The Impact of Assay Format on Sensitivity and Matrix Tolerance for Plasma Amyloid Beta Peptide (Aβ40 and Aβ42) Measurements

Allison Ellington,1 Sid E. O'Bryant,2 Robert Barber,2 Jill Dunty,1 David Stewart,1 Robert Umek,1 Paula Eason,1 Nyssa Puskar,1 Pankaj Oberoi,1 and Jacob N. Wohlstander1
1Meso Scale Discovery (MSD), Gaithersburg, MD; 2University of North Texas Health Sciences Center, Fort Worth, TX

Abstract

Background: 60 peptide pairs in total were measured with sensitivity and selectivity, yet absolute peptide concentrations differ widely from matrix to matrix. Assay format, antibody orientation, and matrix elements such as albumin, interferon, and antinuclear antibodies can all influence the determination of multiple assay formats with respectivity and selectivity and matrix tolerance.

Methods: The format of the assay was evaluated against a total of 70 peptide pairs. Human EDTA plasma was diluted 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold in base diluent and then spiked with either 250 pg/mL or 1000 pg/mL Aβ40. The opposite format is most sensitive for the Aβ40 assay. We evaluated the addition of blockers to our base diluent to determine if high peptide concentrations were reduced in matrix tolerant assays. We evaluated different diluents to reduce matrix interference. We evaluated matched serum and cerebrospinal fluid samples to determine if the same antibody pairs were tested in two different orientations: 6E10 capture antibody, is a more sensitive for the Aβ40 assay.

Results: The 6E10 assay with the peptide-specific antibody observed higher sensitivity than the assay using a 6E10 capture antibody (100 pg/mL, 247 pg/mL). The opposite format using 350 pg/mL Aβ42 captures a sample 40 fold higher for Aβ40 for 6E10 capture plasma samples with 100 pg/mL Aβ42 peptide. The 6E10 capture format, the level of plasma Aβ42 peptide in the normal samples were detectable using the 6E10 capture format. Only four normal samples and four diseased samples had Aβ42 levels in the plasma samples. Assay sensitivity is about 10 times more sensitive. The opposite orientation, using 6E10 capture antibody, is more sensitive for the Aβ42 assay. The opposite orientation is about 10 times more sensitive. The opposite format is most sensitive for the Aβ40 assay.

Matrix Interference: Dilutional Linearity

To assess matrix interference, from human EDTA plasma samples were spiked with 100 pg/mL Aβ42 and Aβ42 peptides, then diluted 1-fold. The other format, and reveals the effect of plasma matrix on the primary antibody capture format. The matrix interference was greater when using the peptide capture antibody. The matrix interference was greater when using the peptide-specific antibody. The matrix interference was greater when using the antibody capture antibody. The matrix interference was greater when using the antibody-specific antibody.