

# Simultaneous Detection of NF-L and Total Tau in CSF and Blood Using the FemtoQuest™ Platform and Millipore® Automated Assay Workstation (AAW™)



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## Introduction

The quantification of neuronal protein biomarkers is essential for advancing our understanding of brain health, particularly in neurodegenerative disease research. Current methodologies primarily focus on biomarkers in cerebrospinal fluid (CSF), but the invasive nature of sample collection limits its practicality for routine analysis. The newly developed SMC® Neurofilament Light Chain (NF-L) and Total Tau dual-plex high sensitivity immunoassay kit addresses this gap by enabling the accurate quantification of these 2 critical biomarkers in human blood samples as well.

Verified for use in CSF, serum, and plasma, this assay exhibits a high degree of specificity with < 5% cross-reactivity between analytes and precision with a coefficient of variation (CV) < 15%. Achieving LLOQs of 0.87 pg/mL and 2.31 pg/mL for NF-L and Total Tau, respectively, NF-L was 100% detectable in all sample types tested while Total Tau was detectable in all CSF and plasma samples with >80% detectability in serum samples. Statistically significant differences between presumed healthy vs Alzheimer's disease CSF samples were also observed. The NF-L and Total Tau sample ranges for healthy samples were 3.53-21.4 pg/mL and 0.639-10.2 pg/mL, respectively, and 6.03-42.7 pg/mL and 1.76-26.3 pg/mL for disease samples.

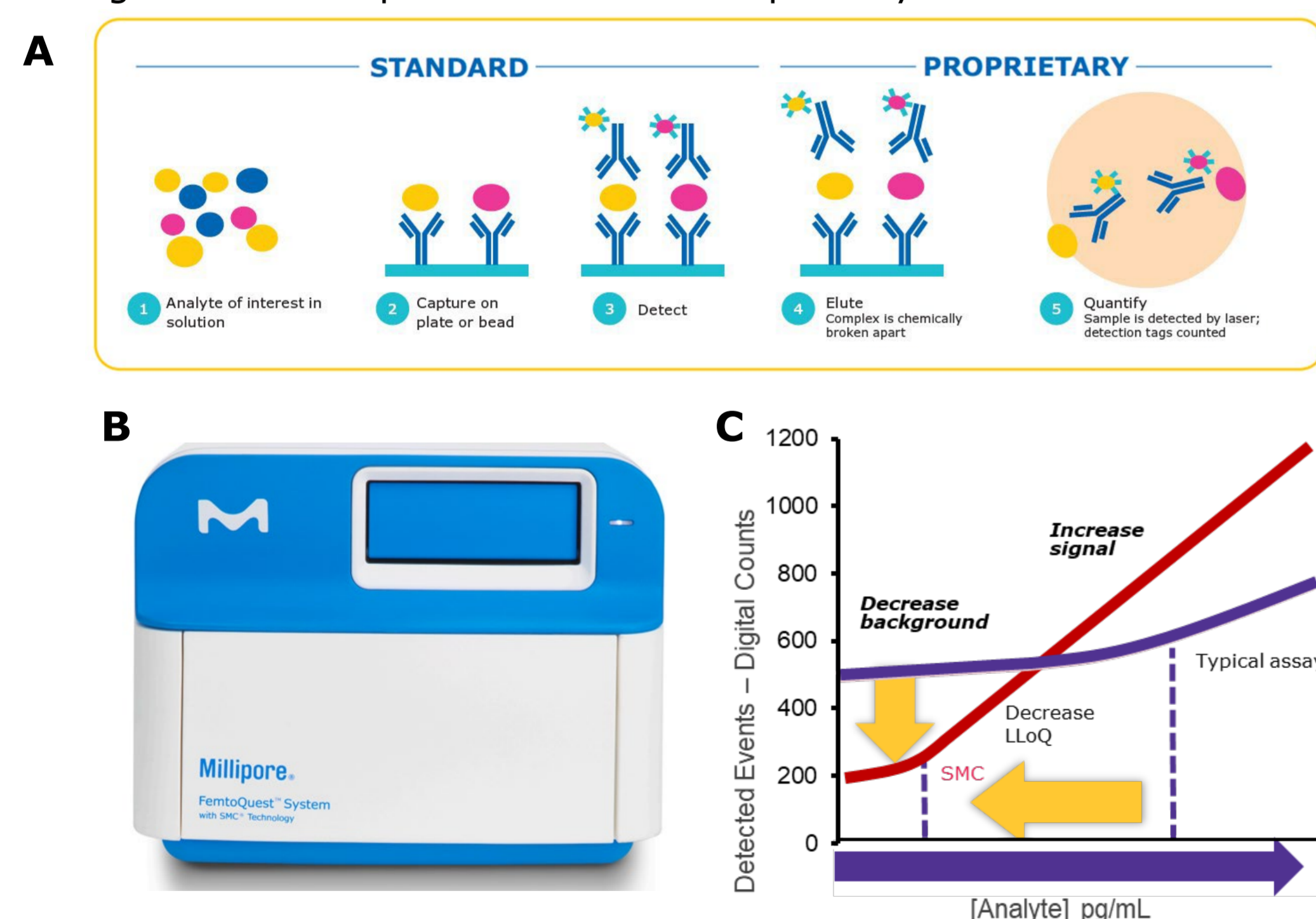
Integration of the AAW™ Automated Assay Workstation into the dual-plex SMC® assay workflow enables enhanced reproducibility, minimizes hands-on time, and reduces the potential for human error. The automated assay workflow using the AAW™ workstation resulted in a lower LLOQ than manual assays with NF-L achieving 0.43 pg/mL and Tau achieving 0.58 pg/mL. Finally, performance on the AAW™ workstation results in a 57% decrease in hands-on time compared to manual assay performance.

In summary, this robust immunoassay enables the generation of accurate and reproducible data in multiple sample types and facilitates more efficient research of neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and Multiple Sclerosis.

## Methods

### SMC® Technology

SMC® immunoassays achieve high sensitivity assay performance while following a workflow like that of a traditional ELISA, as shown in **Figure 1A**. By combining a unique assay elution step and robust digital counting, researchers achieve improved signal-to-noise ratios over traditional immunoassay methods, as shown in **Figure 1C**. The FemtoQuest™ instrument (**Figure 1B**) provides enhanced quantification at both low and high levels of expression on one complete system.



**Figure 1. Ultrasensitive SMC® Technology for the FemtoQuest™ Platform.** (A) SMC® immunoassays follow a simple assay protocol. Magnetic beads conjugated to a capture antibody bind to the analyte. A fluorescently-labeled detection antibody then forms a sandwich complex with the analyte and capture beads. Using a proprietary elution step, individual detection antibodies are counted to allow for ultrasensitive measurement. (B) SMC® immunoassays are available for the FemtoQuest™ instrument, which employs a scanning confocal laser to perform digital molecular counting. (C) SMC® immunoassays show broader dynamic range and greater sensitivity than standard ELISAs.

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### Pairing SMC® Technology with the AAW™ Automated Assay Workstation

The AAW™ workstation, powered by Opentrons®, was used to automate the SMC® dual-plex high sensitivity immunoassay. Verified assay protocols were adapted into Python code and imported onto the workstation using the Opentrons® App. The flexible workstation performed all liquid handling steps, including serial curve dilutions, on-deck shaking, and magnetic bead pull-down.

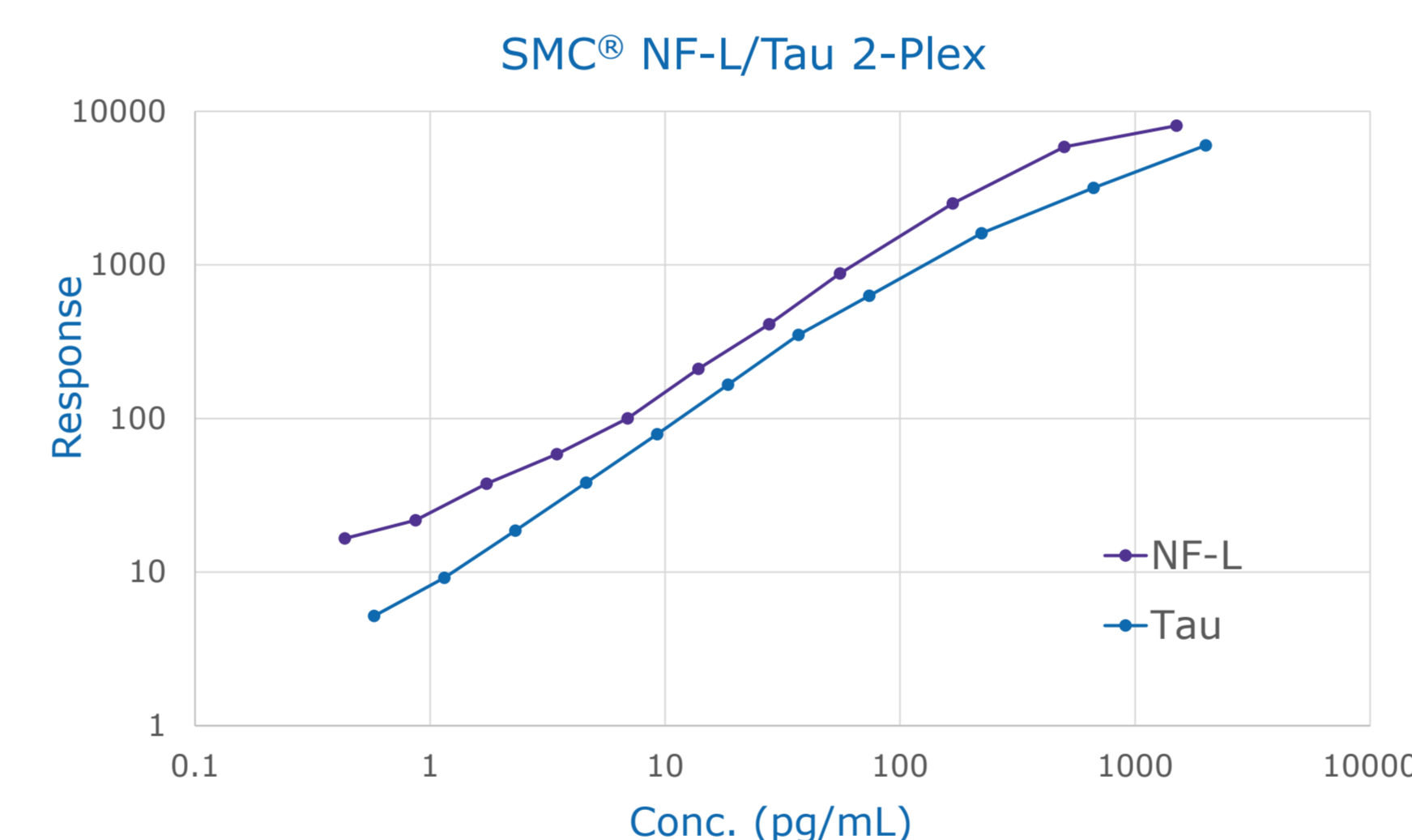


**Figure 2. AAW™ Automated Assay Workstation.**

The AAW™ workstation is a user-friendly, flexible, automated workstation. Millipore® verified protocols were confirmed to reproducibly perform SMC® assays as well as or better than manual assay handling. In addition, automated assays minimize hands-on time.

## Results

SMC® immunoassay technology enables accurate measurement of low-abundant biomarkers in biological samples through a proprietary assay mechanism involving the interrogation of individual molecular binding events. Coupled with the FemtoQuest™ platform, researchers are empowered to confidently study key neurodegenerative markers, including those in blood-based biomarker studies.

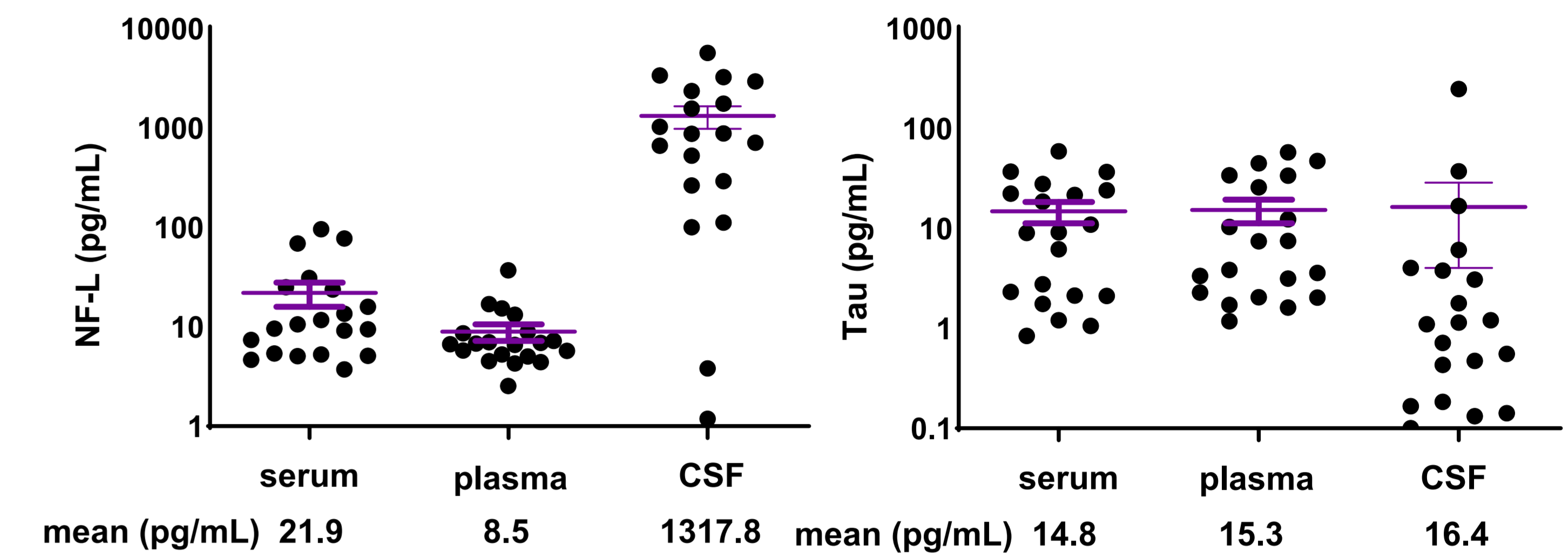


**Figure 3. SMC® Dual-Plex Standard Curves.** The SMC® NF-L/Tau dual-plex immunoassay kit was run according to kit protocol. Standard curves were generated using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122). Response represents arbitrary fluorescent units.

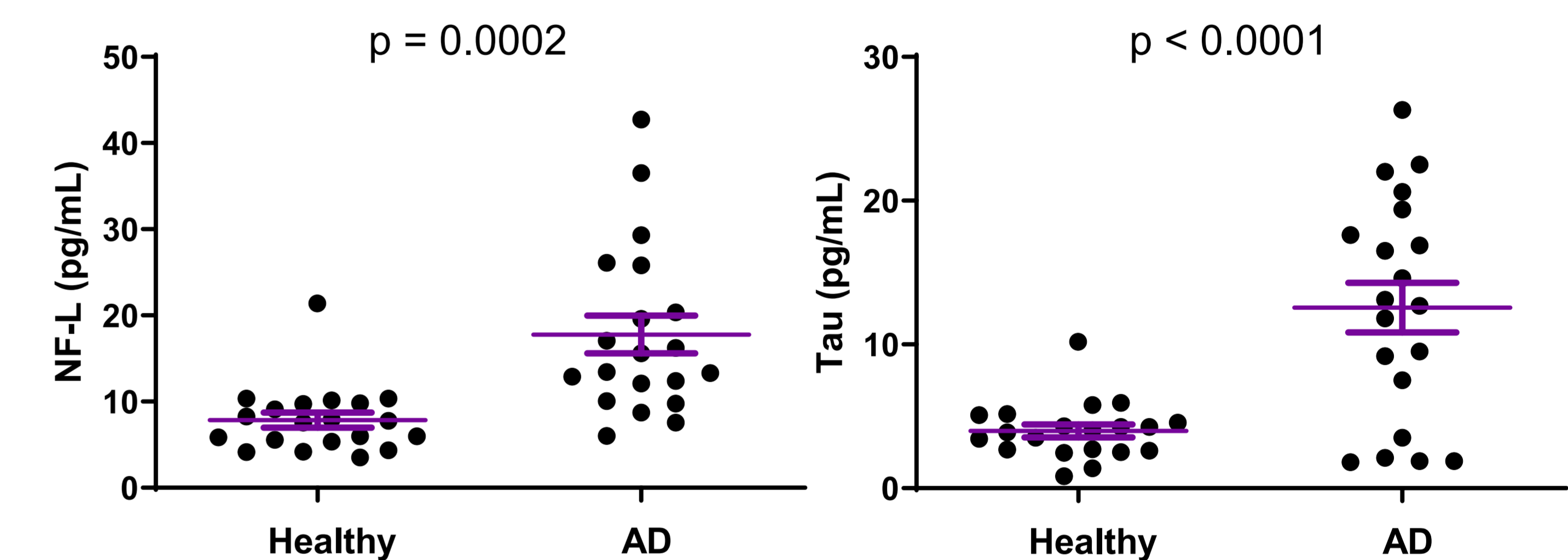
Analyte	Standard Curve Range (pg/mL)	Sensitivity LLOQ (pg/mL)	Precision		Accuracy		
			Avg. Intra-Assay (% CV)	Avg. Inter-Assay (% CV)	Mean % Recovery (Serum)	Mean % Recovery (Plasma)	Mean % Recovery (CSF)
NF-L	0.43 – 1,500	0.87	<10	<15	84	86	99
Tau	0.58 – 2,000	2.31	<10	<15	105	77	88

Analyte	Sample Values (pg/mL)			Linearity / Parallelism		
	Mean / Range (Serum)	Mean / Range (Plasma)	Mean / Range (CSF)	Mean % (Serum)	Mean % (Plasma)	Mean % (CSF)
NF-L	21.9 / 3.7-95.9	8.5 / 2.6-37.1	1318 / 1.2-5690	110 / 102	106 / 111	92 / 85
Tau	14.8 / 0.8-58.9	15.3 / 1.2-57.9	16.4 / 0.1-247.7	110 / 113	106 / 118	102 / 86

**Table 1. Summary of SMC® NF-L/Tau immunoassay kit performance characteristics.** Values for this kit's performance were generated during assay development using serum, plasma, and CSF samples from apparently healthy individuals.



**Figure 4. Biomarker Concentrations in Control Samples by Sample Type.** Dot plots representing distribution of interpolated, dilution-corrected concentrations for sample cohorts corresponding to human serum, EDTA plasma, and CSF. Purple bars correspond to mean and standard deviation values.



**Figure 5. Plasma Measurement of AD Biomarkers.** SMC® NF-L/Tau dual-plex immunoassay kit was run according to vendor protocol. Analyte concentrations for apparently healthy and AD plasma samples are reported. NF-L and Tau showed statistically significant differences,  $p=0.0002$  and  $p<0.0001$ , respectively. Apparently healthy and AD plasma samples were purchased from BioIVT.

Single Analyte SMC® Neuroscience Biomarker Kits		
NF-L (03-0202-00)	Tau (03-0185-00)	NPTX2 (03-0199-00)
Ab 1-40 (03-0145-00)	p-Tau T181 (03-0184-00)	TDP-43 (03-0205-00)
Ab 1-42 (03-0146-00)	p-Tau T217 (03-0210-00)	UCHL1 (03-0183-00)
α-synuclein (03-0196-00)	p-Tau T231 (03-0211-00)	SNAP-25 (03-0206-00)
p-α-synuclein (S129) (03-0188-00)	GFAP (03-0203-00)	BDNF (03-0171-00)

**Table 2. Verified Neuroscience Biomarker Kits for the SMC® Platform.** Assays for the listed analytes (kit catalog numbers noted in parentheses) are available for use with the FemtoQuest™ ultrasensitive immunoassay platform. All kits are for research use only, not for use in diagnostic procedures.

## Summary

The SMC® dual-plex human NF-L, Total Tau high sensitivity kit coupled with the FemtoQuest™ platform enables the accurate quantification of critical neurological biomarkers in human blood samples, overcoming the limitations of CSF analysis. With high specificity and low limits of quantification, this assay shows significant detectability across various sample types. The integration of the AAW™ workstation enhances reproducibility and reduces hands-on time, facilitating efficient research into neurodegenerative diseases like Alzheimer's and Parkinson's disease. By supporting the accurate measurement of low-abundant proteins, SMC® technology advances blood-based biomarker studies.

