-blood are collected for serum (3) and plasma (1) isolation, using the same blood collection and processing protocols described previously for TRACK-TBI.**

- **To be counted as a complete sample, whole blood must be collected and processed for BOTH time points as described below.**

1. **The first blood draw must be within 24 hours of injury**.
This is a **single** additional vacutainer tube of blood in addition to what is normally collected on TRACK-TBI patients at baseline.

2. **The second blood draw is 3-6 hours after the first blood draw**.
For this blood draw, collect **three** (3) vacutainer tubes of whole blood. One serum and one plasma sample will be for the Abbott study, and one serum sample for TRACK-TBI.

Abbott Biospecimen Collections (Baseline and 3-6 hours later):

Sample Type	Blood draws (A, B, C, D)	3 red-top and 1 purple top Vacutainer tubes will be provided in each kit	Processing/ Aliquotting	Cryovial labels
Whole blood for isolation of serum	**A**: first blood draw must be within 24 hours of injury	**6 ml Red Top A** Baseline draw for Abbott	**SERUM: 0.5ml serum aliquots per 1.5 ml cryovial. Top with orange caps.**	**A-01S - A-06S**
	B & C: second blood draws are 3-6 hours after the first blood draw.	**6 ml Red Top B** 2nd draw for Abbott **6 ml Red Top C** 2nd draw for TRACK		**B-07S - B-12S C-13S - C-18S**
Whole blood for isolation of plasma	**D**: second blood draws are 3-6 hours after the first blood draw.	**6 ml Purple Top D** 2nd draw for Abbott	**PLASMA 0.5ml plasma aliquots per 1.5 ml cryovial. Top with purple caps.**	**D-19P - D-24P**

ALL SAMPLES COLLECTED UNDER THIS PROTOCOL ARE TO BE STORED IN THE PROVIDED CRYOBOXES MARKED "**ABBOTT STUDY**". THIS INCLUDES THE "C" SAMPLES COLLECTED DURING THE 2ND TIME POINT.

IT IS CRITICALLY IMPORTANT WHEN COMPLETING THE ELECTRONIC MANIFEST TO INCLUDE THE **CORRESPONDING TRACK-TBI SUBJECT ID** FOR EACH PARTICIPANT IN THE ABBOTT PROTOCOL.

Specimen Collection Kit Contents:

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject.

3	Red top vacutainer tubes
1	Purple top vacutainer tube
24	1.5 ml cryovials
18	orange caps
6	Purple caps
3	transfer pipettes
1	biohazard pouch

2 81-cell cryoboxes will be sent for every 10 kits shipped. These boxes will be labeled "Abbott Study" and should contain ALL the serum and plasma samples collected for this protocol.

Preprinted cryovial labels will be provided.

Blood Collection and Processing Procedures

Labeling Samples

- Each kit is supplied with 4 preprinted labels for the vacutainer tubes. This will help identify these blood collecting tubes as separate from the TRACK-TBI study tubes.

Baseline: Red Top A **Time 2: Red Top B** **Purple Top D** **Red Top C (for TRACK)**	AL-03-1010-**Red Top A** Baseline draw for **Abbott**	AL-03-1010- **Red Top B** Time 2 draw for **Abbott**	AL-03-1010- **Purple Top D** Time 2 draw for **Abbott**	AL-03-1010- **Red Top C** Time 2 draw for **TRACK**

- Each kit is supplied with labels for the specimens shipped to the TRACK-TBI BioRepository (24 labels for four (4) completed blood draws).
- Each cryovial will be labeled with an alphanumeric string. For example:
  AL-01-1001-A-01S (represents the first serum cryovial label from the first (baseline) Abbott blood draw on the first subject at TRACK-TBI site 1)
- The code used in the labeling is as follows:

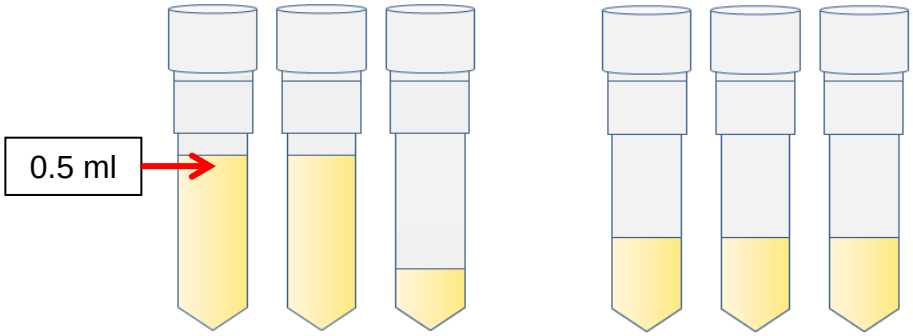
Study	Site	Subject ID	Blood Draw	Specimen Type and Vial
AL	**01** (Baylor) **03** (UCSF) 04 (Cincy) **07** (Pitt) **08** (Austin) **10** (Wash) **11** (VCU)	**1001 to 1020**	A thru **D** **A**: Day 1 (baseline) **B**: 3-6 hours after baseline (**Abbott**) **C**: 3-6 hours after baseline (**TRACK**) **D**: 3-6 hours after baseline (**Abbott**)	A (serum): **A-01S toA-06S** B (serum): **B-07S to B-12S** C (serum): **C-13S to C-18S** D (plasma): **D-19P to D-24P**

An example of one complete set of cryovial labels for an Abbott subject:

AL-03-1012-A-01S	AL-03-1012-A-02S	AL-03-1012-A-03S	AL-03-1012-A-04S	AL-03-1012-A-05S
AL-03-1012-A-06S	AL-03-1012-B-07S	AL-03-1012-B-08S	AL-03-1012-B-09S	AL-03-1012-B-10S
AL-03-1012-B-11S	AL-03-1012-B-12S	AL-03-1012-D-19P	AL-03-1012-D-20P	AL-03-1012-D-21P
AL-03-1012-D-22P	AL-03-1012-D-23P	AL-03-1012-D-24P		AL-03-1012-C-13S
AL-03-1012-C-14S	AL-03-1012-C-15S	AL-03-1012-C-16S	AL-03-1012-C-17S	AL-03-1012-C-18S

Filling Aliquot Cryovials

In order to ensure that the TRACK-TBI BioRepository receives a sufficient amount of the sample for processing and storage, and to avoid cracking of the cryovials prior to shipment, each aliquot tube should ideally be filled to 0.5 milliliters (see picture below) with serum or plasma after processing is completed. If there is biologic material remaining that will not fill a subsequent aliquot vial, that remaining amount should still be included and shipped to the TRACK-TBI BioRepository. Essentially, all material should be shipped to the TRACK-TBI BioRepository, ensuring maximum amount in as many aliquot cryovials as will allow after processing the sample. You do not have to fill all cryovial tubes provided; you should attempt to fill as many tubes as possible with 0.5 ml of sample. For example, if 2.7 ml of sample is obtained, you should fill 5 cryovial tubes each with 0.5 ml, and one additional cryovial tube with the remaining 0.2 ml.



Whole Blood (er Tube (for processing of serum aliquots). One red top tube is collected at baseline and two (2) red top tubes are collected at time 2, 3-6 hours after the baseline collection.

1. Place pre-printed "**SERUM**" labels on the 1.5 ml cryovial tubes.

2. Using a blood collection set and a holder, collect blood into the **6 ml red top tube** using your institution's recommended procedure for standard venipuncture technique

   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

4. **Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

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

6. After 30 minutes of clotting, **centrifuge the balanced vacutainer tube for 15 minutes at 1500 rcf (x g)**. It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation.

7. **Remove the serum**, being careful not to disturb the clot at the bottom of the tube, by tilting the tube and placing the disposable graduated pipette tip along the lower side of the glass wall without touching the pellet. **Using a disposable graduated transfer pipette, transfer 0.5 ml aliquots into each pre-labeled cryovial** (5-6). The red top tube should yield, on average, 3 ml of blood serum for a total of 5-6 aliquot cryovial tubes per subject.

   Be sure to only place **serum** in cryovials labeled with the suffix

   A-01S - A-06S (Baseline blood draw)

   B-07S - B-12S (2nd blood draw 3-6 hours after baseline, for Abbott)

   C-13S - C-18S (2nd blood draw 3-6 hours after baseline, for TRACK)

8. Place an **ORANGE** cap on each cryovial filled with serum.

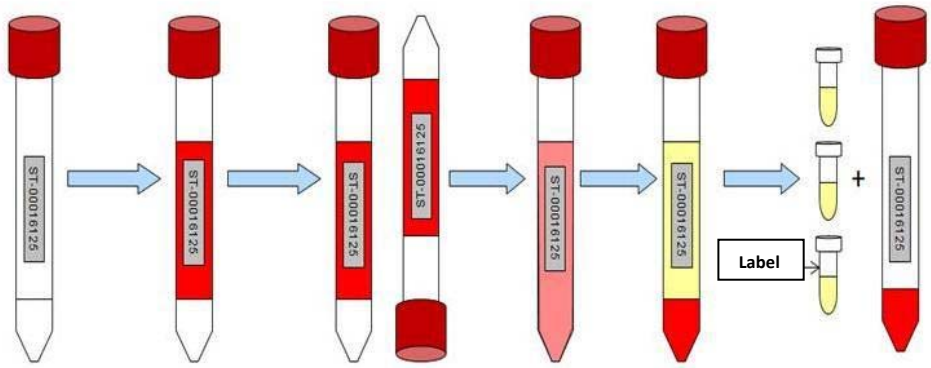
9. Place cryovials in an 81 grid cryovial box and freeze samples immediately in **-80°C Freezer**. **Record vial locations within the cryovial box and freezer on batch record**.

   STORE THESE SAMPLES IN THE PROVIDED CRYOBOXES LABELLED "ABBOTT STUDY". KEEP THESE SAMPLES SEPARATE FROM THE TRACK-TBI SAMPLES OF THE PARENT STUDY

10. Dispose of vacutainer with clotted blood in the bottom of the tube.

IT IS CRITICALLY IMPORTANT WHEN COMPLETING THE ELECTRONIC MANIFEST TO INCLUDE THE CORRESPONDING TRACK-TBI SUBJECT ID FOR EACH PARTICIPANT IN THE ABBOTT PROTOCOL.

6ml Red Top Tubes for Serum



1: Store tubes at room temp, label tube with subject ID prior to blood draw.

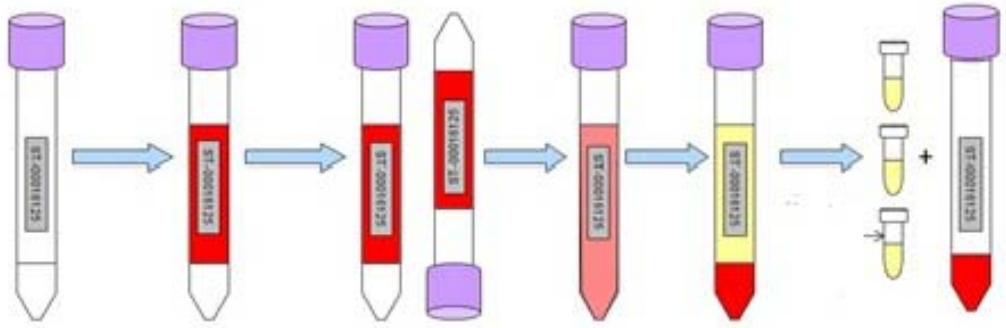
2: Collect blood into red top tube, allowing blood to flow for 10 sec and ensuring blood has stopped flowing.

3: Immediately after blood draw, invert tube gently 5 times to mix sample.

4: Allow blood to clot for 30 mins. Then centrifuge tubes at 1500 x g for 15 minutes.

5: Label cryovials with Abbott "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.

6ml Purple Top Tubes for Plasma



1: Store tubes at *room* temp, label cryovial tubes with preprinted "plasma" " labels prior to blood draw.

2: Collect blood into the purple top tube, allowing blood to flow 10 sec and ensuring blood has stopped flowing.

3: Immediately after blood draw, invert tube gently 8-10 times to mix sample.

4: Centrifuge sample for 15 min at 1500 x g.

5: Label cryovials with Abbott "plasma" labels. Using graduated pipette, aliquot 0.5 ml plasma into "plasma" cryovials . Cap plasma cryovials with purple caps; . Store at -80°C until shipment.

Whole Blood Collection for Isolation of Plasma: 6 ml EDTA Purple Top Tube (for processing of plasma aliquots).

1. Place the pre-printed "**PLASMA**" labels on the 1.5 ml cryovial tubes (6).

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 standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

3. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

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

5. Within 30 minutes of blood collection, centrifuge balanced tube for 15 minutes at 1500 RCF (x g) with no brake. **It is critical that the tube 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.

6. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the vacutainer tube, by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material. Using a disposable graduated transfer pipette, transfer plasma into the pre-labeled cryovials. 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).
   Be sure to only place **PLASMA** in cryovials labeled with the suffix

   D-19P - D-24P (2nd blood draw 3-6 hours after baseline, for Abbott)

7. Place a **PURPLE** cap on each cryovial filled with PLASMA.

8. Place cryovials in 81 grid cryovial box and freeze samples immediately in **-80°C Freezer**.
   Record vial locations within the cryovial box and freezer on batch record.
   STORE THESE SAMPLES IN THE PROVIDED CRYOBOXES LABELLED "ABBOTT STUDY".
   KEEP THESE SAMPLES separate FROM THE TRACK-TBI SAMPLES OF THE PARENT STUDY.

9. Dispose of the vacutainer with cell pellet.

Packaging & Shipping Instructions

THE FILLED ABBOTT STUDY CRYOBOXES ARE TO BE SHIPPED TO THE TRACK-TBI BIOREPOSITORY IN PITTSBURGH.
BE SURE THAT:

1. **<u>ALL</u> SAMPLES COLLECTED FOR THE ABBOTT PROTOCOL ARE IN CRYOBOXES LABELLED "ABBOTT STUDY'**
2. **PROVIDE A LOG OF SAMPLES FOR SPECIMENS COLLECTED UNDER THE ABBOTT PROTOCOL (see the sample log at end of this protocol)**
3. **INCLUDE THE CORRESPONDING <u>TRACK-TBI SUBJECT ID</u> FOR EACH PARTICIPANT IN THE ABBOTT PROTOCOL.**

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Contact Information:
Miri Rabinowitz, Manager TRACK-TBI BioRepository
Email: rabinowitzmk@upmc.edu
Phone: 412-648-2031

IT IS CRITICALLY IMPORTANT WHEN COMPLETING THE ELECTRONIC MANIFEST TO INCLUDE THE TRACK-TBI SUBJECT ID FOR THE PARTICIPANT IN THE ABBOTT PROTOCOL

***SAMPLE*- BioRepository Electronic Manifest Form**

Sample ID (Subject)	Time-point	Material Type	Material Modifier	Date/Time Drawn	Date/Time Processed	Date/Time Frozen	Date Shipped	Site
Corresponding TRACK-TBI Subject ID: TR-01-__ __ __ __								
AL-01-1001-A-01S	BASELINE	SERUM	SST					Baylor
AL-01-1001-A-02S	BASELINE	SERUM	SST					Baylor
AL-01-1001-A-03S	BASELINE	SERUM	SST					Baylor
AL-01-1001-A-04S	BASELINE	SERUM	SST					Baylor
AL-01-1001-A-05S	BASELINE	SERUM	SST					Baylor
AL-01-1001-A-06S	BASELINE	SERUM	SST					Baylor
AL-01-1001-B-07S	TIME 2	SERUM	SST					Baylor
AL-01-1001-B-08S	TIME 2	SERUM	SST					Baylor
AL-01-1001-B-09S	TIME 2	SERUM	SST					Baylor
AL-01-1001-B-10S	TIME 2	SERUM	SST					Baylor
AL-01-1001-B-11S	TIME 2	SERUM	SST					Baylor
AL-01-1001-B-12S	TIME 2	SERUM	SST					Baylor
AL-01-1001-C-13S	TIME 2	SERUM	SST					Baylor
AL-01-1001-C-14S	TIME 2	SERUM	SST					Baylor
AL-01-1001-C-15S	TIME 1	SERUM	SST					Baylor
AL-01-1001-C-16S	TIME 2	SERUM	SST					Baylor
AL-01-1001-C-17S	TIME 2	SERUM	SST					Baylor
AL-01-1001-C-18S	TIME 2	SERUM	SST					Baylor
AL-01-1001-D-19P	TIME 2	PLASMA	EDTA					Baylor
AL-01-1001-D-20P	TIME 2	PLASMA	EDTA					Baylor
AL-01-1001-D-21P	TIME 1	PLASMA	EDTA					Baylor
AL-01-1001-D-22P	TIME 2	PLASMA	EDTA					Baylor
AL-01-1001-D-23P	TIME 2	PLASMA	EDTA					Baylor
AL-01-1001-D-24P	TIME 2	PLASMA	EDTA					Baylor