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Solution Brief
Neuroscience

Advancing Neuroscience Discovery

Raising the Bar in Analytical Speed and Precision

Protein biomarkers have become central to neuroscience research, especially for developing therapeutic targets in neurodegenerative and neuroinflammatory conditions.

Recently, biomarkers found in blood and cerebrospinal fluid (CSF), including phospho-tau 217, GFAP, and amyloid beta 40/42, have emerged as promising indicators across a range of neurological disorders like Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and traumatic brain injury.

Despite these advances, many biomarkers remain challenging to detect in the early stages of disease, delaying crucial clinical intervention. Traditional methods like ELISA, with their manual workflows, are labor-intensive, prone to variability, and often lack the sensitivity required for certain biomarker analyses.

While next-generation platforms offer improved sensitivity, they can still suffer from complex workflows and variability, which can hinder reproducibility.

Detecting Low-Abundance Proteins with Precision

To overcome these challenges, next-generation automation platforms have come to the forefront as a highly efficient and precise alternative.

By enabling miniaturization and automation of assays, these innovations help reduce costs and increases reproducibility. These advanced platforms allow researchers to perform multiplex analysis of biomarkers simultaneously, minimizing concerns about antibody cross-reactivity.

Additionally, the automation inherent in these systems has opened the door for large-scale studies that were previously impractical. With access to larger datasets, researchers can identify new biomarkers and unlock deeper insights into the molecular underpinnings of neurological diseases.

For instance, neurofilament light (NF-L) has proven to be a critical biomarker for various neurodegenerative disorders, including ALS, Huntington’s disease, and multiple sclerosis. Elevated levels of NF-L in blood and CSF signal axonal damage, a hallmark of these conditions.

NF-L: a Game-Changing Biomarker for Neurological Disorders

The utility of NF-L as a neurodegeneration biomarker is enhanced by its ability to be measured in less invasive blood samples rather than CSF. However, because NF-L exists in very low concentrations in blood (in the low picogram per milliliter range), a highly sensitive assay is required to deliver accurate, reproducible data.

In response, researchers have turned to advanced immunoassay platforms that combine sensitivity with efficiency. These systems incorporate enhanced detection methods, enabling the detection of low-abundance biomarkers that traditional assays like ELISA might miss.

The ability to quantify NF-L in blood derivatives like plasma and serum has unlocked new possibilities for more accessible and less invasive research methodologies.

Evaluating Advanced Platforms for NF-L Measurement

Recent studies[1-5,8] have demonstrated that the Ella™ microfluidic immunoassay offers the sensitivity required to reliably measure NF-L in serum, plasma, and CSF across neurological conditions. Its effectiveness in blood samples has been confirmed through several head-to-head platform comparisons. In a seminal **commutability study**[3] (from the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden), 40 paired serum and plasma samples were analyzed across four different platforms and six assay configurations. The results showed strong correlations in NF-L concentrations between the platforms (Spearman's ρ≥0.96, Figure 1). Interestingly, while some calibration biased was noticed across the various platforms, the correlation between paired plasma and serum concentrations was consistently strong (r2 ≥0.95). The strong correlations across these platforms suggest similar selectivity between the assays. These results are encouraging and support further efforts to develop certified reference materials (CRMs) for NF-L in blood.

FIGURE // 01

Serum Concentrations for NF-L

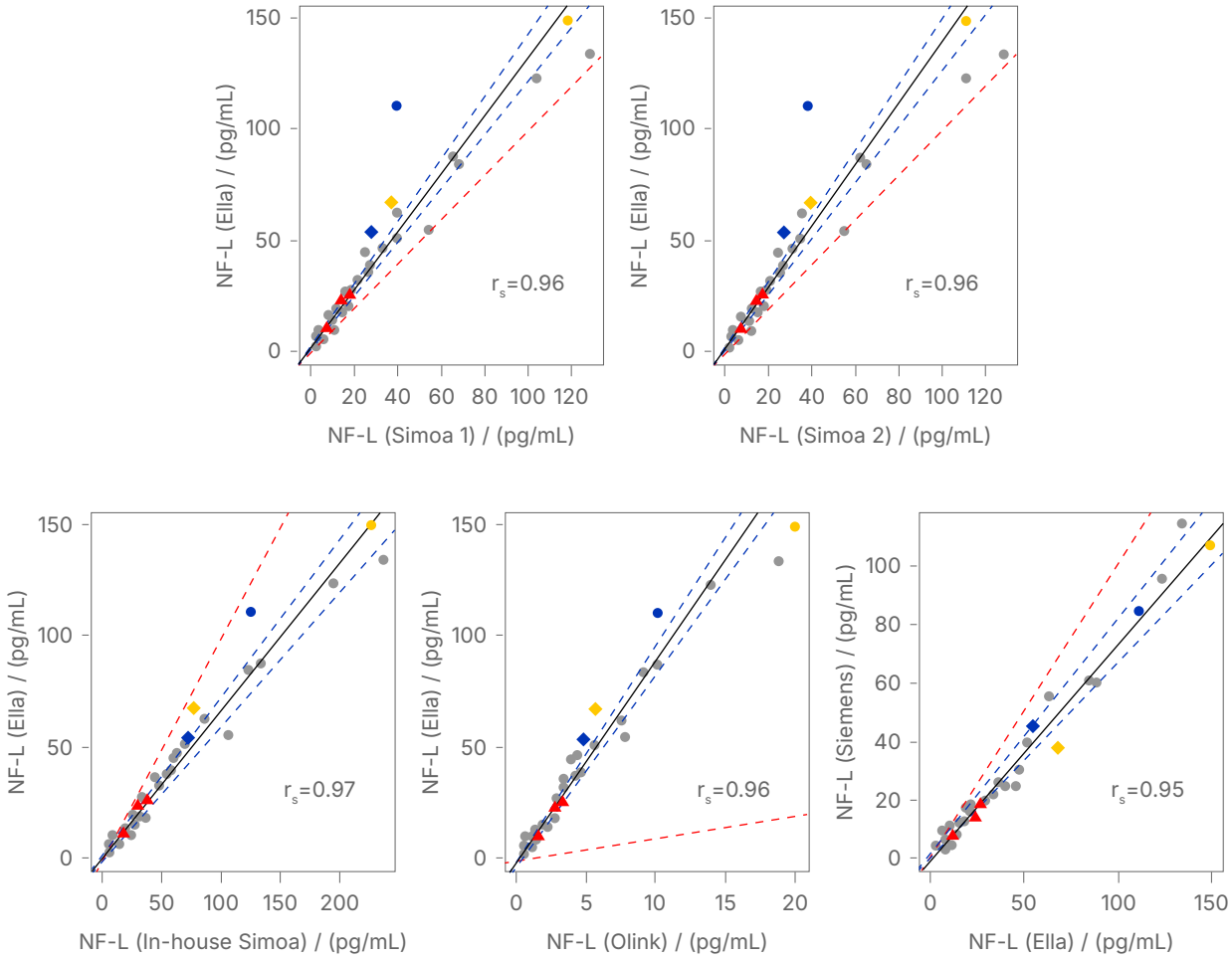

Figure 1. Forty paired serum and plasma samples were analyzed using four platforms and six assay configurations. Results showed strong correlations in NF-L concentrations across platforms (Spearman's ρ≥0.965). While some calibration differences were observed across platforms, plasma and serum NF-L concentrations remained highly correlated. These findings highlight comparable assay selectivity across platforms. (Figure recreated from Fazeli et al., under a creative commons license).

One key advantage of the Ella platform over other next-generation biomarker platforms is its hands-off workflow, which utilizes factory-calibrated cartridges to ensure reproducibility across different operators and locations. In multi-site tests, Ella maintained high precision, with a coefficient of variation (CV) of 9.7% (Figure 2), demonstrating its reliability under varying experimental conditions.

Additional studies[1-2,4-5] comparing Ella to other platforms in various conditions (e.g., MS, ALS, dementia) further support the platform's suitability for this application. Across multiple studies, Ella consistently performed on par with other assays, reinforcing its reliability and utility in NF-L measurement.

FIGURE // 02

Inter-Assay Precision

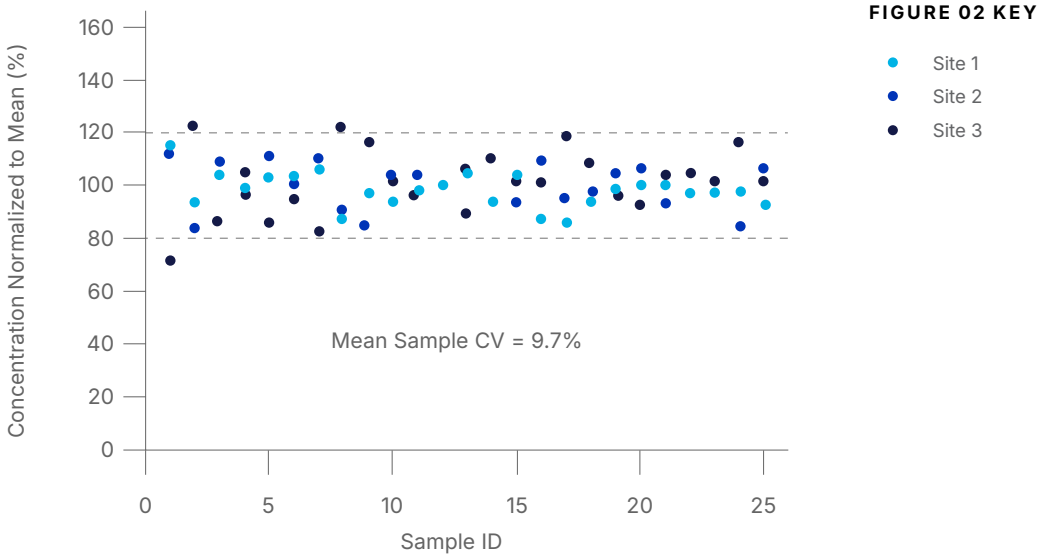

Figure 2. To assess inter-assay precision, plasma and serum samples, obtained from healthy donors, were prepared and measured at three different sites across multiple operators and Ella analyzers. Even at relatively low picogram levels, the results were reproducible regardless of operators, cartridge, or cartridge lot, with an overall coefficient of variability (CV) of 9.7%

GFAP as a Biomarker for Neurodegeneration

In addition to neurodegeneration biomarkers, neuroinflammatory markers in CSF and blood have proven highly informative for various neurological conditions.

Astrocytic biomarkers, such as glial fibrillary acidic protein (GFAP), offer critical insights into the diagnosis, prognosis, and monitoring of conditions like Multiple Sclerosis and Alzheimer's disease. However, low GFAP levels in blood present challenges for assay development, similar to NF-L.

A **recent study**[6] utilizing the Simple Plex™ microfluidic immunoassay running on Ella confirmed the assay's ability to reliably detect GFAP with greater than 3.5 logs of dynamic range and sensitivity at the low pg/mL range. Importantly, GFAP levels in this study were significantly elevated in Alzheimer's and MS patients, reinforcing its role as a valuable blood-based biomarker for these diseases.

In this study, the 2nd-generation Ella GFAP assay was thoroughly validated for serum analysis in a clinical sample cohort. Importantly, significant elevation of serum GFAP was found among Alzheimer's disease and multiple sclerosis patients, as well is in meningitis (Figure 3). Further analytical validation of this novel GFAP assay demonstrated high precision (single-digit %CV), strong performance, and solid correlation with a bead-based biomarker platform (Figure 4). Additionally, the recovery rate of spiked GFAP protein was within an acceptable range of 80-120%, indicating minimal interference from serum matrix effects.

These results underscore GFAP's utility as a blood-based astrocytic biomarker for neurodegeneration and highlight the microfluidic GFAP immunoassay as a sensitive, user-friendly method for GFAP detection in biofluids.

FIGURE // 03

GFAP Concentrations of Diagnostic Groups

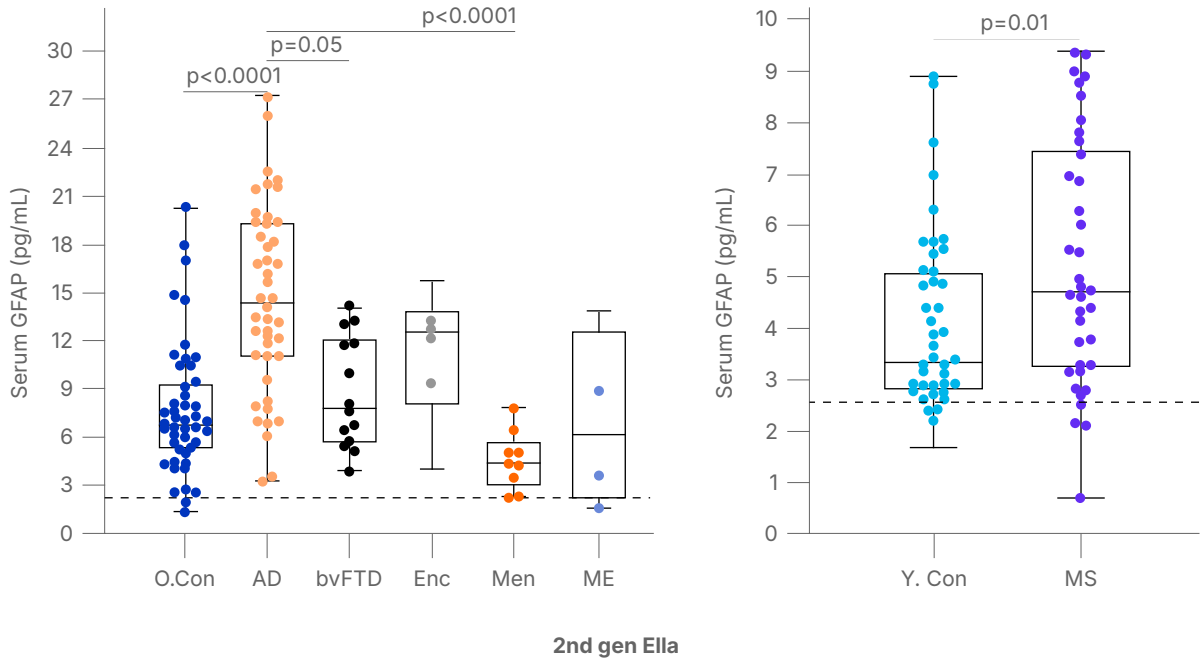

Figure 3. Left panel: GFAP concentrations of diagnostic groups Alzheimer's Disease (AD); behavioral variant frontotemporal dementia (bvFTD); encephalitis (Enc); meningitis (Men); meningoencephalitis (ME); multiple sclerosis (MS); and Frontotemporal dementia (FTD) in the same age range were measured using the 2nd-generation Ella GFAP assay. GFAP concentration patterns across diagnostic groups demonstrated the robustness and reliability of the novel assay. Notably, GFAP levels in AD patients were significantly higher than in the control group (O. Con) ($p<0.0001$ for all assays) and considerably higher than in meningitis patients across all evaluations. Right panel: GFAP levels in a multiple sclerosis (MS) cohort were significantly elevated compared to an aged-matched control cohort (Y. Con) when measured using the 2nd generation Ella assay ($p=0.01$, Mann-Whitney test). (Figure recreated from Fazeli et al., under a creative commons license).

Overall, the findings highlight the robustness and reliability of the 2nd-gen Simple Plex assay for the quantification of serum GFAP in blood and CSF. The assay displays a good correlation (Figure 4) with other available GFAP blood assays, though it may offer slightly lower sensitivity compared to bead-based approaches. Nevertheless, its cost-effectiveness and ease of use position it as a promising alternative for GFAP analysis, with strong potential for routine clinical applications.

FIGURE // 04

Correlations of Serum GFAP

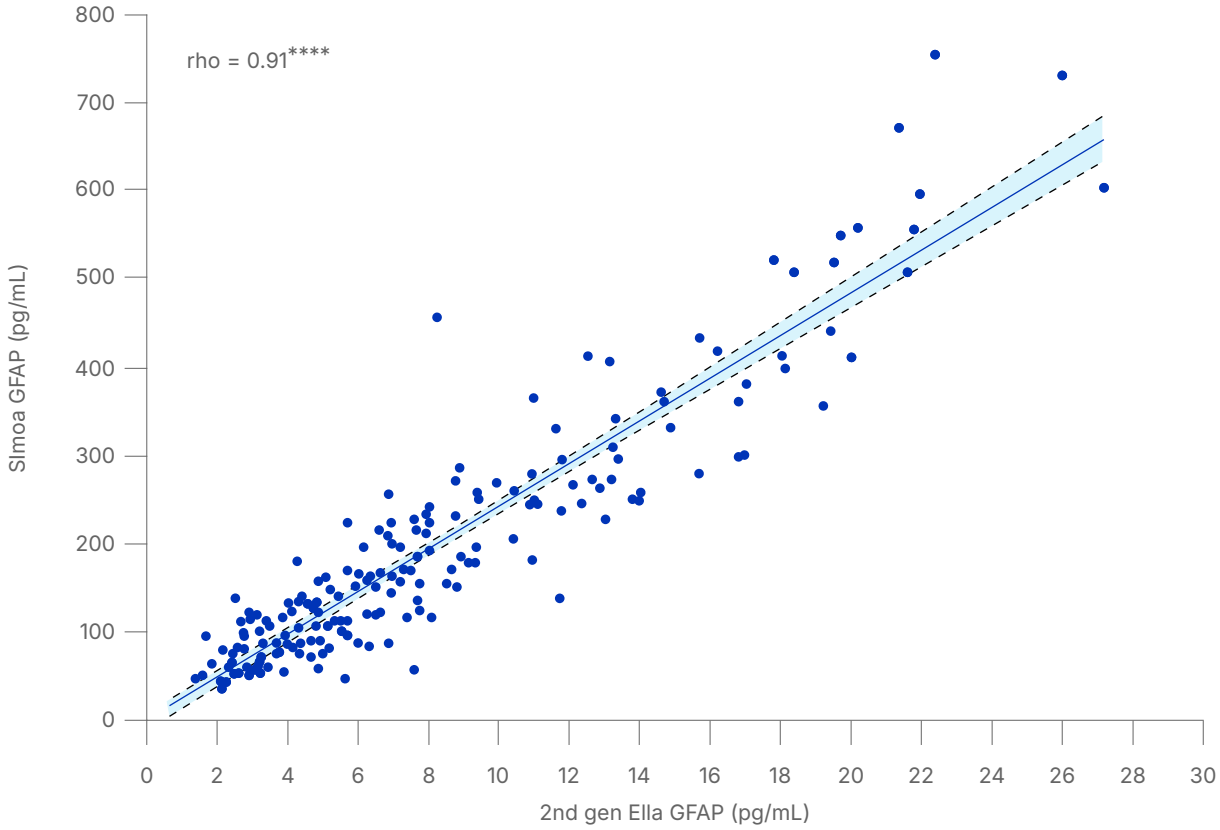

Figure 4. Pairwise correlations of serum GFAP between the 2nd-gen Ella assay and a bead-based assay (Simoa) were measured in 210 samples, showing a strong correlation (r=0.91; 95% CI: 0.88–0.93; p<0.0001). These results underscore the robustness and reliability of the Ella assay for GFAP quantification in blood and CSF, demonstrating good alignment with existing blood assays. (Figure recreated from Fazeli et al., under a creative commons license).

pTau-217: Transforming Alzheimer's Disease Diagnostics

Phosphorylated tau protein (pTau-217) has emerged as one of the most promising biomarkers in Alzheimer's disease (AD) research. As evidence grows for its use in diagnosing AD, developing reliable blood-based assays remains a challenge. Many existing methods are costly and time-consuming, limiting their clinical accessibility. Expanding access to this highly accurate biomarker is essential for broader implementation of AD blood tests.

In a **recent study**[7], the Simple Plex pTau-217 ALZpath assay on the Ella platform demonstrated robust utility in detecting Alzheimer's amyloid pathology. Plasma levels of pTau-217 were significantly elevated in Alzheimer's patients, consistent with prior findings. Both plasma and CSF pTau-217 levels were elevated in ATN-positive AD samples ($p<0.001$, Mann-Whitney test, (Figure 5), in line with earlier reports[9]. The assay's limit of detection was determined to be 0.09 pg/mL, with limits of quantitation ranging from 0.32 to 1,200 pg/mL.

These results support the potential of this novel immunoassay as a valuable tool for measuring pTau-217 in both research and clinical settings. Its ability to reliably inform amyloid pathology from blood samples alone makes it a powerful diagnostic resource for Alzheimer's disease.

FIGURE // 05

pTau-217 Comparisons

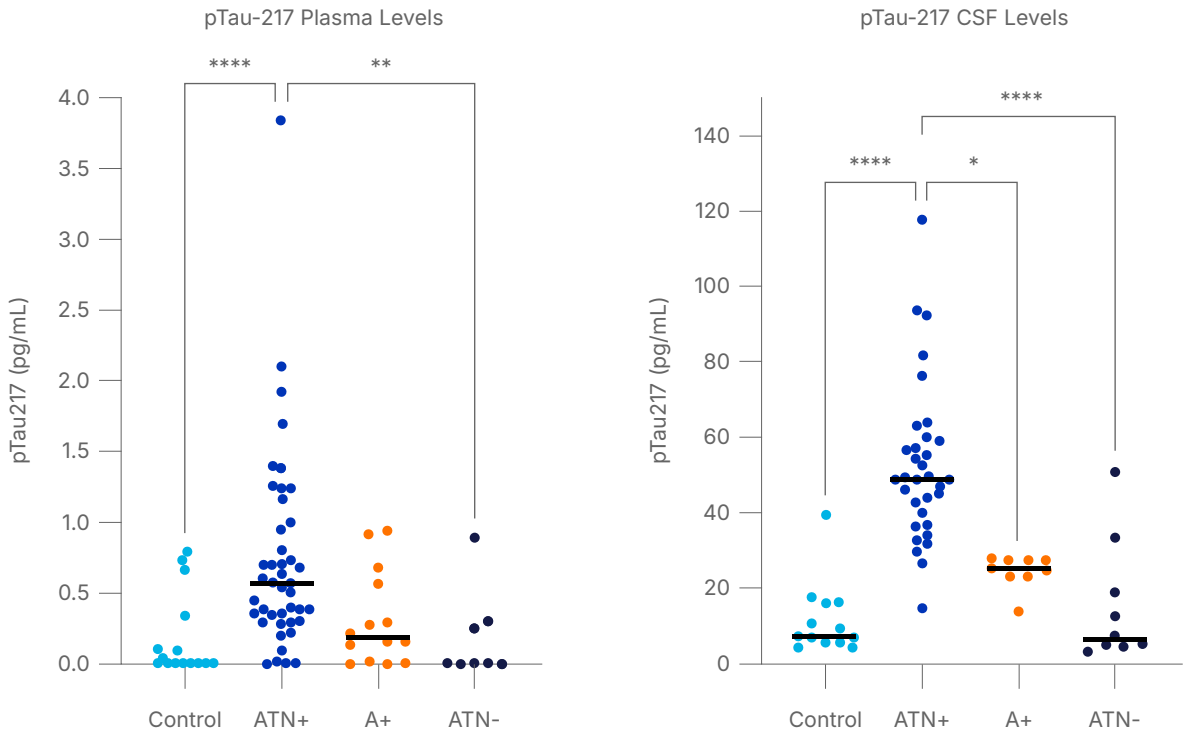

Figure 5. Plasma (left) and CSF (right) pTau-217 levels were compared between healthy control donors and clinically diagnosed Alzheimer's disease patients. ATN positive patients (ATN+) had significantly elevated plasma and CSF pTau-217 levels when compared to both healthy controls as well as ATN negative patients. Significant difference in CSF pTau levels was also found upon comparison of ATN+ and Amyloid positive (A+) patient groups.

The Future of Biomarker Detection in Neuroscience

Advances in next-generation analytical instruments are reshaping neuroscience research, delivering unparalleled sensitivity and reproducibility. These innovations allow for more precise detection of early-stage neurological disorders, paving the way for improved diagnostics and more effective therapeutic interventions.

As technology continues to evolve, so does our potential to unlock new insights into brain diseases, driving breakthroughs in treatment and discovery.

Learn More About Simple Plex Neuroscience Assays

Scan the QR Code or Visit:

bio-techne.com/simple-plex-neuro

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