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

### Acquisition of CSF from an external ventricular drain (EVD)

1. Document whether continuous or intermittent (catheter opened only in response to intracranial hypertension) fluid drainage is administered. Drainage method has been shown to alter CSF protein concentration (Shore, 2004).
2. Draw CSF directly from ventriculostomy catheter
3. Target CSF collection within the first 24 hours of admission, recording time from TBI and time of day. Ideally, the first collection should be as close to the TBI as feasible (e.g., 6h post TBI), at a minimum frequency of every 6 h for sufficient biokinetic studies.
4. Collect 5 mL of CSF in 1-mL fractions and place in ice bath. The first 1 or 2 fractions are sent for clinical laboratory analysis for cell count, protein and glucose measurements. Blood contamination of CSF is a significant confound. Protein concentrations are 400-fold greater in plasma than CSF (Maurer, 2008). CSF is considered blood contaminated if RBC counts are > 10 cells/µL, or alternatively if hemoglobin levels are > 30 pg/mL of CSF (Zhang, 2007). Brain specific proteins are typically present at low concentrations in CSF, with 80% of normal CSF protein mass originating from plasma (Bergquist, 2002).
5. Appropriate CSF control samples may be available from hydrocephalic patients that undergo ventriculoperitoneal shunt placement and had CSF collected intraoperatively, or unruptured subarachnoid hemorrhage patients who had CSF drawn intraoperatively (Pineda, 2007).

### Acquisition of CSF from a Lumbar Puncture (LP)

1. In patients who are unlikely to receive ventriculostomy. CSF accessed by less invasive lumbar puncture (LP). A greater number of LP collected control samples are available; however, comparison with EVD CSF is discouraged given a 2.5-fold lower protein concentration than in LP CSF (Huhmer, 2006).
2. Atraumatic spinal needle LP kits should be used to minimize risk of post-LP headache. Draw with a sterile polypropylene syringe, or allow flow under gravity.
3. Target CSF collection within the first 24 hours after admission, recording time from TBI and time of day. Ideally, the first collection should be as close to the TBI as feasible (e.g., 6h post TBI), at a minimum frequency of every 6 h for sufficient biokinetic studies.
4. Collect 1-mL fractions and place in ice bath, with a maximum of 25 mL per time point. Send the first 2 mL for clinical laboratory analysis. It is important to match fractions when comparing across patients, as protein concentration varies depending on the draw volume (Blennow, 1993).
5. Patient should rest in a recumbent position for 1 h post-LP receive liberal fluid intake, and avoid exertion for 24-48 h to minimize risk of headache.

### Processing and Storage of CSF

1. Transport CSF on ice and process immediately after collection as significant cell lysis contamination will occur within 1 hour.
2. Collect CSF samples into low protein binding polypropylene tubes (e.g., Eppendorf brand LoBind® tubes). Avoid polystyrene and glass tubes, which will result in significant protein loss (Hesse, 2000).
3. Centrifuge CSF at 2,000 g and 4˚C for 10 min. Draw off supernatant and place in new, low binding tube, and document volume of fluid collected. Centrifugation will only remove RBCs, not serum derived proteins. Samples found to contain > 10 RBCs/µL CSF may be inappropriate for proteomic analysis.
4. Control samples are available; however, comparison with EVD CSF is discouraged given a 2.5-fold lower protein concentration than in LP CSF (Huhmer, 2006).
5. Additives or preservatives may be combined with CSF depending on experimental objectives. Protease and phosphatase inhibitors are recommended.
6. Snap freeze samples in liquid nitrogen, and store at or below -80 ˚C to minimize proteolytic breakdown (Wagner, 2007). Avoid repeated freeze and thawing cycles and storage at -20˚C (Carrette, 2005).

### References

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

### Acquisition of CSF

We agree with the recommendations of the original CDE (i.e., the recommendations for adults on the preceding pages) as it relates to CSF collection. CSF can be obtained from an indwelling catheter in the ventricular space (externalized ventricular drain [EVD]) or via lumbar drain or puncture. In general, CSF specimens obtained via EVD should be collected using established sterile technique. Lumbar drains, infrequently placed in childhood TBI patients, can offer another such reservoir of potential samples. Lumbar puncture is almost never indicated in children with TBI. However, this technique can be used in control subjects (generally undergoing diagnostic procedures) to obtain CSF for analysis. We would, however, recommend that if researchers are considering collection of CSF via lumbar puncture from healthy children who are not undergoing diagnostic procedures, that this be discussed directly with the local IRB. The decision of individual IRBs might be expected to vary in their risk determination of pediatric studies that include CSF collection since healthy children cannot undergo greater than minimal risk procedures in any research study. Investigators should be prepared to provide a rationale that discusses how the risk is justified by the extent of the potential benefit to the involved children and if the procedure does not hold out the potential for direct benefit, that this risk represents only a minor increase over minimal risk. (45 CFR 46, Subpart D, sections 401-409, US Department of Health and Human Services 1993)

## II. 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 250μl. 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.

## III. 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.
2. 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.

### IV. References

US Department of Health and Human Services. (1993 ). Code of Federal Regulations 45 CFR 46

[United States Government Printing Office Fedderal Regulations on Public Welfare Link](http://www.access.gpo.gov/nara/cfr/waisidx_01/45cfr46_01.html).