

## 24-HOUR BIOFLUID SAMPLING STUDY

### Summary of Biomarker Sample Collection

#### Study 1 – Young Healthy Control Subjects

##### 1.1 CSF Sample Collection and Handling

The investigator may use fluoroscopy and/or local anesthesia for the insertion of intrathecal catheter, if required. Upon secure placement of the catheter, CSF samples will be collected by connecting the catheter to a peristaltic roller pump. Six (6) mL samples will be collected at 0 (within 30 minutes of catheterization), 1, 2, 4, 6, 10, 12, 16, 20, 24 and 26 hours. The rate of CSF collection will be approximately 0.5 mL/min. Thus, it is anticipated to take 12 minutes to collect each 6 mL sample. In order to collect the samples close to the identified sample times, the CSF collection will be started approximately 6 minutes prior to each identified sampling time and will be concluded approximately 6 minutes after these timepoints. The residual volume (approximately 2 mL) remaining in the catheter from a previous collection will be collected prior to the collection of subsequent samples using the same rate of 0.5 mL/min and kept separate from the other samples. The residual volume samples will be processed similarly to the timed samples. The 6 mL CSF samples and 2 mL residual samples will be collected, centrifuged (4°C, 1600 x g, 15 minutes). The 6 mL CSF sample supernatants will be transferred first as six 0.5 mL aliquots and then as three 1.0 mL aliquots. The 2 mL residual sample supernatants will be transferred as one 2.0 mL aliquot. All CSF aliquots will be placed into individual, appropriately labeled screw-capped polypropylene tube and stored at approximately -80°C or on dry ice within 1 hour of collection. Each CSF label should contain the word "CSF", the date of collection, the time of collection, the subject's initials, subject number and the visit number. The actual start and stop times of the CSF sample will be recorded in the CRF or data collection tool. The time between collection and processing, processing and freezing, and the time of the last meal will also be recorded.

The quality of each of the CSF samples should be clear and visually colorless. Prior to centrifugation, a 10 µL aliquot will be taken from each of the 6 mL samples and used for microscopic analysis of red blood cell (RBC) concentrations. The CSF microanalysis (and protein assessment) should be performed following gentle mixing of the sample (gentle inversion). The analysis should be done STAT, since the cells are stable in CSF for only a short period of time (1 hour). If the CSF sample is considered to be contaminated with blood (as determined by visual inspection, eg, colored or cloudy), the sample should

not be used and continued participation of the subject in the study should be assessed by the investigator. The quality of the sample as well as the RBC concentration will be recorded. The investigator or designee will examine the CSF sampling procedure regularly to monitor the quality of the sample, as well as the subject.

## **1.2 Serum and Plasma Sample Collection and Handling**

Serum and plasma samples will be collected at 0 (within 30 minutes of catheterization), 1, 2, 4, 6, 10, 12, 16, 20, 24 and 26 hours. Approximately 10 mL of whole blood will be collected in a red top vacutainer and 10 mL of whole blood will be collected in an EDTA purple top vacutainer. The samples will then be centrifuged at 1350 x g for 15 minutes at 4°C. The serum and plasma will then be transferred first as six 0.5 mL aliquots and then as three 1.0 mL aliquots into individual cryotubes. Each label should contain the word "serum" or "plasma", the date of collection, the time of collection, the subject's initials, subject number and the visit number. The time of processing and freezing will also be collected. Serum and plasma samples will be stored at approximately -80°C (or approximately -20°C if -80°C is unavailable), until shipment on dry ice.

## **Study 2 – Parkinson's Disease subjects and elderly age-matched controls**

### **2.1 CSF Sample Collection and Handling**

The investigator may use fluoroscopy and/or local anesthesia for the insertion of intrathecal catheter, if required. Upon secure placement of the catheter, CSF samples will be collected by connecting the catheter to a peristaltic roller pump. Six (6) mL samples will be collected at 0 (within 30 minutes of catheterization), 1, 2, 4, 6, 10, 12, 16, 20, 24 and 26 hours. The rate of CSF collection will be approximately 0.5 mL/min. Thus, it is anticipated to take 12 minutes to collect each 6 mL sample. In order to collect the samples close to the identified sample times, the CSF collection will be started approximately 6 minutes prior to each identified sampling time and will be concluded approximately 6 minutes after these timepoints. The residual volume (approximately 2 mL) remaining in the catheter from a previous collection will be collected prior to the collection of subsequent samples using the same rate of 0.5 mL/min and kept separate from the other samples. The residual volume samples will be processed similarly to the timed samples. The 6 mL CSF samples and 2 mL residual samples will be collected, centrifuged (4°C, 1600 x g, 15 minutes). The 6 mL CSF sample supernatants will be transferred first as six 0.5 mL aliquots and then as three 1.0 mL aliquots. The 2 mL residual sample supernatants will be transferred as one 2.0 mL aliquot. All CSF aliquots will be placed into individual, appropriately labeled screw-capped polypropylene tube and stored at approximately -80°C or on dry ice within 1 hour of

collection. Each CSF label should contain the word “CSF”, the date of collection, the time of collection, the patient/subject's initials, and patient/subject number. The actual start and stop times of the CSF sample will be recorded in the CRF or data collection tool. The time between collection and processing, processing and freezing, and the time of the last meal will also be recorded.

The quality of each of the CSF samples should be clear and visually colorless. For the 0 hour collection, the microscopic analysis of red blood cell (RBC) concentrations in CSF will be measured from the 2 mL safety sample. For the 12 and 26 hour sample collections, RBC concentrations will be measured from the discard sample collected at those timepoints. The CSF microanalysis (and protein assessment) should be performed following gentle mixing of the sample (gentle inversion). The analysis should be done STAT, since the cells are stable in CSF for only a short period of time (1 hour). If the CSF sample is considered to be contaminated with blood (as determined by visual inspection, eg, colored or cloudy), the sample should not be used and continued participation of the patient/subject in the study should be assessed by the investigator. The quality of the sample as well as the RBC concentration will be recorded. The investigator or designee will examine the CSF sampling procedure regularly to monitor the quality of the sample, as well as the patient/subject.

## **2.2 Serum and Whole Blood Sample Collection and Handling**

Serum and whole blood samples will be collected at 0 (within 30 minutes of catheterization), 1, 2, 4, 6, 10, 12, 16, 20, 24 and 26 hours. For serum, approximately 10 mL of whole blood will be collected in a red top vacutainer. The samples will then be centrifuged at 1350 x g for 15 minutes at 4°C. The serum will then be transferred first as six 0.5 mL aliquots and then as three 1.0 mL aliquots into individual cryotubes. Each label should contain the word “serum”, the date of collection, the time of collection, the patient/subject's initials, and patient/subject number. The time of processing and freezing will also be collected. Serum samples will be stored at approximately -80°C (or approximately -20°C if -80°C is unavailable), until shipment on dry ice.

Whole blood samples of 8 mL will be collected into two 4 mL EDTA purple top vacutainers, mixed thoroughly, and then immediately frozen. Each label should contain the words “whole blood”, the date of collection, the time of collection, the patient/subject's initials, and patient/subject number. The time of freezing will also be collected. Whole blood samples will be stored at approximately -80°C (or approximately -20°C if -80°C is unavailable), until shipment on dry ice.