Adaptation and Validation of a Multiplex Assay Kit for the Quantitative Analysis of Aβ38, Aβ40, and Aβ42 Peptides in Cynomolgus Monkey Plasma

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**Purpose**
A multiplex assay kit, namely, the V-PLEX™ Aβ Peptide Panel 1 (6E10) kit, has been designed and commercialized by Meso Scale Discovery® for the simultaneous quantitative analysis of beta amyloid peptides Aβ38, Aβ40, and Aβ42 in human cerebrospinal fluid (CSF). These Aβ peptides are potential diagnostic biomarkers in neurodegenerative disorders such as Alzheimer’s disease. The assay kit as such, however, is not suitable for measuring Aβ38, Aβ40, and Aβ42 in human plasma. Therefore, we adapted this multiplex assay panel for quantification of these biomarkers in monkey plasma and performed a “fit-for-purpose” method validation prior to using the assay in a preclinical study.

**Methods**
Preliminary experiments revealed that reconstitution of calibrators in the assay diluent (as recommended by the manufacturer) gave erroneous results in the quality control (QC) samples prepared in monkey plasma. We found that spiking calibrators and QCs in pooled normal monkey plasma (instead of assay diluent) dramatically improved assay performance. Normal monkey plasma was screened to determine the endogenous levels of each Aβ peptide. To evaluate precision and accuracy, 6 batches of validation samples at 5 concentration levels, namely, at the lower limit of quantification (LLOQ), low QC (LQC), medium QC (MQC), high QC (HQC), and upper limit of quantification (ULOQ), were assayed. Additional batches of validation samples were also analyzed to evaluate other parameters including selectivity, specificity, dilution integrity, stability, and ruggedness.

**Results**
The minimum required dilution for monkey plasma was determined to be a 2-fold dilution with assay diluent. A 4 parameter logistic curve fit for calibration standards yielded a mean correlation coefficient (R2) > 0.99 for each Aβ peptide. The dynamic ranges of quantifications (LLOQ to ULOQ) for Aβ38, Aβ40, and Aβ42 were established as 127 to 16,240 pg/mL, 97 to 12,400 pg/mL, 17 to 2208 pg/mL, respectively, in monkey plasma. The inter assay %CV ranged from 1.1% to 6.5% and the percent analytical recovery (%AR) from 97.9% to 113.7%; the intra-assay %CV ranged from 0.2% to 12.4% and %AR from 95.1% to 118.9%. Selectivity was established by spiking each Aβ peptide near the respective LQC level in an individual lot of monkey plasma, and no significant matrix effect was observed. Dilution integrity was demonstrated up to 100-fold dilution. Specificity tests showed that two closely related peptides Aβ43 and Tau did not interfere with the measurement of the 3 Aβ peptides in monkey plasma. Stability of each Aβ peptide was also established in monkey plasma after 4 freeze/thaw (−80°C/room temperature) cycles, 5 hours at benchtop, 16 hours at 4°C, and long-term frozen (−80°C) storage for up to 2 weeks for Aβ38 and Aβ40 and for up to 1 week for Aβ42.

**Conclusion**
We successfully adapted and validated the V-PLEX™ Aβ Panel 1 multiplex assay kit for the simultaneous quantitative analysis of 3 Aβ peptides in cynomolgus monkey plasma. Reconstitution of calibrators in the test matrix was the critical step in adapting this kit for use in monkey plasma; this change maintained the performance characteristics of the method. The assay exhibited acceptable precision, accuracy, selectivity, specificity, dilution integrity, stability, and ruggedness.