



# **NINDS Common Data Element (CDE) Project**

## **Traumatic Brain Injury Version 3.0**

### **Internal Review / Public Review**

#### **Neurodiagnostic Technologies: Biomarkers Subgroup**

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- Guidance for Protein Analysis from Blood Samples of TBI Participants
- Guidance for Protein Analysis from Cerebrospinal Fluid (CSF) Samples of TBI Participants



## **NINDS CDE Project Traumatic Brain Injury Version 3.0 Neurodiagnostic Technologies: Biomarkers Subgroup Summary**

The NINDS TBI v3.0 Common Data Element (CDE) Neurodiagnostic Technologies: Biomarkers Subgroup (Biomarkers) reviewed and updated CDEs based on the state of neuroscientific clinical research.

The Biomarkers Subgroup focused on molecular biomarkers that can be measured in biological fluids or tissues. This includes proteins, nucleic acids, lipids, polysaccharides, and other metabolites, from fluids including plasma, serum whole blood, cerebrospinal fluids, microdialysate, urine, saliva, sweat, and potentially others. While the Biomarkers Subgroup acknowledges that molecular biomarkers obtained from tissues or cells—such as brain tissue from biopsy or autopsy, or cells isolated from blood, CSF, lymph, or skin—are likely to hold significant value, their application in neurotrauma research remains in an early stage, making the development of CDEs for this emerging area premature.

Biomarkers have the potential to be useful across multiple contexts. These include, but are not limited to, the following:

1. Diagnostic biomarkers: Used to detect and confirm the presence of a disease or condition, or to identify individuals with a subtype of a disease.
2. Prognostic biomarkers: Used to inform about expected trajectory of a disease, such as improvement or progression, in patients who have the disease or condition of interest.
3. Predictive biomarkers: Used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable response to exposure to a therapeutic product or environmental agent.
4. Monitoring biomarkers: Used repeatedly to assess the status of a disease or condition, such as the progression, resolution, or response to an exposure.
5. Response biomarkers: Used to show that a biological response, potentially beneficial or harmful, has occurred in an individual who has been exposed to a therapeutic intervention or environmental agent.
  - a. Pharmacodynamic biomarkers: A response biomarker that indicates biologic activity of a medical product of environmental agent without necessarily drawing conclusions about efficacy or disease outcome or necessarily linking this activity to an established mechanism of action. Pharmacodynamic biomarkers can be useful in early-stage proof-of-concept studies, assisting in dose selection, or as a measure of potential harm. Such measures may be secondary endpoints in clinical trials.
  - b. Safety biomarkers: Measured before and after an exposure to a medical product of environmental agent to indicate the likelihood, presence, or extent of toxicity or an adverse event.
  - c. Surrogate endpoint biomarkers: A response biomarker that is an endpoint in a clinical trial as a substitute for a direct measure of how the patient feels, functions, or survives. Surrogate endpoint biomarkers do not measure the clinical benefit of primary interest but are expected to predict the clinical benefit based on epidemiologic, pathophysiologic, or other scientific evidence. Surrogate endpoint biomarkers can be characterized based on the level of clinical validation:
    - i. Validated surrogate endpoint
    - ii. Reasonably likely surrogate endpoint
    - iii. Candidate surrogate endpoint
6. Multicomponent biomarker. A combination or defined set of two or more individual biomarkers whose values, when considered together in a specified way, provide an indicator of normal biological processes, pathogenic processes or biological responses to an exposure or intervention.

The subgroup reassessed the Laboratory Tests CRF, the TBI Biospecimen Collection Protocol SAMPLE, as well as the following guidance documents: DNA Guidance for Genomic Analysis (Table 1), Plasma and Serum Guidance for Proteomic Analysis (Table 2), Cerebral Spinal Fluid Guidance (Table 3), and Cerebral



Microdialysis Guidance (Table 4). The Biomarkers Subgroup also notes that although most neurotrauma biomarker research has focused on the acute and subacute phases, emerging advances across neuroscience underscore the need to prioritize biomarker collection in the chronic period after injury, spanning months to years.

#### REFERENCES

FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Silver Spring (MD): Food and Drug Administration (US); 2016-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK326791/>. Co-published by National Institutes of Health (US), Bethesda (MD).

#### Summary of Recommendations

Subdomain	CRF/Guidance Document Name	Classification	Population
Laboratory Tests and Biospecimens/Biomarkers	Guidance for Analyte Analysis from Microdialysate from TBI Participants	N/A	Adult; Pediatric
	Guidance for DNA and RNA Analysis of Blood Samples from TBI Participants	N/A	Adult; Pediatric
	Guidance for Protein Analysis from Blood Samples of TBI Participants	N/A	Adult; Pediatric
	Guidance for Protein Analysis from Cerebrospinal Fluid (CSF) Samples of TBI Participants	N/A	Adult; Pediatric
	Laboratory Tests	Pending Classification	Adult; Pediatric

## Laboratory Tests

[Study Name/ID pre-filled]

Site Name:  
Participant ID:

Visit Date:  
Visit Name:

1. Age:
2. Time of injury:
3. BMI:
4. Glomerular filtration rate (GFR):
5. Suspected alcohol use:
6. Was a transfusion received immediately before the blood draw?
7. Time of processing of sample/specimen:
8. Duration of time before storage at -80°C:
9. Platform and assay information:
  - a. Type:
  - b. Manufacturer:
  - c. Kit lot number:
  - d. Limit of detection:
  - e. Limit of quantitation:

**Where feasible, the collection of these additional tests may help with the interpretation of the levels of TBI biomarkers: BUN, creatinine, INR.**

Indicate the appropriate result for each test.

**Table 1 Table for Type of Specimen and Date and Time Collected**

Lab Panel (Choose one):	Lab Specimen Type (Choose one):	Date and Time Collected:
<input type="checkbox"/> Chemistry <input type="checkbox"/> Hematology <input type="checkbox"/> Urinalysis <input type="checkbox"/> Other, specify:	<input type="checkbox"/> Blood <input type="checkbox"/> Venous blood – serum <input type="checkbox"/> Arterial blood – serum <input type="checkbox"/> Venous blood – plasma <input type="checkbox"/> Arterial blood – plasma <input type="checkbox"/> Venous blood – platelets <input type="checkbox"/> Arterial blood – platelets <input type="checkbox"/> Venous blood – buffy coat <input type="checkbox"/> Arterial blood – buffy coat <input type="checkbox"/> Venous blood – whole <input type="checkbox"/> Arterial blood – whole <input type="checkbox"/> Newborn cord blood <input type="checkbox"/> Urine <input type="checkbox"/> Cerebral spinal fluid <input type="checkbox"/> Buccal swab <input type="checkbox"/> Brain tissue <input type="checkbox"/> Saliva <input type="checkbox"/> Amniotic fluid <input type="checkbox"/> Placenta <input type="checkbox"/> Hair <input type="checkbox"/> Other, specify:	<del>//-yyyy-mm-dd</del> <del>:(24-hour clock)-hh-mm</del> Data to be filled in by site

## Laboratory Tests

[Study Name/ID pre-filled]

Site Name:

Participant ID:

**Table for Lab Results**

Test Lab test other text	Result	Units for Result	Was test result abnormal?	If abnormal, clinically significant?
†GlucoseFasting Glucose	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Random Glucose	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	
†Urea	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Creatinine	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Amylase	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Aspartate Aminotransferase (ASAT/ SGOT)	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Alanine Aminotransferase (ALAT/ SGPT)	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Lactate Dehydrogenase (LDH)	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Alkaline Phosphatase	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Total Bilirubin	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant

## Laboratory Tests

[Study Name/ID pre-filled]

Site Name:

Participant ID:

Test Lab test other text	Result	Units for Result	Was test result abnormal?	If abnormal, clinically significant?
Hemoglobin	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Sodium	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Potassium	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Hematocrit	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†White blood cell	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Neutrophils	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Lymphocytes	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Eosinophils	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Platelet	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant
†Prothrombine Time (PTT)	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> -Clinically significant <input type="checkbox"/> -Not clinically significant

## Laboratory Tests

[Study Name/ID pre-filled]

Site Name:

Participant ID:

Test Lab test other text	Result	Units for Result	Was test result abnormal?	If abnormal, clinically significant?
†International Normalized Ratio (INR)	Data to be filled in by site	Data to be filled in by site	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal <input type="checkbox"/> Unknown	<input type="checkbox"/> Clinically significant <input type="checkbox"/> Not clinically significant

CDE details also include the following additional tests:

8-isoprostanes	GAA repeat-expansion	Pre-Albumin
8-OH-2-deoxyguanosine	mutation	Serum N-terminal atrial
Albumin	Gamma-glutamyl	natriuretic peptide (ANP-N)
Anion Gap	transpeptidase (GGT)	Serum osmolality (OSM)
Apolipoprotein B (apo B)	Glucose D-stick	Total Cholesterol (TCHOL)
apolipoprotein E (apo E)	Glucose serum	Total Iron Binding Capacity
Apolipoprotein-I (apo A1)	Glycosylated Hemoglobin	(TIBC)
Atrial natriuretic peptide (ANP)	(HgBA1C)	Total protein
Bicarbonate (HCO <sub>3</sub> )	Hemoglobin Electrophoresis	Triglycerides (TGs)
Blood Urea Nitrogen (BUN)	High Density Lipoprotein (HDL)	Troponin I
B-type natriuretic peptide (BNP)	Ionized Calcium	Troponin T
Calcium (Ca)	Iron	Urine 8-OH-2-
Carbon Dioxide (CO <sub>2</sub> )	Lactate	deoxyguanosine/creatinine ratio
Chloride (Cl)	Lipoprotein (a) [Lp(a)]	Urine Creatinine
Cortisol	Low Density Lipoprotein (LDL)	Urine Nitrite
C-Reactive Protein	Magnesium (Mg)	Urine pH
Creatine Kinase	Monocyte Count	Urine White Blood Cells (WBC)
Creatine Kinase-Myocardial	<del>Oral glucose tolerance</del>	Very Low Density Lipoprotein
Bands (CK-MB)	Phenobarbital	(VLDL)
Fasting Insulin	Phenytoin (Dilantin)	Vitamin B12
Ferritin	Phosphate (PO <sub>4</sub> )	Vitamin E
Frataxin Level	Plasma malondialdehyde	

### Additional Supplemental Elements:

These elements may be included if relevant to the study. ~~For additional details like permissible values, see the data dictionary associated with this CRF.~~

- Lab test LOINC code:
- Lab test result unit of measure UCUM code:
- Pregnancy test date and time:
- Pregnancy test specimen type  
Pregnancy Test Type:  
☐ Blood  
☐ Urine
- Pregnancy test qualitative result  
Pregnancy test qualitative result:  
☐ Positive  
☐ Negative
- Pregnancy test not applicable reason  
Pregnancy test: Reason Test N/A:  
☐ Post-menopausal  
☐ Surgically sterile

## Laboratory Tests

[Study Name/ID pre-filled]

Site Name:

Participant ID:

☐ Non-surgically sterile

☐ Male

- Alcohol blood test performed indicator:

☐ Yes

☐ No

☐ Unknown

- Alcohol blood level measurement:

- Drug screen qualitative result:

☐ Negative

☐ Positive

☐ Inconclusive

☐ Not available

- Drug screen positive substance type

Drug screen positive type:

☐ Cocaine metabolite

☐ Marijuana metabolites

☐ Opiates (codeine and morphine)

☐ Phencyclidine

☐ Barbiturates

☐ Benzodiazepines

☐ Methadone

☐ Propoxyphene

☐ Amphetamines

☐ Other, specify:

- Drug screen sample type:

☐ Serum

☐ Urine

☐ Hair

☐ Saliva

☐ Other, specify:

~~†Core or Basic for adult only~~

Recorder Signature:

Date:



## Laboratory Tests CRF Module Instructions

### GENERAL INSTRUCTIONS

TBI v3.0 classification pending.

Additional details regarding classification definitions are available: [Link to be added once available.]

Please see the Data Dictionary for element classifications.

~~Important note: None of the data elements included on this CRF Module are classified as Core (i.e., strongly recommended for all TBI clinical studies to collect).~~

~~All the data elements are classified as Supplemental and should only be collected if the research team considers them appropriate for their study.~~

### SPECIFIC INSTRUCTIONS

Please see the Data Dictionary for definitions for each of the data elements included in this CRF Module.

- Age –
- Time of injury –
- BMI –
- Glomerular filtration rate (GFR) –
- Suspected alcohol use –
- Was a transfusion received immediately before the blood draw? –
- Time of processing of sample/specimen –
- Duration of time before storage at -80°C –
- Type –
- Manufacturer –
- Kit lot number –
- Limit of detection –
- Limit of quantitation –
- Lab specimen collection date and time – Date/time should be recorded to the level of granularity known (e.g., year, year and month, complete date plus hours and minutes, etc.) and in an unambiguous format acceptable to the study database like DD-MMM-YYYY. When date/time data are prepared for aggregation or sharing, they should be converted to the format specified by [ISO 8601](#); YYYY-MM-DD T:hh:mm:ss.
- Lab panel – Choose one.
- Lab specimen type – Choose one
- Lab test name – Choose one.
- Lab test LOINC code – Code the laboratory test with Logical Observation Identifiers Names and Codes (LOINC).
- Result – Enter the alphanumeric result.
- Units for Result – Choose or specify the appropriate response.
- Lab test result unit of measure UCUM code – Code the lab test result unit of measure using UCUM codes.
- Was test result abnormal? – Choose one. Indicate if the laboratory test result is abnormal. Abnormal means the test result falls outside the normal range.
- ~~If abnormal, clinically significant? – Choose one. If the laboratory test result is abnormal, indicate if the physician considers the result clinically significant.~~
- Pregnancy test date and time – Date/time should be recorded to the level of granularity known (e.g., year, year and month, complete date plus hours and minutes, etc.) and in an unambiguous format acceptable to the study database like DD-MMM-YYYY. When date/time data are prepared for aggregation or sharing, they should be converted to the format specified by [ISO 8601](#); YYYY-MM-DD T:hh:mm:ss.
- Pregnancy test specimen type – Choose one.

## Laboratory Tests CRF Module Instructions

- Pregnancy test qualitative result – Choose one.
- Pregnancy test not applicable reason – Choose one. Response is obtained from report by participant/~~subject~~, reliable proxy or caretaker, attending medical health professional or medical records.
- Alcohol blood test performed indicator – Choose one. Response is obtained from report by participant/~~subject~~, reliable proxy or caretaker, attending medical health professional or medical records.
- Alcohol blood level measurement –
- Drug screen qualitative result –
- Drug screen positive substance type – Choose all that apply.
- Drug screen sample type – Choose all that apply.

### REFERENCES

A.D.A.M. Medical Encyclopedia [Internet]. Johns Creek (GA): Ebix, Inc., A.D.A.M.; c1997-2020. Pregnancy test; [updated 2022 Nov 11]; Available from: <https://medlineplus.gov/ency/article/003432.htm>

Logical Observation Identifiers Names and Codes (LOINC) - <https://loinc.org/>

Rai AJ, Gelfand CA, Haywood BC, Warunek DJ, Yi J, Schuchard MD, Mehig RJ, Cockrill SL, Scott GB, Tammen H, Schulz-Knappe P, Speicher DW, Vitzthum F, Haab BB, Siest G, Chan DW. HUPO Plasma Proteome Project specimen collection and handling: towards the standardization of parameters for plasma proteome samples. Proteomics. 2005 Aug;5(13):3262-77.

Substance Abuse and Mental Health Services Administration drug testing information - <http://workplace.samhsa.gov/Dtesting.htm> <https://www.samhsa.gov/workplace/drug-testing-resources>

Unified Code for Units of Measure (UCUM) - <https://ucum.org/>

## Guidance for Analyte Analysis from Microdialysate from TBI Participants

**This document has been updated for TBI v3.0. The TBI v2.0 guidance document is available here: [Cerebral Microdialysis Guidelines \(Table 4\)](#)**

### PROBE PLACEMENT

The microdialysis probe must be inserted by personnel credentialed to perform burr holes and intracranial monitor placement (typically a neurosurgeon or neurosurgery trainee).

The preferred insertion site is the **right frontal lobe**, unless contraindicated by prior craniotomy, depressed skull fracture, or focal parenchymal injury (e.g., contusion or intracerebral hematoma). In such cases, placement in the **left frontal lobe** is recommended. The goal is to position the probe at an appropriate distance from focal lesions to allow sampling of metabolically representative tissue.

At some sites, the probe may be inserted by **subgaleal tunneling** rather than a bolt, especially when a craniectomy is present. Regardless of technique, the probe should be positioned **remote from visible contusions**.

In select participants, with asymmetric injuries, **bilateral probe placement** may provide additional metabolic information.

### Checking Probe Placement

If ICP and PbtO<sub>2</sub> probes inserted through the same multi-lumen bolt produce reliable values (including appropriate FiO<sub>2</sub> response), the microdialysis probe is generally presumed to be correctly positioned **2–3 cm into the parenchyma**.

A **non-contrast head CT** should be obtained when probe malfunction is suspected or when clinical concerns require confirmation of placement.

### PROBE CHARACTERISTICS

Commercially manufactured **concentric microdialysis probes** are preferred.

Low molecular weight (MW) cut-off membranes remain the standard for routine metabolic monitoring (Shores & Knapp, 2007). High MW cut-off membranes can support protein or large-molecule sampling for research applications, though recovery may be low or inconsistent and regulatory approval varies by jurisdiction.

For all studies, the **MW cut-off, manufacturer, and probe model** must be documented in reporting and metadata.

### MICRODIALYSATE CHARACTERISTICS

Acceptable perfusates include:

- Artificial CSF
- Sterile medical-grade normal saline

For specialized research, particularly when using high MW cut-off membranes, **carrier proteins** (e.g., dextran, albumin) may be added to reduce nonspecific adsorption of hydrophobic or high-MW analytes (Hillered 2014).

The **composition and source** of the perfusate must be documented.

### SAMPLE ACQUISITION

A flow rate of **0.3 µL/min** is recommended, yielding ~18 µL/hour and ensuring high recovery of low MW analytes.

## Guidance for Analyte Analysis from Microdialysate from TBI Participants

Because sample volumes are extremely small, they are susceptible to **evaporation** even when sealed. Characteristics of the collection vials must be reported, and samples should be **processed or frozen immediately** to minimize loss.

For analytes known to be **labile or hydrophobic** (e.g., drug candidates, lipophilic metabolites), stabilization measures—such as rapid freezing, carrier protein supplementation, or validated pH adjustments—may be required to prevent degradation or adsorption.

### ANALYTES

The **minimal essential data set** includes:

- Glucose
- Pyruvate
- Lactate
- Lactate/Pyruvate (L/P) ratio

These markers are typically analyzed at the bedside for real-time clinical decision-making.

Measurement of **glycerol** and **glutamate** is recommended.

Other analytes—including cytokines, nitrate/nitrite, GFAP, neurofilament, tau,  $\beta$ -amyloid, and exploratory biomarkers—remain **investigational** due to limited or inconsistent recovery, especially with high MW cut-off membranes. These analyses should be encouraged when scientifically justified, with appropriate method validation and reporting of recovery efficiency.

### ANALYTE STABILITY DURING STORAGE

Essential low MW analytes are stable at **−70°C**. Samples should be stored at this temperature in vials designed to minimize evaporative loss.

Storage at **−20°C** is inadequate for most analytes—particularly pyruvate—except for temporary holding of **≤3 days**.

For large or experimental analytes, stability varies widely; dedicated validation studies or method-specific stabilization protocols should guide storage conditions.

### HANDLING OF SAMPLES AT THE TIME OF ANALYSIS

Frozen samples must be fully **thawed before analysis**. During thawing, initial meltwater may contain concentrated solutes due to partial ice melt; therefore, samples must be **mixed or centrifuged** after complete thaw to ensure homogeneity.

Batch analysis is acceptable, but microdialysate's small volume makes it vulnerable to **evaporative concentration artifacts** if samples remain in the analyzer too long. Calibration and QC samples should be interspersed throughout each analytical run to detect systematic shifts.

For low-abundance or experimental biomarkers, additional QC measures—such as replicate runs, matrix-matched controls, and reporting of recovery efficiency—are required.

### MICRODIALYSIS DATA REPORTING

Analyte concentrations should be reported in **SI units (kg/m<sup>3</sup>)** whenever possible.

Ratios (e.g., L/P ratio) are **unitless**.

All datasets must include standardized **metadata**, including:

- Probe manufacturer, model, MW cut-off
- Perfusate composition and source

## Guidance for Analyte Analysis from Microdialysate from TBI Participants

- Flow rate
- Collection interval and sample volume
- Storage conditions and duration
- Analytical method, calibration data, and QC performance
- Recovery efficiency data when high MW cut-off membranes or protein analytes are used

This level of documentation is essential for reproducibility and multicenter comparability.

### REFERENCES

Falcone JA, Chen JW. Technical notes on the placement of cerebral microdialysis: A single center experience. Front Neurol. 2023 Jan 9;13:1041952.

Hillered L, Dahlin AP, Clausen F, Chu J, Bergquist J, Hjort K, Enblad P, Lewén A. Cerebral microdialysis for protein biomarker monitoring in the neurointensive care setting - a technical approach. Front Neurol. 2014 Dec 3;5:245.

Shores KS, Knapp DR. Assessment approach for evaluating high abundance protein depletion methods for cerebrospinal fluid (CSF) proteomic analysis. J Proteome Res. 2007 Sep;6(9):3739-51.

## Guidance for DNA and RNA Analysis of Blood Samples from TBI Participants

**This document has been updated for TBI v3.0. The TBI v2.0 guidance document is available here: [DNA Guidelines for Genomic Analysis \(Table 1\)](#)**

### DNA

Genomic DNA is obtained primarily from the leukocyte-rich buffy coat isolated after centrifugation of EDTA-anticoagulated venous (or arterial) blood, as leukocytes in the buffy coat are the primary source of high-quality DNA. If buffy coat is unavailable, validated alternative sources of leukocyte DNA—such as EDTA whole blood processed by direct leukocyte lysis or a leukocyte pellet obtained after red blood cell lysis—may be used. Cell-free DNA from plasma or serum is not an acceptable substitute for germline genomic DNA.

#### **Acquisition of Blood Biospecimens (DNA)**

Collect venous or arterial blood in **EDTA tubes (purple top)**. Heparin or citrate tubes *can* be used, but heparin can inhibit some downstream enzymatic reactions, so EDTA is the standard. Fill EDTA tubes to at least 80% to ensure proper anticoagulant-to-blood ratio for downstream DNA yield and purity.

#### **Local Processing (DNA)**

Gently **invert 8–10 times** to ensure thorough mixing with anticoagulant. Whole blood may be kept at **room temperature** until transported to the laboratory for processing. If time to processing will be delayed >24 hours, freezing at -80°C is recommended. Centrifuge whole blood at **1100-1500 × g for 10-20 minutes at room temperature (18-24°C)**. Exact speed is not critical for buffy coat separation. (If cell-free applications are planned, perform a second centrifugation at 2000xg for 10 minutes to remove residual platelets and cellular debris). After centrifugation, carefully pipette off plasma without disturbing the buffy coat and aliquot plasma per protocol. Aspirate **~0.5 mL of buffy coat**—leave 0.5-1mm of plasma layer above the buffy coat to reduce RBC contamination. Some RBC contamination is expected and does not impair DNA extraction. Divide equally into **two cryovials** and freeze immediately at -80°C.

### RNA

#### **Acquisition of Blood Biospecimens (RNA)**

Collect venous or arterial blood for RNA extraction in **PAXgene Blood RNA Tubes**.

#### **Local Processing (RNA)**

Invert gently **8–10 times** and incubate **upright at room temperature for 2 hours**. PAXgene tubes must not be frozen before the 2-hour incubation, **as premature** freezing compromises RNA integrity. Freeze at **-80°C** for long-term storage. Record time from blood draw to initial incubation and time from incubation to freezing, as deviations may affect downstream transcriptomic analyses. Ship tubes **frozen and unprocessed**.

### FOR BOTH DNA AND RNA

#### **Local Documentation and Storage**

Accurate documentation of biospecimen collection, processing, and storage is essential for high-quality data. Use **barcode identification** with automated date/time stamping. Maintain an **inventory system** tracking sample provenance, including collection, processing, storage, and QC procedures. All freeze–thaw events must be logged, and samples with more than one freeze–thaw cycle should be flagged for limited downstream use.

#### **Shipping**

Ship frozen samples on dry ice and ensure tubes remain upright and cushioned to prevent cracking during transport. RNA is particularly sensitive to fluctuations in temperature. Regulations for biospecimen shipping are detailed by International Air Transportation Association (IATA) (<https://www.iata.org>) and U.S. Department of

## Guidance for DNA and RNA Analysis of Blood Samples from TBI Participants

Transportation (DOT) (<https://www.transportation.gov/>). Most samples fall under **Category B Infectious Substances** or **Exempt Participant Specimens**, which have lower infection-risk classifications.

### Central Storage

Comprehensive documentation from individual sites is critical to ensure sample quality and reliable multicenter data. Use **barcoded sample IDs** with automated timestamps. Establish a **formal sharing plan** for central biospecimen distribution. Maintain records of laboratories receiving samples to prevent duplicative genotyping or redundant data reporting. Track any **informed consent stipulations**, including restrictions specific to TBI-related research. The central repository should perform periodic QC analysis (e.g., DNA integrity number for a random subset of samples) to confirm long-term storage stability.

### REFERENCES

Betsou F, Lehmann S, Ashton G, Barnes M, Benson EE, Coppola D, DeSouza Y, Eliason J, Glazer B, Guadagni F, Harding K, Horsfall DJ, Kleeberger C, Nanni U, Prasad A, Shea K, Skubitz A, Somiari S, Gunter E; International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Standard preanalytical coding for biospecimens: defining the sample PREanalytical code. *Cancer Epidemiol Biomarkers Prev.* 2010 Apr;19(4):1004-11.

Goossens W, Van Duppen V, Verwilghen RL. K2- or K3-EDTA: the anticoagulant of choice in routine haematology? *Clin Lab Haematol.* 1991;13(3):291-5.

Lehmann S, Guadagni F, Moore H, Ashton G, Barnes M, Benson E, Clements J, Koppandi I, Coppola D, Demiroglu SY, DeSouza Y, De Wilde A, Duker J, Eliason J, Glazer B, Harding K, Jeon JP, Kessler J, Kokkat T, Nanni U, Shea K, Skubitz A, Somiari S, Tybring G, Gunter E, Betsou F; International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Standard preanalytical coding for biospecimens: review and implementation of the Sample PREanalytical Code (SPREC). *Biopreserv Biobank.* 2012 Aug;10(4):366-74.

National Cancer Institute, National Institutes of Health. (2016). *NCI Best Practices for Biospecimen Resources* (March 2016). National Cancer Institute.

Parry-Jones, A. (2018). *ISBER best practices: recommendations for repositories* (4<sup>th</sup> edition.). Cryobiology.

Preanalytical Standards (SPREC) for biospecimen classification



## Guidance for Protein Analysis from Blood Samples of TBI Participants

**This document has been updated for TBI v3.0. The TBI v2.0 guidance document is available here: [Plasma and Serum Guidelines for Proteomic Analysis \(Table 2\)](#)**

### FOR ADULTS

#### Acquisition of Blood Biospecimens

For severely injured participants, collect blood through vascular access catheters placed for clinical care. For others, obtain samples via venipuncture. Typically, collect 5–10 mL of whole blood using a vacutainer system. Use polypropylene vacutainers and cryovials rather than glass, as proteins and peptides may adhere to glass surfaces.

#### Local Processing

Serum: Collect samples in vacutainers without anticoagulant (e.g., BD serum separator tubes; red or gold-topped). Gently invert tubes five times after collection. Allow blood to clot upright at room temperature for 30–60 minutes. Centrifuge within 90 minutes of blood draw.

Plasma: Collect samples in vacutainers with anticoagulant (e.g., BD lavender-top [EDTA] tube). Centrifuge within 30–45 minutes of blood draw. See Appendix for when to use EDTA tubes supplemented with protease and/or phosphatase inhibitors.

Centrifuge at 1300–2000 relative centrifugal force (*g*) for 10 minutes at 4°C. This lower temperature provides improved stability and reduced protease activity required for most brain proteins, cytokines, complement factors, and oxidative metabolites. (Exceptions include GFAP, S100B, and NSE for which room temperature centrifugation is acceptable.) Aliquot serum/plasma into pre-labeled 500 µL cryovials. Check for hemolysis using the CDC reference palette; redraw if > 20 mg/dL free hemoglobin. Record aliquot volumes. Because biomarkers such as GFAP, UCH-L1, tau species, and cytokines degrade with repeated cycles, allow no more than one freeze–thaw cycle before analysis.

#### Local Documentation and Storage

Accurate documentation of collection, processing, and storage is essential. Use barcode labeling for studies with more than 100 samples. Store samples at or below –70°C in ultra-low freezers with backup power, alarms, and a contingency plan for equipment failure. Maintain an inventory system tracking sample origin, time of collection, processing, storage, and quality control measures carried out on each sample.

#### Shipping

Ship frozen specimens on dry ice, minimizing transport time. Dry ice sublimates at 5–10 lbs per 24 hours; use insulated containers. Shipments should be scheduled Monday through Wednesday only to avoid weekend delays. Follow International Air Transportation Association website (<https://www.iata.org>) and the U.S. Department of Transportation website (<https://www.transportation.gov/>) requirements for packaging and labeling. Classify shipments as Category B Infectious Substances or Exempt Participant Specimens. Each shipment should include an itemized specimen manifest listing participant ID, timepoint, sample type, number of aliquots, and key timestamps. Use temperature loggers to verify temperature integrity. Upon arrival, receiving sites should confirm package integrity and immediately store all specimens at –80°C.

#### Central Storage for Multicenter Studies

For multicenter studies, ensure consistent documentation and sample handling. Barcode all samples. The central repository should track sample distribution to prevent duplicate testing and record consent restrictions, such as approval for TBI-specific use. Maintain a transparent sharing and access plan.



## Guidance for Protein Analysis from Blood Samples of TBI Participants

### FOR CHILDREN

#### **Acquisition of Blood Biospecimens**

For pediatric participants, adhere to IRB-approved blood volume limits. (**Table 1**) For example, in a 4 kg infant, the total allowable draw (clinical and research) is 8 mL per visit and 16 mL per 30 days. Use leftover clinical serum for research when possible, provided processing and storage meet study standards.

#### **Site of Sample Collection**

Record the collection site. Venous or arterial samples are preferred, though capillary collection may be acceptable in pre-mobile children when performed by trained staff after warming the heel. Up to 1 mL can be collected via heel stick. Comparative data between capillary and venous samples are lacking.

#### **Hemolysis**

Hemolysis is common in pediatric samples due to small needle gauges and capillary draws. Hemolysis may falsely elevate biomarkers such as neuron-specific enolase (NSE). Quantitative assessment with adjustment factors can correct NSE values but not other biomarkers (Berger & Richichi, 2009).

#### **Sample Processing**

Follow adult procedures with smaller aliquots. Because most assays require <100 µL, consider aliquot volumes of 250-500 µL per vial.

#### **Documentation and Storage**

Record the exact time after injury for each sample. For biomarkers with short half-lives, such as S100B, document both time of injury and collection. When the time of injury is unknown, standardize it to the time of first medical contact (e.g., EMS call or hospital arrival). Store samples as for adults.

Because informed consent is ongoing under Office for Human Research Protection (OHRP) regulations, re-consent is required when participants reach age 18 unless waived by the IRB. If re-consent is not obtained, samples must be anonymized or destroyed. Implement tracking to flag participants' 18th birthdays for timely re-consent, anonymization, or disposal.

### VARIABLES AFFECTING INTERPRETATION OF SERUM AND PLASMA BIOMARKERS

To support accurate biomarker interpretation and harmonization across sites, the following variables should be collected for each specimen:

**Recommended:** age, sex, race/ethnicity, time of injury, time of blood collection, collection method (venous, arterial, capillary), tube type and additives, time from collection to freezing, freezer temperature, and number of freeze–thaw cycles.

**Optional:** Fasting status, recent exertion (within 24 hours), last menstrual period, hormonal contraception use, renal function indices, and hepatic function indices.

**Additional optional variables commonly collected in 2024–2025 biomarker networks:** APOE genotype, BMI, time since last analgesic use (opioids or ketamine), albumin CSF:serum ratio (blood–brain barrier integrity)

### REFERENCES

Berger R, Richichi R. Derivation and validation of an equation for adjustment of neuron-specific enolase concentrations in hemolyzed serum. *Pediatr Crit Care Med*. 2009 Mar;10(2):260-3.

## **Guidance for Protein Analysis from Blood Samples of TBI Participants**

Goossens W, Van Duppen V, Verwilghen RL. K2- or K3-EDTA: the anticoagulant of choice in routine haematology? Clin Lab Haematol. 1991;13(3):291-5.

## Guidance for Protein Analysis from Blood Samples of TBI Participants

**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 participant 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

## Guidance for Protein Analysis from Blood Samples of TBI Participants

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 participant has respiratory/CV compromise
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

### APPENDIX

**Use K<sub>2</sub>EDTA tubes *supplemented with protease ± phosphatase inhibitors*** when collecting plasma for:

- Labile or phosphorylation-sensitive brain proteins, including:
  - Total tau, phosphorylated tau (p-tau181/217/231), BD-tau
  - Other neuronal phosphoproteins
- Broad proteomic analyses, including:
  - Untargeted or targeted mass spectrometry
  - Olink, MSD, or other multiplex inflammatory/complement panels
  - Exosomal/EV protein assays
- Complement or activation-sensitive biomarkers (e.g., C3a, C5a)
- Future, undefined biomarker discovery where stability requirements are unknown.
- When delayed processing is expected, including:
  - Field, athletic, or prehospital collection
  - Any anticipated delay >30 minutes from draw to centrifugation
  - Situations where samples may briefly remain at room temperature

### Handling requirements for inhibitor tubes:

- Keep tubes **on ice** immediately after collection.
- Centrifuge at **4°C within 60 minutes** of draw.
- Aliquot to labeled cryovials and freeze at **-80°C**.

**Standard EDTA tubes without inhibitors are acceptable** for stable biomarkers (GFAP, NfL, UCH-L1, S100B, NSE) **when** processing is cold and completed **within 60 minutes**.

### Do not use inhibitor-containing tubes for clinical chemistry panels.

- If clinical and research testing are both needed, draw a **separate inhibitor tube labeled “Research Only.”**

## Guidance for Protein Analysis from Cerebrospinal Fluid (CSF) Samples of TBI Participants

**This document has been updated for TBI v3.0. The TBI v2.0 guidance document is available here: [Cerebral Spinal Fluid Guidelines \(Table 3\)](#)**

### FOR ADULTS

#### Acquisition of CSF Biospecimens

For participants with an external ventricular drain (EVD), document whether drainage is continuous or intermittent, as drainage mode alters CSF protein concentration (Shore et al., 2004). Continuous drainage typically produces **lower and more stable** protein concentrations than intermittent drainage.

When feasible, collect the first available CSF sample after EVD placement, up to 5 mL. Draw CSF directly from the ventriculostomy sampling port using sterile technique and gentle aspiration. Avoid glass or polystyrene tubes.

CSF obtained via lumbar puncture (LP) may be used for participants without ventriculostomy. Use atraumatic spinal needles to reduce post-LP headache. CSF may be collected in multiple 1 mL polypropylene fractions, up to a maximum of 25 mL per timepoint. Fraction number should be recorded for each participant because protein concentrations vary across the draw volume (Blennow et al., 1993). Participants should rest in a recumbent position for one hour after LP and avoid exertion for 24–48 hours.

Blood contamination is a major confound because plasma protein concentration is approximately 400-fold higher than that of CSF (Maurer, 2010). Evaluate contamination using RBC counts or hemoglobin; contamination is defined as >10 RBCs/ $\mu$ L or >30 pg/mL hemoglobin (Zhang et al., 2007). For cytokines, chemokines, and proteomic assays, exclude samples with >5 RBCs/ $\mu$ L due to platelet- and erythrocyte-related artifacts. Acceptable control CSF may be obtained from hydrocephalus participants undergoing shunt placement or from unruptured subarachnoid hemorrhage participants undergoing intraoperative CSF sampling (Pineda et al., 2007).

Do not directly compare ventricular and lumbar CSF for tau, NfL, or GFAP due to rostrocaudal gradients (Hühmer et al., 2006). Ventricular CSF has lower axonal injury marker concentrations (NfL, tau) than lumbar CSF.

#### Local Processing

Transport CSF on ice to the processing area immediately. Centrifuge at 2,000 g  $\times$  10 min at 4°C. For cytokine or extracellular vesicle studies, add a second high-speed spin at 10,000 g  $\times$  10 min to remove residual platelets.

Carefully aliquot 0.5–1.0 mL of supernatant into pre-labeled polypropylene cryovials, again avoiding glass, polycarbonate or polystyrene tubes to prevent protein loss. Add protease and phosphatase inhibitors when appropriate for downstream applications.

#### Local Documentation and Storage

Document sample appearance (clear, cloudy, or bloody), time of collection, aliquot number and volume, and time of freezing. Store CSF at  $-70^{\circ}\text{C}$  or below; never at  $-20^{\circ}\text{C}$  (Wagner et al., 2007, Carrette et al., 2005). BD-tau, p-tau217, and p-tau231 **should not exceed 2 freeze–thaw cycles** due to demonstrated instability. Liquid nitrogen vapor shippers ( $\leq -130^{\circ}\text{C}$ ) are preferred over dry ice for long-distance transport. Maintain an inventory system that tracks sample collection parameters, laboratory handling steps, and quality assessments.

# Guidance for Protein Analysis from Cerebrospinal Fluid (CSF) Samples of TBI Participants

## FOR CHILDREN

### **Acquisition of CSF Biospecimens**

Pediatric acquisition parallels adult procedures. LP in healthy children requires IRB review because children cannot undergo > minimal-risk procedures without direct benefit (45 CFR 46 Subpart D; US DHHS 2023).

For abusive head trauma or unwitnessed injuries, define time of injury as **first medical contact**. This definition should also be used for unwitnessed adult injuries for consistency.

### **Sample Processing**

Processing is identical to adults but with smaller aliquots to reduce freeze–thaw cycles. Because multiplex assays typically require <100 µL, aliquoting in 250 µL portions is preferred.

### **Documentation and Storage**

Record the exact time after injury for each CSF sample, as this is crucial for biomarkers with short half-lives. Storage conditions follow adult guidance. Track specimens until adulthood. At age 18, samples must be re-consented, anonymized, or discarded in accordance with OHRP requirements. OHRP (2024) requires explicit documentation of whether broad future-use consent is granted at re-consent.

## VARIABLES AFFECTING INTERPRETATION OF CSF BIOMARKERS

To support accurate biomarker interpretation and harmonization across sites, the following variables should be collected for each specimen:

**Recommended:** age, sex, race/ethnicity, time of injury, time of CSF collection, collection method (EVD or LP), fraction number (for LP), tube type, time from collection to freezing, freezer temperature, and number of freeze–thaw cycles.

**Optional:** Fasting status, recent exertion (within 24 hours), last menstrual period, hormonal contraception use, renal function indices, and hepatic function indices.

**Additional optional variables commonly collected in 2024–2025 biomarker networks:** APOE genotype, BMI, time since last analgesic use (opioids or ketamine), albumin CSF:serum ratio (blood–brain barrier integrity)

## REFERENCES

Bergquist J, Palmblad M, Wetterhall M, Håkansson P, Markides KE. Peptide mapping of proteins in human body fluids using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Mass Spectrom Rev.* 2002 Jan-Feb;21(1):2-15.

Blennow K, Fredman P, Wallin A, Gottfries CG, Långström G, Svennerholm L. Protein analyses in cerebrospinal fluid. I. Influence of concentration gradients for proteins on cerebrospinal fluid/serum albumin ratio. *Eur Neurol.* 1993;33(2):126-8.

Carrette O, Burkhard PR, Hughes S, Hochstrasser DF, Sanchez JC. Truncated cystatin C in cerebrospinal fluid: Technical [corrected] artefact or biological process? *Proteomics.* 2005 Aug;5(12):3060-5.

Hesse C, Larsson H, Fredman P, Minthon L, Andreasen N, Davidsson P, Blennow K. Measurement of apolipoprotein E (apoE) in cerebrospinal fluid. *Neurochem Res.* 2000 Apr;25(4):511-7.

Hühmer AF, Biringer RG, Amato H, Fonteh AN, Harrington MG. Protein analysis in human cerebrospinal fluid: Physiological aspects, current progress and future challenges. *Dis Markers.* 2006;22(1-2):3-26.

## **Guidance for Protein Analysis from Cerebrospinal Fluid (CSF) Samples of TBI Participants**

Maurer MH. Proteomics of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF). *Mass Spectrom Rev*. 2010 Jan-Feb;29(1):17-28.

Pineda JA, Lewis SB, Valadka AB, Papa L, Hannay HJ, Heaton SC, Demery JA, Liu MC, Aikman JM, Akle V, Brophy GM, Tepas JJ, Wang KK, Robertson CS, Hayes RL. Clinical significance of alphaII-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. *J Neurotrauma*. 2007 Feb;24(2):354-66.

Shore PM, Thomas NJ, Clark RS, Adelson PD, Wisniewski SR, Janesko KL, Bayir H, Jackson EK, Kochanek PM. Continuous versus intermittent cerebrospinal fluid drainage after severe traumatic brain injury in children: effect on biochemical markers. *J Neurotrauma*. 2004 Sep;21(9):1113-22.

Wagner AK, Ren D, Conley YP, Ma X, Kerr ME, Zafonte RD, Puccio AM, Marion DW, Dixon CE. Sex and genetic associations with cerebrospinal fluid dopamine and metabolite production after severe traumatic brain injury. *J Neurosurg*. 2007 Apr;106(4):538-47.

Zhang J. Proteomics of human cerebrospinal fluid - the good, the bad, and the ugly. *Proteomics Clin Appl*. 2007 Aug;1(8):805-19.