APPLICATION NOTE

Detection of sAPPα in Human CSF:

A Comparison Between AlphaLISA and Standard Sandwich ELISA

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Abstract

Robust, sensitive and reproducible immunoassays for biomolecules are essential for the drug discovery process. We compared the performance of an AlphaLISA® and a standard ELISA assay at detecting the secreted amyloid precursor protein alpha (sAPPα) in human cerebrospinal fluid (CSF). Assay sensitivity, dynamic range and reproducibility were among the performance parameters examined. The AlphaLISA assay showed superior sensitivity and reproducibility while consuming 20 times less sample volume and relying on a faster and simpler assay protocol.

Introduction

Alzheimer's disease is the most prevalent neurodegenerative disorder in the aged population and is characterized by the deposition of the 40/42-residue amyloid β protein (Aβ), a proteolytic fragment of the amyloid precursor protein (APP)[1].

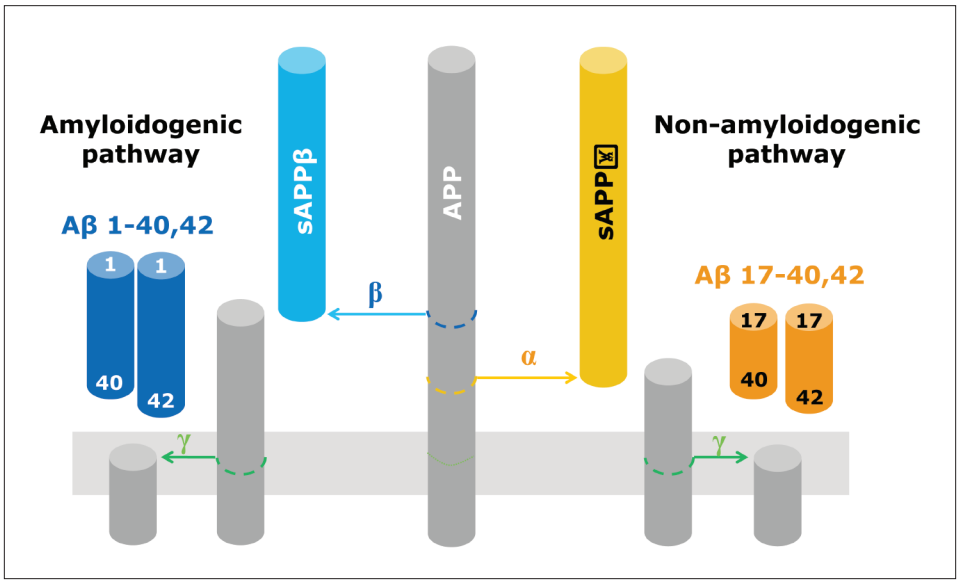

Figure 1. **APP proteolytic processing**

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As shown in Figure 1, APP is a single-transmembrane domain protein containing specific cleavage sites for α-, β-, and γ-secretase enzymes. Non-amyloidogenic processing of APP by α-secretase cleaves APP to generate the N-terminal secreted APPα (sAPPα) fragment and the transmembrane domain-containing α-CTF. The α-CTF is then cleaved by the γ-secretase to produce the non-amyloidogenic p3 and γ-CTF fragments. Amyloidogenic processing of APP by β-secretase produces APPβ and β-CTF. Then γ-secretase can cleave β-CTF at either position 40 or 42 amino-acids C-terminal to the β-secretase site. Therefore, the β/γ-secretase cleavage yields amyloidogenic Aβ40, Aβ42 peptides and γ-CTF.

Substantial research efforts are aimed at developing inhibitors of β-secretase or activators of α-secretase function to treat Alzheimer's disease.[2] Further to measuring Aβ40 and Aβ42 peptides, it is critical to determine the levels of sAPPα and sAPPβ as well. Therefore, robust, sensitive and reproducible immunoassays for the peptides listed above are essential for the drug discovery process.

We compared the performance of an AlphaLISA kit (PerkinElmer) and a standard ELISA kit at detecting the sAPPα in human CSF. The main performance parameters considered in this study included assay sensitivity, dynamic range, variability and % recovery of spiked sAPPα.

Materials and Methods

The AlphaLISA sAPPα (C-term specific) kit and white 96-well ½ Area Plates were supplied by PerkinElmer. The sAPPα sandwich ELISA kit was obtained from a well-known competing manufacturer (Supplier B). Assays were performed according to each manufacturer's recommended protocol (Table 1). The standard curve samples were prepared by serial dilution of the sAPPα standard supplied with each kit in AlphaLISA immunoassay buffer (IAB) + 10% FBS (for AlphaLISA) or ELISA Immunoassay (EIA) buffer (Supplier B). The samples used for the percent recovery determinations were prepared by adding the specified amounts of sAPPα standard to pooled CSF that had been diluted 4-fold in the assay diluents as specified above for each kit.

Material	Vendor	Catalog Number
Pooled human cerebrospinal fluid (CSF)	Bioreclamation Inc	HMCSF, lot BRH305627
Fetal bovine serum (FBS)	HyClone	SH 30071
AlphaLISA sAPPα (C-term specific) Research kit, 500 assay points	PerkinElmer	AL254C, lot D20910
96-well 1/2 area plate	PerkinElmer	6005560

Table 1. **Comparison of immunoassay protocols**

Step	AlphaLISA (PerkinElmer)	Sandwich ELISA (Supplier B)
1	Add **5 µL** of standard diluted in IAB + 10% FBS, or sample in 1/4X CSF	Add **100 µL** of CSF standard diluted in EIA buffer, or sample in 1/4X CSF
2	Add 5 µL mixture of anti- sAPPα Acceptor beads (10 µg/mL final) and biotinylated anti-sAPPα antibody (1 nM final)	Incubate overnight at 4 ˚C
3	Incubate 60 minutes at room temperature	Wash plate 7X
4	Add 40 µL Streptavidin Donor bead (40 µg/mL final)	Add 100 µL labeled antibody
5	Incubate 30 minutes at RT in the dark	Incubate 30 minutes at 4 ˚C
6	Read plate on EnVision® Multilabel Plate Reader	Wash plate 9X
7		Add 100 µL Chromogen solution
8		Incubate 30 minutes at RT in the dark
9		Add 100 µL Stop solution
10		Read plate on EnVision Multilabel Plate Reader
	2.5 hrs overall	**17 hrs overall**

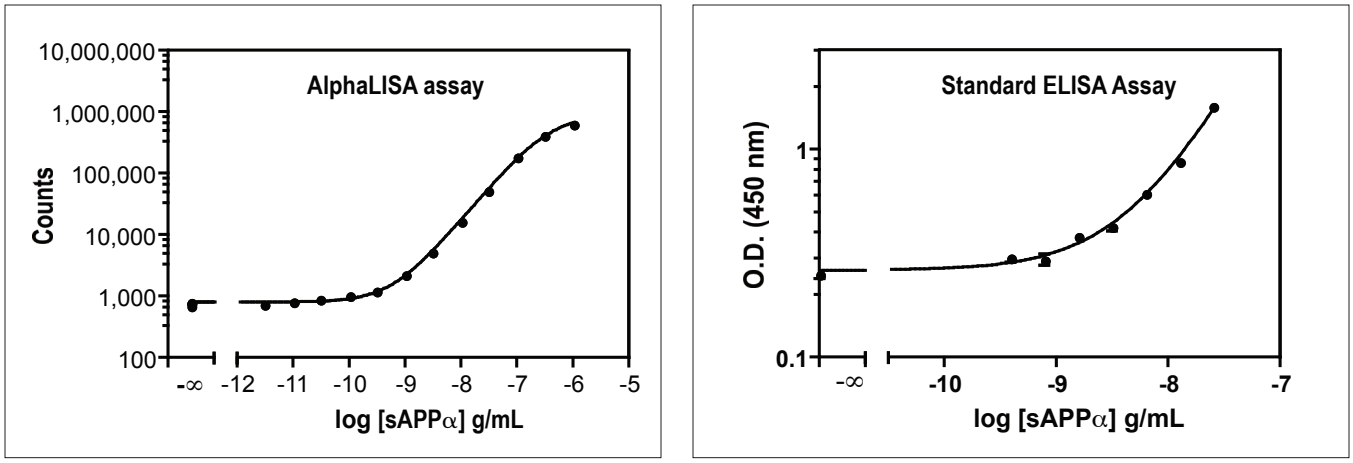

Figure 2. **sAPPα calibration curves**. Left panel: The AlphaLISA assay detected sAPPα over a 4 log range, from 0.1 ng/mL to 1 µg/mL . Right panel: The standard ELISA assay detected sAPPα from 1.5 ng/mL to 25 ng/mL , the highest sAPPα concentration achievable with the calibrator included in this kit.

Table 2. **Comparison of analyte detection accuracy in CSF samples**

sAPPα spiked in diluted CSF (ng/mL)	AlphaLISA recovery (%)	ELISA recovery (%)
50	83	outside detection range
10	82	70
5	114	70

Results and Discussion

We first evaluated the performance of the AlphaLISA and standard ELISA kits at detecting their respective internal sAPPα calibrators. As observed in Figure 2, the AlphaLISA assay provided 15-fold more sensitive detection of sAPPα compared to the standard ELISA, with respective lowest detection limits (LDL) of 0.1 and 1.5 ng/mL. The fact the AlphaLISA assay showed a lower variability between replicates (4% vs. 8% for ELISA) is in part responsible for this observation.

To determine the accuracy of the two assays, sAPPα was spiked into a sample matrix consisting of CSF diluted four-fold, and the percent recovery was then determined. Table 2 shows that the AlphaLISA assay provided better percent recovery values for sAPPα.

Summary

We compared the performance of AlphaLISA and standard sandwich ELISA assays at detecting sAPPα in CSF. Using the respective protocols recommended by the manufacturers, the AlphaLISA assay showed 15-fold higher sensitivity (lower LDL) and better reproducibility. No data indicating the reproducibility between replicates compared to the ELISA assay. Percent sAPPα recovery values were also better with AlphaLISA.

Compared to standard ELISA, the AlphaLISA (C-term specific) assay also offers a number of advantages including fewer protocol steps, less time to execute, and reduced sample consumption.

References

1. Shoji, M, et al. Production of the Alzheimer amyloid α protein by normal proteolytic processing. Science 1992. 258:126-129.

2. Steven L. Wagner and Benito Munoz. Modulation of amyloid α protein precursor processing as a means of retarding progression of Alzheimer's disease. J. Clin. Invest. 104(10): 1329-1332 (1999).

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