**Abstract**

Background: Tau has emerged as a putative therapeutic target for many neurodegenerative disorders. This protein is a major component of paired helical filaments and other large intracellular aggregates in the brains of patients with Alzheimer’s disease (AD) and other neurological disorders. Clinical diagnosis of AD most often occurs several years after neurodegeneration commences. The accumulation of Tau protein in the cerebrospinal fluid (CSF) of AD patients correlates with neurodegeneration, and may also be a useful biomarker for identifying patients with mild cognitive impairment (MCI). However, collection of CSF is invasive, painful, and inconvenient for routine screening for early detection of the disease. Use of blood and urine is greatly preferred, but levels of Tau in blood are largely undetectable with currently available technologies. Therefore, more sensitive detection methods are required to evaluate Tau as a biomarker for AD in blood.

Methods: MSD offers a Human Total Tau assay kit that has been validated for the detection of Tau protein in CSF. The MSD V-PLEX™ Tau assay offers sensitivities competitive with other commercially available assays (Lower limit of quantitation [LOQ] of 30 pg/mL). However, higher sensitivity is needed for the detection of Tau in serum or plasma. We developed a next-generation assay format, S-PLEX™, using MSD’s MULTI-ARRAY™ electrochemiluminescence technology. The S-PLEX technology allows quantification of previously unmeasurable levels of biomarkers with fg/ml sensitivity and a wide dynamic range. S-PLEX assays are compatible with existing MSD instrumentation. An S-PLEX assay was developed for Tau and used to measure Tau levels in serum/plasma samples from both normal and diseased individuals. Recombinant forms of known Tau isoforms were also tested.

Results: The newly developed MSD S-PLEX Tau assay has a limit of detection (LOD) of 6 fg/mL, with lower and upper limits of quantitation of 22 pg/mL (ULOQ) and 160,000 pg/mL (ULOQ), respectively, covering a dynamic range of approximately 5 logs. The S-PLEX Tau assay detects all 6 isoforms of the protein tested. Tau recovery and dilutional linearity were between 80% and 120% for both serum and plasma samples. Levels of Tau in normal serum (n=16), normal plasma (n=16), and plasma from AD patients (n=13) were detectable in all samples, and median concentrations observed were 1.7 pg/mL (interquartile range [IQR] 1.1 - 2.5 pg/mL), 2.6 pg/mL (IQR 1.7 - 4.1 pg/mL), and 3.6 pg/mL (IQR 0.4 - 4.7 pg/mL), respectively. Elevated levels of Tau (n = 2, median concentration 13 pg/mL) were observed in plasma samples from patients with traumatic brain injury (TBI). Levels of Tau in all normal serum and plasma samples were easily measurable using fifty-fold diluted samples. Calibration: MSD has developed a next-generation Tau assay that is up to 1,000x more sensitive than currently available Tau assays. This new assay format can be run on a standard MSD instrument and can be performed within a normal workday using common lab services.

Conclusions: A next-generation assay for human Tau was developed, based on MSD’s ultra-sensitive S-PLEX technology. This novel technology is 100 - 1,000 times more sensitive than the currently available Tau assays. This enables accurate determination of Tau concentrations in serum and plasma samples. This new assay format can be run on a standard MSD instrument and can be performed within a normal workday using common lab equipment. The assay will initially be available through MSD’s assay services.

**Methods**

MSD’s electrochemiluminescence detection technology uses SULFO-TAG™ labels which emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY™ and MULTI-SPOT™ microparticles. We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY™ technology with significantly higher sensitivity.

**Calibration Curve, Assay Range**

The calibration curve was generated by using serial dilutions of recombinant Tau protein. The recombinant Tau protein used for assay calibration represents the longest isoform, Tau 441. Other isoforms showed similar performance to Tau 441. LOD is a calculated concentration corresponding to the average signal at 2.5 standard deviations above the background (zero calibrator). LOQ and ULOQ are established for the assay by measuring multiple levels of calibrator near the expected LOQ and ULOQ. LOQ and ULOQ are the lowest and highest concentrations of calibrator tested which have 4%CV of 20% or less, with recovered concentrations within 70-130%.

**Reproducibility**

To determine reproducibility of quality controls (QC, calibrator in aliquot), 5 replicates of each sample were measured in a run for 8 runs by at least two operators over at least 4 days (n=40). The % CV for each source of variation was determined using analysis of variance (ANOVA) with guidance from NCCLS document EP5-A2. The % CV for QC samples ranged from 3.5% to 4.7% within run and from 2.6% to 4.8% between runs. Average concentration recovery for all QC samples was 94-95%, with individual replicate recovery within 30% of the expected concentration.

**Dilution Linearity, Spike Recovery**

Serum, EDTA plasma, and heparin plasma samples (n=9) were diluted 2x, 4x, and 8x. Average dilution linearies for serum/plasma samples were 104%. Serum, EDTA plasma, and heparin plasma samples (n=9) were spiked with calibrator at three concentrations. Percent recovery was calculated by taking the difference between measured concentration in spiked and native sample, and dividing by the known spike concentration [% Recovery = (Measured Spike – Measured Unspiked/Spiked)]. Average spike recovery for the Tau assay was 103%.

**Human Samples**

Thirty two matched serum and EDTA plasma samples from 16 normal donors, EDTA plasma from AD patients (n=13) and subjects with mild TBI (n=8), and 10 individual CSF samples were tested on the S-PLEX Tau assay. Some TBI and CSF samples were tested 50-fold diluted. Tau was detectable and quantifiable in 100% of the samples. CSF samples are recommended to be run 50-fold diluted.

**Conclusions**

A next-generation assay for human Tau was developed, based on MSD’s ultra-sensitive S-PLEX technology. This novel technology is 100 - 1,000 times more sensitive than the currently available Tau assays. This enables accurate determination of Tau concentrations in serum and plasma samples. This new assay format can be run on a standard MSD instrument and can be performed within a normal workday using common lab equipment. The assay will initially be available through MSD’s assay services.