SpearBio

Distributed By
biotechne

APPLICATION NOTE

Unparalleled precision of SPEAR UltraDetect™ immunoassays with homogeneous format: GFAP and NfL

Abstract

This application note highlights the exceptional sensitivity and precision of the SPEAR UltraDetect platform, demonstrated through two SPEAR UltraDetect™ assays targeting key neurological biomarkers: Glial Fibrillary Acidic Protein (GFAP) and Neurofilament-Light (Nf-L). These assays deliver ultra-sensitivity with high precision, enabling reproducible detection of subtle, clinically meaningful changes—often missed by other ultra-sensitive platforms due to high variability. With remarkably low coefficients of variation (CVs) even at the lowest concentrations, SPEAR sets a new benchmark in analytical performance, making it a powerful tool for early detection and longitudinal study of neurological disease.

Introduction

Accurate biomarker quantification, combining high sensitivity and precision, is vital for neurological research and clinical applications. Biomarkers like GFAP, indicative of astrocytic activation, and Nf-L, reflecting axonal damage, are present at low levels in blood early in disease and can rise subtly over time.[1-3] Quantifying these biomarkers at very low levels in conditions such as Alzheimer's disease, multiple sclerosis, or stroke enables informed disease management and timely intervention. This requires not only detecting low-abundance biomarkers but doing so consistently and reproducibly over time and across samples. High precision of biomarker measurement is essential for distinguishing disease-related changes from assay variability. However, while conventional ultra-sensitive platforms have enabled new discoveries, they have historically struggled with assay precision and variability—especially in blood matrices where concentrations of neuro biomarkers are typically much lower than in CSF. These limitations compromise the accurate tracking of modest but meaningful biomarker shifts, which are essential for detecting early disease signals, evaluating therapeutic response, and monitoring longitudinal disease progression.

Successive Proximity Extension Amplification Reaction (SPEAR) is a homogeneous, ultra-sensitive immunoassay technology designed to overcome the sensitivity limitations and complexity of conventional immunoassays. Utilizing a unique two-factor authentication mechanism, SPEAR ensures that amplifiable signal is only generated through sustained co-

Introduction, continued

ocalization of probes on target proteins. It precisely quantifies protein biomarkers at attomolar concentrations from as little as 1 µl of diluted sample, greatly surpassing the most sensitive heterogenous platforms on the market. The homogeneous nature of SPEAR eliminates non-specific binding associated with solid surface capture which gives it superior specificity over heterogeneous immunoassay platforms. SPEAR requires no error-prone wash steps in the workflow, ensuring its extremely high precision.

Powered by this core technology, SPEAR UltraDetect™ assays offer unparalleled sensitivity in measuring low-abundance biomarkers with exceptional specificity and precision. SPEAR UltraDetect utilizes standard qPCR instruments for readout, enabling highly consistent results across different qPCR platforms and formats. It is easy to implement into existing laboratory settings with unprecedented scalability.

Here we present data demonstrating the exceptional precision of SPEAR UltraDetect GFAP and Nf-L assays, 100% detectability and quantifiability in healthy samples, and excellent concordance with the GFAP and Nf-L assays on the widely used Simoa platform.

Materials and Methods

Samples from healthy donors and diseased individuals were obtained from multiple commercial sources, such as Precision for Medicine and BIOIVT, and tested using SPEAR UltraDetect™ Nf-L (item 90004, Spear Bio) and GFAP (item 90010, Spear Bio) and Simoa® NF-Light® Advantage PLUS (item 104364, Quanterix) and Simoa GFAP Advantage PLUS (item 104619, Quanterix).

SPEAR UltraDetect™ Method

Samples were processed using the Formulatrix® F.A.S.T™ liquid handler and QInstruments® BioShake® iQ, following SpearBio's instructions. Briefly, a 1 µL probe mix was combined with 1 µL of sample or calibrator and incubated for 1 hour. 6 µL reaction mix was added and incubated for 20 minutes, followed by addition of 12 µL of qPCR mix for final analysis. qPCR results were converted to protein concentrations using SPEARview analysis software. (Figure 1).

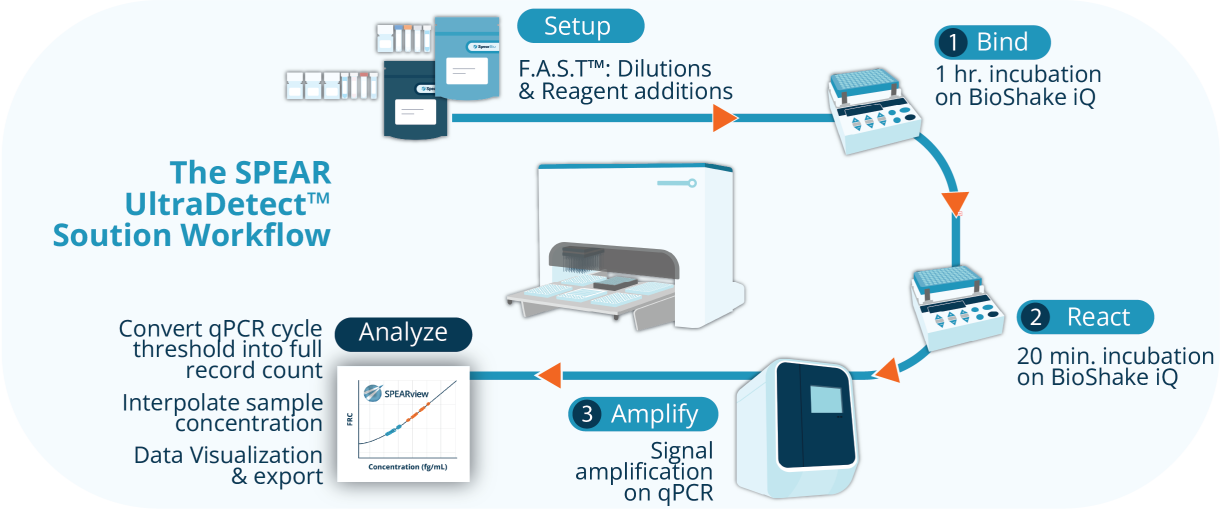


Figure 1. The full SPEAR UltraDetect Assay workflow.

Methods, continued

Quanterix Simoa Method

Samples were run on the Simoa HD-X Analyzer® per the manufacturer's protocol. Briefly, 100 µL of sample or calibrator was mixed with 25 µL bead reagent, followed by washing (3x), addition of 100 µL detection reagent, washing (3x), addition of 100 µL SBG reagent, washing (4x), and addition of 50 µL RGP. Protein measurements were generated using the Quanterix software.

Results

Precision

Intra-plate CVs for both GFAP and Nf-L were consistently below 10%, with average CV 3.0% and 3.2%, (Figure 1A, 2A) respectively—indicating superior precision compared to the respective assays on the Simoa platform as reported[4,5].

Sensitivity

SPEAR UltraDetect achieves detection limits for GFAP and Nf-L down to pg/mL (Table 1), detecting and quantifying 100% samples from healthy individuals, with the lowest endogeneous readings across all tested matrices at least one order of magnitude above the functional LLoQ. (Figure 1B, 2B). This level of sensitivity ensures reliable quantification of baseline and small yet disease-relevant changes of GFAP and Nf-L in broad neurology research applications.

Concordance

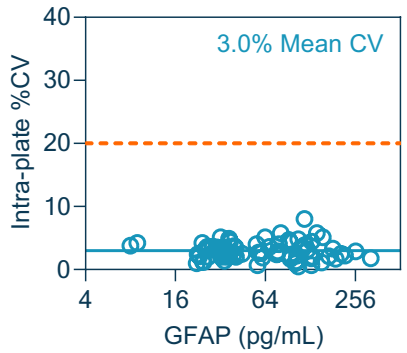
Measurements of GFAP and Nf-L from SPEAR UltraDetect and Simoa are highly concordant across healthy and diseased samples, with R^2 of 0.941 and 0.996, respectively. (Figure 3).

Table 1: SPEAR UltraDetect™ NfL and GFAP Assay specifications in EDTA plasma and serum samples.

Specification	NfL	GFAP
Minimum required dilution (MRD)	4x (EDTA plasma, serum)	4x (EDTA plasma, serum)
Diluted sample volume	1µL	1µL
Raw sample volume to machine*	10µL	10µL
Analytical LLoD (range)	0.024 pg/mL (0.009-0.042)	0.041 pg/mL (0.015-0.067)
Functional LLoD	0.096 pg/mL	0.163 pg/mL
Analytical LLoQ	0.125 pg/mL	0.156 pg/mL
Functional LLoQ	0.500 pg/mL	0.625 pg/mL
Functional Assay Range	0.5 – 4000 pg/mL	0.625 – 20,000 pg/mL

* Can be used for multiple replicates and biomarker measurements.

(A) SPEAR UltraDetect GFAP assay precision [1 µL diluted sample]



(B) SPEAR UltraDetect GFAP assay quantifiability [1 µL diluted sample]

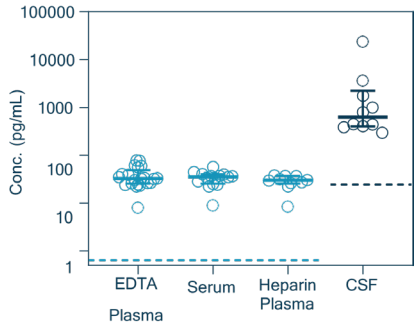
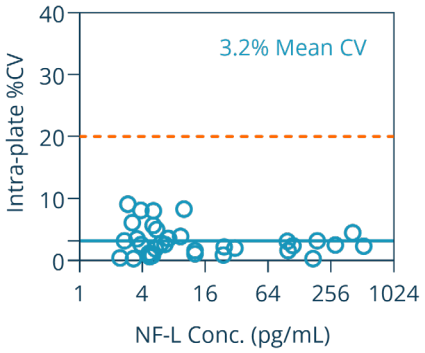


Figure 2: Precision and quantifiability of SPEAR UltraDetect GFAP assay. **(A)** Intra-plate CV plot from total 70 samples, including matched EDTA plasma (n=15) and serum (n=15) healthy controls, amyloid negative plasma (n=6), and amyloid positive plasma (n=34). The 20% CV reference line is marked; **(B)** Endogenous readings of human EDTA plasma

*Heparin plasma and CSF samples are tested for reference only.

(A) SPEAR UltraDetect Nf-L assay precision
[1 µL diluted sample]



(B) SPEAR UltraDetect Nf-L assay quantifiability
[1 µL diluted sample]

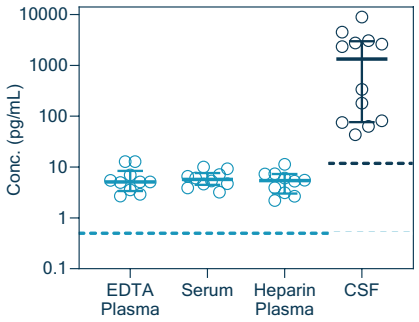
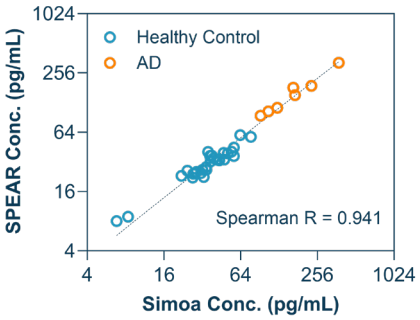


Figure 3: Precision and quantifiability of SPEAR UltraDetect Nf-L assay. **(A)** Intra-plate CV plot from total 34 samples, including matched EDTA plasma (n=10) and serum (n=10), and amyotrophic lateral sclerosis (n=14). The 20% CV reference line is marked; **(B)** Endogenous reading of human EDTA plasma (n=10), serum (n=10), heparin plasma* (n=10), and CSF* (n=10). Functional LLoQ is indicated as dashed lines. Bars indicate mean with interquartile range.

*Heparin plasma and CSF samples are tested for reference only.

(A) SPEAR UltraDetect GFAP concordance
[1 µL diluted sample]



(B) SPEAR UltraDetect Nf-L concordance
[1 µL diluted sample]

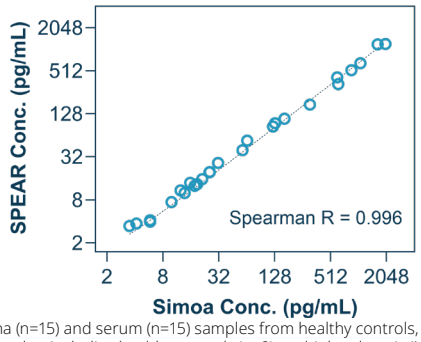


Figure 4: Assay concordance with Simoa platform. **(A)** 37 samples, including matched EDTA plasma (n=15) and serum (n=15) samples from healthy controls, and 7 AD donors were measured using SPEAR UltraDetect GFPA and Simoa GFAP Advantage PLUS; **(B)** 26 plasma samples, including healthy controls (n=8), multiple sclerosis (MS, n=6), AD (n=2), progressive supranuclear palsy (PSP, n=3), amyotrophic lateral sclerosis (ALS, n=1), PSP/ALS spiked (n=6), were measured using SPEAR UltraDetect Nf-L and Simoa NF-Light Advantage PLUS.

Discussion

The unparalleled sensitivity and precision of SPEAR UltraDetect demonstrated in this application note are key advantages for detecting early and small changes of neurodegenerative biomarkers. By enabling detection of GFAP and Nf-L, even at ultra-low levels, with robust precision, SPEAR UltraDetect allows researchers to capture the earliest shift in protein biomarker levels in prodromal and mild stages of neurological conditions.

In clinical research, the high precision of SPEAR UltraDetect further facilitates reliable detection and tracking of biomarker changes along disease progression or in response to treatment, enabling powerful biomarker monitoring for longitudinal studies.

The demonstrated combination of high sensitivity, precision, and scalable implementation positions SPEAR as a breakthrough tool for neurology applications from early disease detection to therapeutic development.

References

1. Wang, X., Shi, Z., Qiu, Y. et al. Peripheral GFAP and NfL as early biomarkers for dementia: longitudinal insights from the UK Biobank. *BMC Med* 22, 192 (2024). https://doi.org/10.1186/s12916-024-03418-8
2. Samadzadeh, S., Sleator, R., The role of Neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) in MS and AQP4-NMOSD: Advancing clinical applications. *eNeurologicalSci* Volume 38, March 2025, 100550. https://doi.org/10.1016/j.ensci.2025.100550
3. Barro, C., Healy, B., et al. Serum GFAP and NfL Levels Differentiate Subsequent Progression and Disease Activity in Patients With Progressive Multiple Sclerosis. *Neurology Neuroimmunology & Neuroinflammation*, January 2023 issue 10 (1) https://doi.org/10.1212/NXI.0000000000200052
4. Simoa GFAP Advantage PLUS data sheet (available at www.quanterix.com)
5. Simoa Nf-L Advantage PLUS data sheet (available at www.quanterix.com)

SpearBio
100 Tradecenter Dr. Suite 510
Woburn, MA 01801
spear.bio

©2025 Spear Bio

SPR DOC80052

Distributed by:
bio-techne
614 McKinley Place NE
Minneapolis, MN 55413
bio-techne.com
1-800-343-7475