



Cerebral Spinal Fluid Biomarkers Support Central Nervous System Studies

The ratios of these important biomarkers for neurological disorders can be very predictive of disease state and potential efficacy of treatment

Alzheimer's Disease (AD) is the most common form of dementia in the elderly and will soon become a public health crisis in the United States. Definitive diagnosis is currently made at autopsy and is characterized by neurofibrillary tangles consisting of hyperphosphorylated microtubule-associated Tau proteins (P-Tau) and extracellular deposits of amyloid beta protein.

Probable diagnosis is determined using neuropsychological tests. Neuroimaging tools, including positron emission tomography and functional magnetic resonance imaging, are being evaluated in current trials to determine diagnostic sensitivity and specificity.

The availability of a serum or cerebral spinal fluid (CSF) diagnostic biomarker would greatly facilitate patient treatment and the development of therapeutic AD drugs. Two obvious candidates are A β 42 (CSF/serum) and total Tau/p-Tau (CSF).

Commercial assays to measure these biomarkers are available from numerous vendors. Immunological and protein products are available to researchers to develop bioanalytical tools.

However, the wide availability of these reagents has resulted in a lack of method standardization. It is often times impossible to compare data between studies.

Below we report on the comparison of two commercial methods used to analyze A β 42 in CSF. The CSF samples are unique in that they were obtained during a continuous sampling procedure over the course of a four-hour period.

METHODS

Normal healthy subjects were admitted to the CEDRA Clinic Research LLC in San Antonio one day prior to the sampling procedure. On the morning of the procedure, under sterile conditions, an anesthesiologist inserted an epidural catheter into the intrathecal space at L3-L4 or L4-L5.

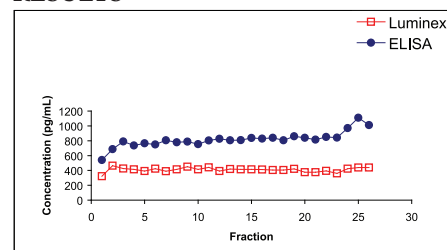
The distal end of the epidural catheter was connected via a luer lock connector to a system of tubing, which was then fitted into a peristaltic pump. Continuous CSF sampling occurred for four hours at a rate of 3cc per 30 minutes.

Samples were collected in 1 mL aliquots using a fraction collector. The samples were maintained at near-freezing temperatures of 1° to 9° C during collection to minimize the degradation of potentially useful CNS biomarkers.

Samples were subsequently stored at -70° C and thawed just prior to analysis. Samples were analyzed with validated

methods for A β 42 using the INNO-BIA® AlzBio3 multiplex assay on a Luminex 200 Analyzer (Method 1) and by INNOTEST® β -Amyloid(1-42) test ELISA (Method 2).

RESULTS



Comparison of A β 42 CSF concentrations from a healthy male collected by continuous sampling for four hours using the INNO-BIA® AlzBio3 multiplex assay on a Luminex 200 Analyzer and by INNOTEST® β -Amyloid(1-42) test ELISA.

Analytical Method

- The performance of both methods during validation was acceptable for accuracy, precision, and stability.
- The determination of A β 42 from CSF samples yields method-dependent results.
- The ELISA method is approximately two-fold higher than the Luminex method.
- The use of a single method is recommended to make appropriate conclusions from the data.

Continuous CSF Collection

- The initial CSF fractions may contain A β 42 concentrations that are significantly different than continuous-flowing fractions.
- The A β 42 concentration in normal patients is constant during the collection period.
- Continuous CSF sampling for 4 hr at 6 mL/hr provides consistent A β 42 concentrations.

CEDRA CORPORATION/WCT SERVICES

CEDRA Corporation/Worldwide Clinical Trials (WCT) is a leader in supporting clinical trials and bioanalytical services for CNS studies.¹ In addition to the measurement of analytes to support neurodegenerative diseases, we offer bioanalytical services to support other CNS studies.¹

The list of biogenic amines and their metabolites available for quantitation from CSF, plasma, and urine include norepinephrine, dihydroxyphenylglycol, serotonin, 5-hydroxyindoleacetic acid, dopamine, and homovanillic acid. The ratios of these important biomarkers for neurological disorders can be very predictive of disease state and potential efficacy of treatment.

The analysis of this class of compounds

is difficult because of their low abundance in some matrices and their potential for instability due to oxidation. Traditionally, these compounds have utilized HPLC-EC detection; however, LC/MS/MS improves the selectivity and sensitivity of analysis

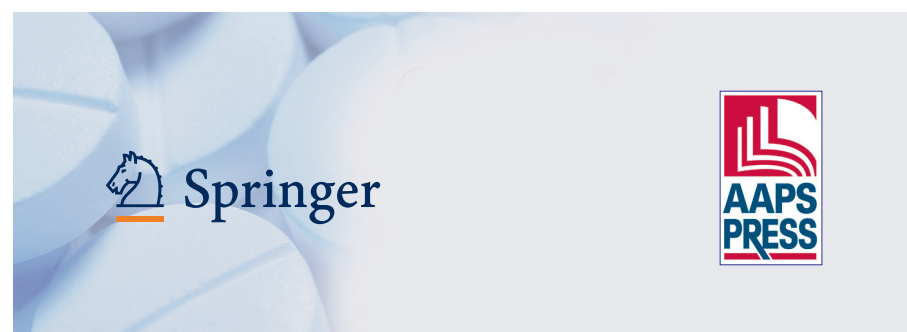
and can shorten sample preparation and analysis time. Improved sensitivity of LC/MS/MS leads to a conservation of some very difficult-to-acquire samples, such as CSF. The ranges of quantitation for these methods are listed in the table below.

RANGES OF QUANTITATION

Cerebral Spinal Fluid		
5-HT/5-HIAA*	DA/HVA [^]	NE/DHPG*
5-250 pg/mL 5-HT	40-2000 pg/mL DA	40-2000 pg/mL NE
2-100 ng/mL 5-HIAA	5-250 ng/mL HVA	200-10,000 pg/mL DHPG
K2-EDTA Human Plasma		
5-HT/5-HIAA*	DA/HVA [^]	NE/DHPG*
50-25,000 pg/mL 5-HT	10-1000 pg/mL DA	40-2000 pg/mL NE
200-20,000 pg/mL 5-HIAA	1-100 ng/mL HVA	200-10,000 pg/mL DHPG
Human Urine		
5-HT/5-HIAA*	DA/HVA [^]	NE/DHPG*
10-1000 ng/mL 5-HT	1-100 ng/mL DA	1-200 ng/mL NE
100-10,000 ng/mL 5-HIAA	100-10,000ng/mL HVA	10-2000 ng/mL DHPG

*5-HT/5-HIAA=serotonin/5-hydroxyindoleacetic acid. [^] DA/HVA=dopamine/homovanillic acid.

*NE/DHPG=norepinephrine/dihydroxyphenylglycol.



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