Collection, Processing, Storage and Shipment from Adult Participants/Subjects:

### Acquisition of Blood Biospecimens

1. For severely injured patients, blood is collected via vascular access catheters that have been placed as part of the patients’ routine medical care. For other patients, trained personnel collect blood through venipuncture.
2. For most purposes, 5 – 10 mL of whole blood will be collected using a vacutainer system. It should be noted that the use of glass tubes can lead to low values for certain analytes. For the most generalizable purposes, polypropylene vacutainers and subsequent storage tubes are recommended.

### Local Processing (Serum)

* + 1. Blood samples should be collected in vacutainers that contain no anticoagulant for the processing of serum.
    2. Samples should be sat upright at room temperature for 30 minutes to allow for clotting. If clot is not formed, allow to set for up to 30 additonal minutes. DO NOT exceed 60 minutes for clotting.
    3. Label cryovials before processing sample.
    4. Once clotted, centrifuge at 1100 - 1500 rcf (xg) at room temperature for 10-20 minutes (http://www.bd.com/vacutainer/pdfs/VDP40161.pdf). Transfer serum to cryovials and store in 500 uL aliquots.
    5. Record the volume of each aliquot.

### Local Processing (Plasma)

* + 1. Blood samples should be collected in vacutainers that contain K2EDTA when preparing plasma. Previous research suggests that K2EDTA is the preferred anticoagulant since other may interfere with analyte detection (Vanderstichele, 2000).
    2. The distinction between K2EDTA and K3EDTA and their concentrations should be assessed. See Reference Article for more details (Goossens, et al, 1991).
    3. Transport the original, unfrozen blood sample on ice to the designated local proteomics lab as soon as possible. Freezing has significant adverse effects on plasma and its proteomic elements.
    4. Label cryovials before processing sample.
    5. Centrifuge at 1100 - 1500 rcf (xg) at room temperature for 10-20 minuteshttp://www.bd.com/vacutainer/pdfs/VDP40161.pdf). Transfer plasma to cryovials and store in 500ul aliquots.
    6. Record the volume of each aliquot.

### Local Documentation and Storage

* + 1. Appropriate and complete documentation surrounding biospecimen collection, processing, and storage is essential and will influence the quality of research data.
    2. Bar code identification of samples is necessary.
    3. Samples should be placed in non-frost free freezers at or below -80 ˚C. Frost-free freezers go through freeze-thaw cycles that further damage the specimen.
    4. Sample storage centers should institute a back-up plan for freezer failure (e.g. dry ice or liquid nitrogen). An appropriate alarm system to support freezers for storage is essential.
    5. An inventory system should be established for tracking provenance of samples, including the time of collection, processing, storage, and QC procedures carried out on each sample.

### Shipping

* + 1. Dry ice pellets must accompany the frozen specimen during shipment. The transport time should be minimized given that **dry ice sublimates at a rate of 5-10 lbs per 24 hours**, depending on the insulation of the shipment container.
    2. Consult the local agency for proper shipping options and certified transport materials. The International Air Transportantion Association website (<http://www.iata.org/Pages/default.aspx>) and the U.S. Department of Transportation website (<https://www.transportation.gov/>) have legal requirements governing the packaging, labeling, and shipping of biospecimen.
       - 1. Category B Infectious Substances (also “diagnostic specimens” or “clinical specimens” could be infectious but do not meet the standard for Category A inclusion.
         2. Exempt Patient Specimens have a minimal likelihood of containing pathogens.
    3. Temperature loggers can be used to monitor temperature in shipments of samples to provide confirmation and assurance that samples have been maintained at appropriate temperatures.

### Central Storage

Appropriate and complete documentation surrounding biospecimen collection, processing, storage, and shipping from the individual sites is essential and will influence the quality of the multicenter research data to be obtained.

Bar code identification of samples is necessary.

A formal plan for sharing the central biospecimen resource is recommended.

* + 1. The Central Bank should maintain information of laboratories where the samples have been sent to avoid duplicative genotyping and inadvertent repetitive reporting of data from the same patient.
    2. The Central Bank should also maintain information regarding any stipulations regarding informed consent for the use of the samples. For example, in some studies participants may provide permission for their samples to be used only for studies on TBI.

### References

Goossens W, Van Duppen V, Verwilghen RL (1991) K2- or K3-EDTA: the anticoagulant of choice in routine haematology? Clin Lab Haematol 13:291-5.

Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E. (2007) Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. Amyloid. 7:245-58.

## Collection, Processing, Storage, and Shipment from Pediatric Participants/Subjects:

### Acquisition of Blood Biospecimens

* + 1. **Sample volume:**

The original CDE Workgroup (i.e., the recommendations for adults on the preceding pages) recommended that 5-10ml of blood be collected for each sample. For pediatric subjects, we recommend that the volume collected be consistent with a pre-defined standard that meets at least the minimal IRB requirements. This is particularly important for serial sampling and for infants and young children. A standard table of allowable volumes is publically available and has been accepted by several IRBs including University of Pittsburgh, Children’s Hospital in Los Angeles, Baylor College of Medicine in Dallas, and Cincinnati Children’s Hospital (Table 1). It is important to recognize that the blood volumes in the table include blood collected both for research and clinical purposes. In a 4kg child, for example, the total allowable blood volume for research and clinical care in a single blood draw is 8ml; the total allowable blood volume for research and clinical care in a 30-day period is 16ml. Because of the limitations on blood volume, it can be very helpful to obtain IRB approval to use serum that is leftover from clinical samples for research purposes. This blood would otherwise be discarded and can be used for biomarker analysis as long as the processing and storage is appropriate as described below.

* + 1. **Risks of phlebotomy:**

In a child with severe TBI, it is likely that arterial or central venous access will be available and phlebotomy will not be necessary. However, in children with mild to moderate TBI and in control subjects, phlebotomy may be necessary. While phlebotomy is considered a minimal risk procedure from an IRB-perspective, the pain/discomfort for a child undergoing phlebotomy must be taken into account and stated in any consent/assent. We would recommend that as part of the protocol/consent, there is a limit to the number of phlebotomy attempts which can be performed (i.e. preferably 1 or 2) in a child who cannot assent to the procedure (generally children <7 yrs of age). In cases in which time permits, we would recommend that consideration be given to the use of a topical anesthetic prior to phlebotomy. Some IRBs require the use of a topical anesthetic as a way to minimize the risk of the pain associated with blood draws.

* + 1. **Site of sample collection:**

We agree with the original CDE that documentation of collection site is important. While adult samples are generally limited to venous and arterial samples, capillary specimens are also a possibility in pre-mobile children. If the heel is pre-warmed and collection is done by an experienced phlebotomist, it is possible to collect 1ml of blood from a heel stick in a premobile child. However, it should be noted that no published data have directly compared biomarker concentrations in capillary and venous specimens.

* + 1. **URINE COLLECTION:**

Although not discussed as part of the recommendations of the original CDE, urine is another potential biospecimen that may be useful for biomarkers which are renally excreted. Urinary S100B concentrations have been evaluated in one pediatric TBI study and extensively in neonates with hypoxic-ischemic encephalopathy. (Berger and Kochanek 2006, Gazzolo, et al. 2001, Gazzolo, et al. 2003a)

* + 1. **Hemolysis:**

Sample hemolysis is not discussed in the original CDE recommendations, but is an important issue in the pediatric patient because of the small needle gauge often used for phlebotomy. In addition, capillary specimens are frequently hemolyzed. Hemolysis can have an important effect on the concentrations of brain biomarkers, mostly notably neuron-specific enolase (NSE). NSE is found in small quantities in red blood cells and thus sample hemolysis can result in falsely elevated serum NSE concentrations. Previous research has demonstrated that qualitative assessment of the amount of hemolysis is not accurate. (Berger and Richichi 2009) In the case of NSE, it is possible to adjust the NSE concentration to account for the amount of hemolysis using a quantitative assessment of the amount of hemolysis and adjustment factor. (Berger and Richichi 2009) This type of adjustment factor has not been derived for other biomarkers.

### Sample Processing

We agree with the recommendations of the original CDE as it relates to sample processing with one small change related to sample volume. With multiplex bead technology, the volumes required for biomarker measurement are often <100μl and therefore, an aliquot of 1-2ml could result in multiple freeze thaw cycles before the sample would be exhausted. We therefore recommend aliquots of 500μl. This recommendation is based on an assessment of an acceptable balance between the need to limit the number of freeze thaw cycles and the need to limit the amount of freezer space which is necessary as more aliquots are made for each subject sample.

### Documentation and Storage

* + 1. **Documentation:**

One of the key CDE that must be recorded for each sample is the ‘time after injury’ when the sample is collected. This is particularly important for biomarkers with a short half-life such as S100B. In order to calculate the ‘time after injury,’ it is necessary to have both a ‘time of injury’ and ‘time of sample collection.’ In cases of abusive head trauma, an important cause of TBI in infants and young children, the time of injury is rarely known. As a result, we would recommend that in cases of suspected abuse or in other cases in which the time of injury is not known, the time of injury be set in a consistent fashion to the time of first contact with medical personnel (e.g. a call to EMS, EMS arrival or arrival to a hospital in cases in which EMS is not activated). Estimating the time of injury in this way provides consistency and allows for comparison between centers.

* + 1. **Storage:**

We agree with original CDE recommendations related to sample storage with one important difference. Because the Office for Human Research Protection (OHRP) considers informed consent to be an ongoing process, unless the IRB determines that requirements for obtaining informed consent can be waived, investigators are required to obtain consent from the subject when he or she reaches age 18. This regulation applies to research with biospecimens because this type of study may involve the continued analysis of identifiable specimens (e.g, by linkage code). Thus, biospecimens obtained before the age of 18 must be discarded unless the subject either re-consents for the samples to be kept or the samples are rendered anonymous, meaning that all links to identifiable data are removed. As a result of this regulation, we recommend that at the time of sample collection, there be a plan in place for tracking subject age so that it is clear when each subject reaches his/her 18th birthday and for re-consenting, anonymizing or discarding all specimens at that time.

### References

Berger RP and Kochanek PM. (2006). Urinary S100B concentrations are increased after brain injury in children: A preliminary study. Pediatr Crit Care Med. 7:557-561.

Berger R and Richichi R. (2009). Derivation and validation of an equation for adjustment of neuron-specific enolase concentrations in hemolyzed serum. Pediatr Crit Care Med. 10:260-263.

Gazzolo D, Bruschettini M, Lituania M, Serra G, Bonacci W and Michetti F. (2001). Increased urinary S100B protein as an early indicator of intraventricular hemorrhage in preterm infants: correlation with the grade of hemorrhage. Clin Chem. 47:1836-1838.

Gazzolo D, Marinoni E, Di Iorio R, Bruschettini M, Kornacka M, Lituania M, Majewska U, Serra G and Michetti F. (2003a). Measurement of urinary S100B protein concentrations for the early identification of brain damage in asphyxiated full-term infants. Arch Pediatr Adolesc Med. 157:1163-1168.

Table 1 MAXIMUM ALLOWABLE TOTAL BLOOD DRAW VOLUMES

| Body Wt (Kg) | Body Wt (lbs) | Total blood volume (mL) | Maximum allowable volume (mL) in one blood draw  ( = 2.5% of total blood volume) | Maximum volume (clinical + research) (mL) in a 30-day period | Minimum Hgb required at time of blood draw | Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | 2.2 | 100 | 2.5 | 5 | 7.0 | 9.0-10.0 |
| 2 | 4.4 | 200 | 5 | 10 | 7.0 | 9.0-10.0 |
| 3 | 6.3 | 240 | 6 | 12 | 7.0 | 9.0-10.0 |
| 4 | 8.8 | 320 | 8 | 16 | 7.0 | 9.0-10.0 |
| 5 | 11 | 400 | 10 | 20 | 7.0 | 9.0-10.0 |
| 6 | 13.2 | 480 | 12 | 24 | 7.0 | 9.0-10.0 |
| 7 | 15.4 | 560 | 14 | 28 | 7.0 | 9.0-10.0 |
| 8 | 17.6 | 640 | 16 | 32 | 7.0 | 9.0-10.0 |
| 9 | 19.8 | 720 | 18 | 36 | 7.0 | 9.0-10.0 |
| 10 | 22 | 800 | 20 | 40 | 7.0 | 9.0-10.0 |
| 11-15 | 24-33 | 880-1200 | 22-30 | 44-60 | 7.0 | 9.0-10.0 |
| 16-20 | 35-44 | 1280-1600 | 32-40 | 64-80 | 7.0 | 9.0-10.0 |
| 21-25 | 46-55 | 1680-2000 | 42-50 | 64-100 | 7.0 | 9.0-10.0 |
| 26-30 | 57-66 | 2080-2400 | 52-60 | 104-120 | 7.0 | 9.0-10.0 |
| 31-35 | 68-77 | 2480-2800 | 62-70 | 124-140 | 7.0 | 9.0-10.0 |
| 36-40 | 79-88 | 2880-3200 | 72-80 | 144-160 | 7.0 | 9.0-10.0 |
| 41-45 | 90-99 | 3280-3600 | 82-90 | 164-180 | 7.0 | 9.0-10.0 |
| 46-50 | 101-110 | 3680-4000 | 92-100 | 184-200 | 7.0 | 9.0-10.0 |
| 51-55 | 112-121 | 4080-4400 | 102-110 | 204-220 | 7.0 | 9.0-10.0 |
| 56-60 | 123-132 | 4480-4800 | 112-120 | 224-240 | 7.0 | 9.0-10.0 |
| 61-65 | 134-143 | 4880-5200 | 122-130 | 244-260 | 7.0 | 9.0-10.0 |
| 68-70 | 145-154 | 5280-5600 | 132-140 | 264-280 | 7.0 | 9.0-10.0 |
| 71-75 | 156-185 | 5680-6000 | 142-150 | 284-300 | 7.0 | 9.0-10.0 |
| 76-80 | 167-176 | 6080-6400 | 152-160 | 304-360 | 7.0 | 9.0-10.0 |
| 81-85 | 178-187 | 6480-6800 | 162-170 | 324-340 | 7.0 | 9.0-10.0 |
| 86-90 | 189-198 | 6880-7200 | 172-180 | 344-360 | 7.0 | 9.0-10.0 |
| 91-95 | 200-209 | 7280-7600 | 182-190 | 364-380 | 7.0 | 9.0-10.0 |
| 96-100 | 211-220 | 7680-8000 | 192-200 | 384-400 | 7.0 | 9.0-10.0 |