Development and Analytical Validation of a Novel Assay for Detection of β42 Peptide in Human CSF

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Abstract

The objective of this study was to develop and validate a novel sandwich immunoassay for the detection of amyloid β 6-42 peptide (A-beta) in cerebrospinal fluid (CSF). The assay was designed to recognize the Aβ42 peptide specifically, and to minimize interferences in Aβ42 measurement. The assay was validated using three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

Peptide Calibrator Development

The assay was developed using calibrators prepared using synthetic peptides. The calibrators were selected based on their ability to accurately map the dynamic range of the assay. The calibrator levels were determined using a standard curve methodology. The assay was validated using three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

Assay Protocol and Standard Curve

The assay was developed using calibrators prepared using synthetic peptides. The calibrators were selected based on their ability to accurately map the dynamic range of the assay. The calibrator levels were determined using a standard curve methodology. The assay was validated using three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

Assay Protocol and Standard Curve

The assay was designed to measure β 42 in the native state and was optimized for human CSF. The assay was validated with three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

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Measurement of Validation Samples

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Specificity and Interference

The assay was designed to measure β 42 in the native state and was optimized for human CSF. The assay was validated with three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

Conclusions

The β 42 assay is a novel, high-throughput, and accurate method for the detection of β 42 in human CSF. The assay is designed to measure β 42 in the native state and was optimized for human CSF. The assay was validated with three independently built kit lots tested by multiple analysts across multiple runs and days. Each lot was built using an optimized assay design with matrix tolerance using the optimized diluent, Diluent 35, was evaluated. Recovery of Aβ42 peptide and Amyloid β (Aβ)1-42 peptide from well-curated CSF samples from normal and Alzheimer’s disease (AD) patients were quantitated. The assay demonstrated excellent linearity, reliability, and robustness over a wide range of concentrations. The assay has been used to analyze CSF samples from clinical trials for the development of AD therapeutic agents. The results of this study provide a foundation for the development of a novel assay for the detection of Aβ42 peptide in CSF.

References
