Abstract

Recent studies have indicated that β-amyloid (Aβ) is a hallmark protein of Alzheimer’s disease (AD). However, the majority of assays currently available for detection of Aβ are limited due to high variability and lack of standardization. Here we describe the development of a novel immunoassay for the specific detection and quantification of Aβ. The assay is designed to be sensitive, specific, reproducible, and scalable, with the ability to detect very low levels of Aβ in a wide range of sample types.

Peptide Calibrator Development

We selected 42 synthetic peptides (Aβ1-37, 1-38, 1-39, 1-40, 1-42) as calibrators for the assay. These peptides were characterized by gel electrophoresis to determine their degree of aggregation. Differences in the degree of aggregation were observed among the different peptides, and the Aβ1-42 peptide was optimized to minimize aggregate formation and maximize stability and consistency.

Assay Sensitivity

We investigated the minimum level of detection (LOD) of the Aβ1-42 peptide using various dilution factors. The LOD was determined to be 0.35 pg/mL, with a quantitative range of 3 to 2000 pg/mL. The precision, accuracy, and total error were determined from human CSF control samples with typical inter- and intra-assay % Recovery.

Peptide Calibrator Robustness

We evaluated the robustness of the assay by comparing the measured concentrations of Aβ1-42 from different kit lots. The assay demonstrated excellent sensitivity, performance, and inter-lot reproducibility.

Real Time Stability of Controls

Aβ1-42 levels were measured in control samples (n=10) over the range of the assay. The assay exhibited tolerance of CSF contamination with a minimum of 12 runs conducted by three analysts across at least three days of testing (N=42 runs across 3 kit lots). In-well concentrations are adjusted for sample dilution.

Conclusion

The development of this novel immunoassay for the detection and quantification of Aβ represents a significant advancement in the field of AD research. The assay is highly sensitive, specific, and reproducible, and can detect very low levels of Aβ in a wide range of sample types.

Keywords: Alzheimer's disease, immunoassay, Aβ, peptide calibrator, LOD, stability, reproducibility.