

APPLICATION NOTE

SPEAR UltraDetect™ Neurofilament

light exceptional precision with F.A.S.T.™

automated workflow

Abstract

Developed at the Wyss Institute at Harvard, the Successive Proximity Extension Amplification Reaction (SPEAR) technology redefines ultra-sensitive protein detection, achieving unprecedented precision with minimal sample volume (1 μ L or less) in a wash-free assay. Spear Bio offers a complete solution for biomarker analysis, pairing SPEAR UltraDetect™ assay kits with the Formulatrix F.A.S.T.™ liquid handler and proprietary software to streamline workflow and deliver unparalleled accuracy. This integrated approach results in hands-on time under 20 minutes while enabling high-precision qPCR readouts for quantification. Together, these components provide researchers with a robust platform for scaling biomarker discovery and validation in neurology and other research areas.

Introduction

The advent of ultra-sensitive technologies for protein detection has transformed biomarker research in neurology by facilitating deeper insights and higher accessibility through minimally invasive testing methods. Quantifying low concentrations of neurofilament light chain (NfL) in blood, rather than cerebrospinal fluid (CSF), has enhanced the ability to study and monitor neurodegenerative diseases such as Multiple Sclerosis (MS)^{1,2}, Amyotrophic Lateral Sclerosis (ALS)^{3,4}, Alzheimer's disease (AD)⁴, Frontotemporal Dementia (FTD)⁴, and Parkinson's Disease (PD)⁵.

Spear Bio's proprietary Successive Proximity Extension Amplification Reaction (SPEAR)

employs a novel 2-factor authentication mechanism minimizing background from non-specific interactions. In just 1 μ L of diluted sample, proteins in blood are detectable at very low fg/mL concentrations with a wash-free, homogeneous format allowing free antigen-antibody interactions to maximize binding specificity and precision. SPEAR UltraDetect™ cost-effective assays are paired with the F.A.S.T.™ liquid handler and proprietary software that converts qPCR results into quantitative concentrations. This unified platform simplifies complex workflows, enhances precision, and accelerates time-to-result, making it the ideal choice for both discovery and clinical translation.

Materials and Methods

The SPEAR UltraDetect™ Neurofilament Light (NfL) immunoassay was optimized for serum and EDTA plasma, utilizing proprietary DNA-antibody probes and 1 μ L of diluted sample. Automated reagent preparation and addition were developed with the Formulatrix F.A.S.T.™ liquid handler and Q Instruments temperature-controlled plate shaker. Results were analyzed with Spear Bio's software for direct conversion of qPCR data to quantifiable concentrations (Figure 1).

SPEAR UltraDetect™ NfL was assessed for analytical sensitivity over multiple runs for its lower limit of detection (LLoD) as the concentration corresponding to 2.5 standard deviations above the blank and lower limit of quantification (LLoQ) as the concentration with $\leq 20\%$ CV and known concentration between 80-120% recovery. Performance in EDTA plasma and serum was tested for spike recovery, dilution linearity, and concordance to existing technologies Quanterix Simoa NF-Light™ (item #104364) and MSD S-Plex (item #K151AKGS).



Figure 1. The full SPEAR solution workflow. SPEAR UltraDetect™ assay and reaction kits contain all required reagents with minimal preparation before loading into custom reagent reservoir for F.A.S.T.™ automated liquid handling. F.A.S.T. prepares calibration curve, sample dilutions, and combines 1 μ L of probe mix and sample or calibrator for a 1-hour incubation on Q Instruments shaker. Reaction mix (6 μ L) is added directly to the plate by F.A.S.T. without any wash steps for an 8-minute reaction automatically followed with a 20-minute inactivation on the shaker. Final addition of qPCR master mix (12 μ L) is directly added to the plate by F.A.S.T. and transferred to qPCR instrument. Proprietary SPEAR Analysis Software converts qPCR results to quantitative concentrations.

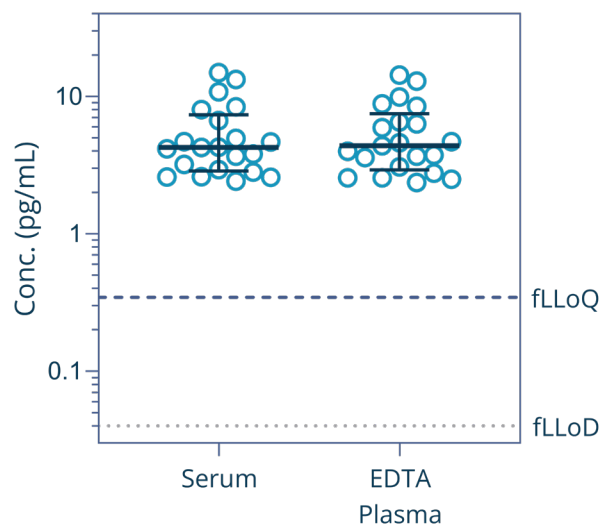


Figure 2. Apparently healthy donors (n=21) Neurofilament light concentrations in matched EDTA plasma and serum. Dotted and dashed line represents functional LLoD (0.04 pg/mL) and LLoQ (0.345 pg/mL).

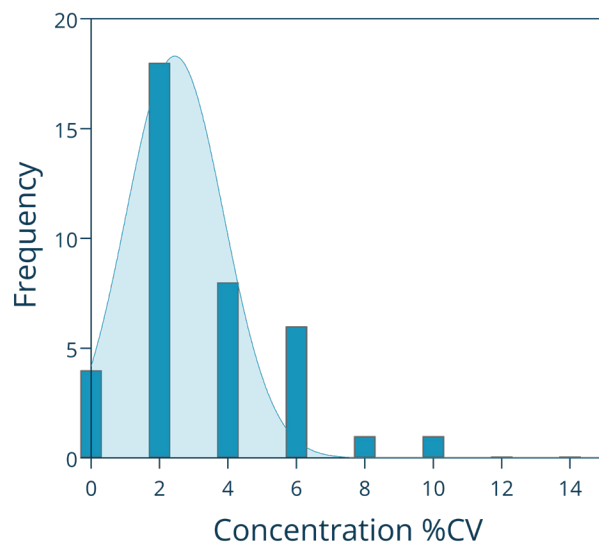


Figure 3. Histogram of concentration %CVs of apparently healthy serum and EDTA plasma with Gaussian fit overlaid.

Results

The SPEAR UltraDetect™ Neurofilament Light immunoassay exhibited excellent detectability and quantification of baseline levels (Figure 2) with spike recovery and dilution linearity (Table 1) illustrating accuracy of measurement. Concentration CVs from apparently healthy control samples on average were 3.1% (Figure 3). Concordance to existing methodologies, Quanterix Simoa and MSD S-Plex (Figure 4), demonstrated highly comparable readings with R2 of 0.993 to Simoa and 0.997 to S-Plex.

	EDTA Plasma (range)	Serum (range)
Spike Recovery	4 donors	4 donors
Low Spike	94% (89 - 97%)	91% (86 - 94%)
Mid Spike	79% (75 - 82%)	78% (74 - 79%)
High Spike	78% (76 - 80%)	87% (85 - 89%)
Mean	84%	85%
Dilution Linearity	4 donors	4 donors
2x	109% (107 - 112%)	108% (104 - 112%)
4x	109% (107 - 113%)	111% (110 - 114%)
8x	117% (113 - 121%)	113% (109 - 118%)
16x	115% (113 - 117%)	114% (106 - 120%)
32x	120% (116 - 123%)	119% (113 - 125%)
Mean	114%	113%

Table 1. Spike recovery and dilution linearity for SPEAR UltraDetect™ Neurofilament light in serum and EDTA plasma.

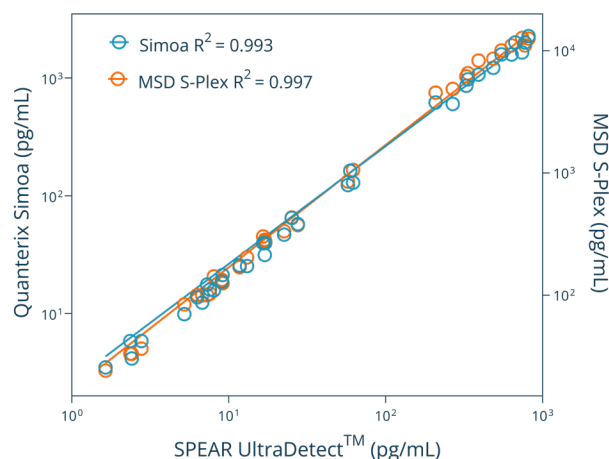


Figure 4. Neurofilament light EDTA plasma and serum concentrations measured with SPEAR UltraDetect™ (x-axis) vs. Simoa (left y-axis) and MSD S-Plex (right y-axis). Linear correlation with R2 shown.

Discussion

Spear Bio's full immunoassay solution, combining SPEAR UltraDetect™ kits, Formulatrix F.A.S.T.™ liquid handler, and SPEAR proprietary analysis software, empowers researchers with a high-throughput, ultra-sensitive solution. The ability to measure protein levels significantly above the functional LLoQ enables improved discrimination in longitudinal studies analyzing marginal changes and facilitates the use of less invasive sampling methods for which analytes must often be diluted during reconstitution. The platform workflow eliminates bottlenecks, reduces user error, and ensures reliable results, making it a critical tool for advancing biomarker research.

References

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