## Probe Placement

1. In TBI, placement in peri-contusional at risk tissue is recommended and if the option for placement of a second probe is available, the second probe should be placed in normal tissue.
2. Probe placement directly into contusions is of no value
3. In case of diffuse injury, probe placement should be in the right frontal lobe.

## Probe Characteristics

* 1. Concentric configuration commercially available probes are preferable to locally fabricated probes.
  2. Low molecular weight cut-off probes have been in use for many years and are available worldwide (Shores and Knapp, 2007).
  3. Higher molecular weight cut-off probes are available but have not passed regulatory clearance in all jurisdictions including the USA.
  4. Probe molecular weight cut-off, manufacturer and model must be specified in all data reporting.

## Microdialysate Characteristics

* 1. Any of the following microdialysate fluids are acceptable:
     1. Artificial CSF
     2. Sterile medical grade normal saline
  2. The composition and source of the microdialysate must be specified in all data reporting

## Sample Acquisition

1. The microdialysate flow rate should be 0.3 μL/min so that it is theoretically possible to collect 18 μL during one hour. This flow rate assures near 100% analyte recovery for common low molecular weight analytes.
2. Due to the extremely small sample volumes, the samples are sensitive to evaporation even though they are “sealed ” in the microvials and since microvials may contain different volumes, the effect of evaporation can vary between the samples. Characteristics of the collecting containers must be reported.
3. Samples should be analyzed and/or stored promptly.

## Analytes

1. The minimal essential data set consists of glucose, pyruvate, lactate, with use of the calculated L/P ratio.
2. Measurement of glycerol and glutamate is recommended.
3. Determination of other analytes including markers of neuroinflammation such as cytokines, nitrate and nitrite as surrogates for nitrous oxide, structural proteins such as GFAP, neurofilament, tau and stress reactants such as beta-amyloid are all experimental. Analysis of these and other potential biomarkers should be encouraged but not required.

## Analyte Stability During Storage

1. The essential low molecular weight analytes are stable at -70 ºC. The samples should be stored at this temperature in containers designed to minimize evaporative losses
2. Analytes, particularly pyruvate, may not be stable at -20 ºC. Storage at this temperature is not acceptable with the exception of temporary holding for not more than 3 days.
3. Determination of other analytes including markers of neuroinflammation such as cytokines, nitrate and nitrite as surrogates for nitrous oxide, structural proteins such as GFAP, neurofilament, tau and stress reactants such as beta-amyloid are all experimental. Analysis of these and other potential biomarkers should be encouraged but not required.

## Handling of Samples at the Time of Analysis

1. If the samples have been frozen, then they must be thawed prior to analysis. It is important to recognize physicochemical events that may occur in the process of thawing that might cause analytical errors. As the samples thaw, the liquid phase will initially contain a very high concentration of salt and analytes. As the thawing progresses, the solution will be diluted by the melting ice. During this process there is a risk that the thawed samples are non-homogeneous; therefore recommended that the samples be thawed and then agitated or centrifuged to assure homogeneous distribution of analyte.
2. It may be desirable to thaw the samples rapidly in a heating cupboard at +40 ºC for about 10 minutes. Longer times and/or higher temperatures, however, should not be used as these may result in a risk for unacceptable evaporation.
3. Stored samples may be assayed using the batch analysis systems. However if the low volume samples sit for too long in the analyzer prior to analysis, unacceptable evaporation may occur. Calibration samples should be interspersed in the batch to detect a systematic elevation in analyte levels due to evaporative loss.

## Microdialysis Data Reporting

1. Analyte concentrations should be reported in SI units.
2. Ratios such as the L/P ratio are devoid of units.

## References

Shores KS, Knapp DR. (2007) Assessment approach for evaluating high abundance protein depletion methods for cerebrospinal fluid (CSF) proteomic analysis. J. Proteome Res. 6:3739-3751.