R&D systems by biotechne

Immunoassays

Precise & Ultra-Sensitive Detection of

NFL, GFAP, pTau217, & Amyloid Beta (1-42)

Using Simple Plex Assays on Ella

Accurate quantification of protein biomarkers in biological fluids has become increasingly important for diagnosing, monitoring, and understanding neurodegenerative disorders. Advances in biomarker research have identified several proteins strongly associated with key pathological processes in neurological disease. Neurofilament light chain (NFL) is widely recognized as a general marker of axonal degeneration [1], while glial fibrillary acidic protein (GFAP) reflects neuroinflammation and astrocyte activation [2]. Amyloid beta (Aβ), together with phosphorylated Tau (217), is strongly associated with amyloid-related pathology in Alzheimer's disease (AD) [3–5].

Traditionally, neurological biomarkers have been measured in cerebrospinal fluid (CSF), which provides a close representation of biological processes occurring in the brain. However, CSF collection is more invasive and less practical for large clinical studies or routine testing. In contrast, blood-based biomarkers offer a more accessible and scalable alternative and are already a clinical reality, with clinically approved tests for conditions such as Alzheimer's disease and multiple sclerosis (MS) [6–8]. Despite this progress, many neurological biomarkers are present at extremely low concentrations in blood. Reliable detection therefore requires analytical methods that combine ultra-high sensitivity with robust precision and standardized, low-variability workflows.

Simple Plex™ Assays on the automated Ella™ system are designed to address these challenges through an integrated, microfluidic approach. Each cartridge contains preloaded reagents and a built-in calibration curve, minimizing user intervention and reducing the potential for human error. Following a brief sample preparation step, the system performs all assay processes automatically, including incubation, washing, and detection, delivering fully analyzed results in under three hours. With over

Highlights:

- Ultra-Sensitive Simple Plex Assays on the Ella system detect and quantify NFL, GFAP, pTau217, and Aβ1-42 in blood at femtogram-per-milliliter concentrations
- All four biomarkers were detectable in 100% of healthy donor plasma samples, with intra-assay CVs below 8% across 8 operators, 23+ instruments, and 68 cartridges
- NFL and GFAP distinguished healthy from AD samples with ROC AUC of 0.90; pTau217 and the pTau217/Aβ1-42 ratio achieved AUCs of 0.88–0.89
- Strong cross-platform agreement (Spearman r = 0.91–0.92) and high commutability with standard Simple Plex assay formats confirmed

Why This Workflow Matters for Blood-Based Neurological Biomarker Research

Reliable blood-based detection of neurological biomarkers requires femtogram-level sensitivity, minimal matrix interference, high analytical performance, and cross-laboratory reproducibility — a combination most platforms struggle to deliver together.

The Ultra-Sensitive Simple Plex Assays on the Ella system meet all three requirements within a single automated, standardized workflow, enabling consistent differentiation of Alzheimer's disease patients from healthy controls across instruments, operators, and sites.

400 validated Simple Plex Assays, the Ella system has been successfully and broadly utilized for neurological disease studies by many research groups, across pathologies [9–13]. To enable reliable detection of low-abundance neurological biomarkers in blood, Simple Plex Assays were further optimized for Ultra-Sensitive quantification of pTau217, GFAP, NFL, and Aβ1-42 at femtogram-per-milliliter levels. These novel assays were developed to deliver this sensitivity while maintaining strong analytical performance.

In this application note, we demonstrate how the novel Ultra-Sensitive Simple Plex Assays on the Ella platform expand the system's utility to enable precise and reliable biomarker quantification in blood, at femtogram-per-milliliter levels.

Methods

Ultra-Sensitive Detection of Neurological Biomarkers

Ultra-Sensitive 32x4 Simple Plex cartridges were run according to product instructions. Plasma, serum, and CSF samples were diluted to the Minimum Required Dilution (MRD = 1.25x) using the provided sample diluent and loaded into the cartrdge; wash buffer was added to the wash reservoir. Cartridge labels were scanned prior to loading, and all assay processing was performed automatically by the Ella instrument, including incubation, washing, and detection, with a total instrument runtime of approximately 2 hours and 35 minutes. Results were exported to the Simple Plex Explorer software for further analysis.

12-point standard curves were generated using multiple preparation steps across users and cartridges. The final curves were fitted using a four-parameter logistic (4PL) model. Limits of quantitation were determined as specified in the assay specification sheet, demonstrating 80-120% recovery of all curve points within the limits of quantitation and a coefficient of variance (CV) below 20%. The Limit of Detection (LOD) was set as 3 standard deviations above the mean background signal as determined from multiple runs.

Analytical performance was evaluated across multiple parameters including detectability, precision, parallelism, and accuracy, followed by assessment of clinical utility in disease samples. Plasma samples from 24 healthy donors (aged 55 and older) and a cohort of Alzheimer's disease (AD) patients were analyzed. Amyloid/Tau/Neurodegeneration (ATN)-positive AD status was confirmed by standard diagnostic criteria through orthogonal methods. Cross-platform comparisons were performed against an independent bead-based immunoassay platform.

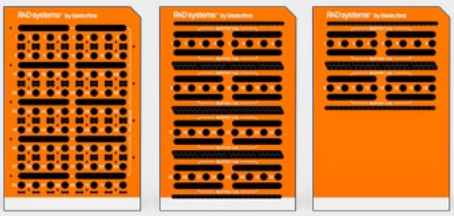
Simple Plex Ultra-Sensitive Assays

- ✓ NFL, GFAP, pTau217 (ALZpath), amyloid β1-42
- ✓ Validated in Plasma, Serum, CSF
- ✓ Available both as single plex or duplex
- ✓ 40 µl required sample volume
- ✓ <2:50 hr to results (15min sample prep + 2:35 hrs instrument time)

Featured Products:
Simple Plex Assays

Neurodegeneration Panels
NFL, GFAP

Alzheimer's Disease Panels
pTau 217, Aβ1-42



Results

Analytical Performance and Sample Quantification

Across multiple cartridges and cartridge lots, standard curves demonstrated strong reproducibility, with dynamic ranges spanning approximately 3–4 logs. All points within the limits of quantitation (LOQ) recovered 80–120% to their target concentrations and had coefficients of variance (CV) below 20%, indicating robust assay performance across the working range. When accounting for the plasma and serum dilution factor (x1.25), functional limits of detection (fLOD) were 0.46pg/mL (NFL); 0.24 pg/mL (GFAP); 0.05 pg/mL and (pTau217); 0.28 pg/mL (Aβ1-42); with corresponding functional lower limits of quantitation (fLLOQ) of 2.49, 0.78, 0.13, and 1.15 pg/mL, respectively (Table 1). Further validation of the LLOQs was carried out by running replicate natural plasma/serum samples at three different dilutions to achieve values both above and below the LLOQ. Under these conditions, the results demonstrated robust precision above the LLOQ (Figure 1), further substantiating the assays' quantitation limits at ultra-low concentrations.

TABLE 1

Ultra-Sensitive Simple Plex Assays Limits of Detection

	NFL	GFAP	p-Tau217	Aβ1-42
Analytical LOD (pg/mL)	0.37	0.19	0.04	0.22
Functional LOD (pg/mL)	0.46	0.24	0.05	0.28
Analytical LLOQ (pg/mL)	1.99	0.62	0.1	0.92
Functional LLOQ (pg/mL)	2.49	0.78	0.13	1.15
Curve Recovery at LLOQ	96.9%	101%	100%	116%
Curve CV at LLOQ	12.6%	10.6%	17.0%	8.9%
Pooled Sample CV Above LLOQ	4.9%	4.6%	7.1%	7.8%

Table 1. Limits of Detection and Quantitation for the four Ultra-Sensitive Simple Plex assays. All assays demonstrated good recovery (97-116%) and precision (CV<17%) at the lower limit of quantitation.

FIGURE 1

Validation of Lower Limits of Quantification

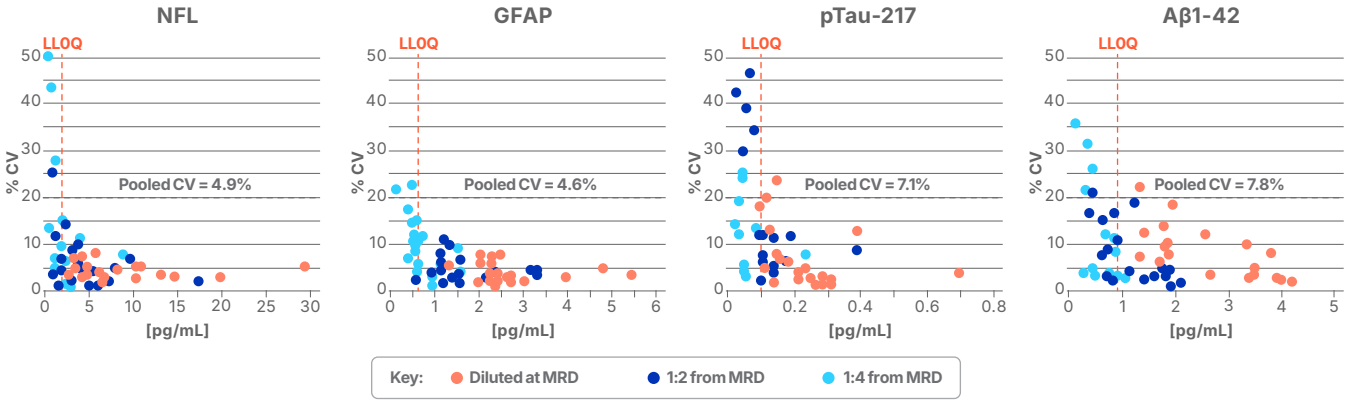


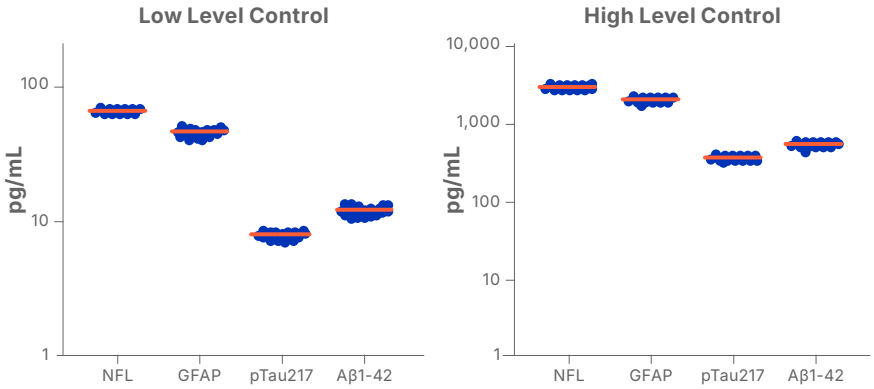
Figure 1. The lower limits of quantitation (LLOQ) were confirmed through serial dilution of plasma and serum samples spanning concentrations above and below the LLOQ threshold. Precision was evaluated using three replicates per dilution, allowing determination of CV for each sample, as well as above the LLOQ (MRD: Plasma and serum diluted 1.25x in sample diluent).

Precision and Reproducibility

To evaluate assay precision, two control samples representing high and low concentrations were tested across multiple replicates. Across all four assays, intra-assay precision demonstrated coefficients of variance at 1.8-5.8%, indicating excellent repeatability within cartridges (Figure 2).

FIGURE 2

Intra-Assay Precision



Intra-Assay %CV		
	Low	**High**
NFL	4.6%	4.9%
GFAP	1.8%	2.6%
pTau217	4.1%	3.2%
Aβ1-42	5.8%	4.5%

Figure 2. To assess the intrinsic precision across different points along the dynamic range of the assays, two controls (high and low) were run across 16 wells for each assay. The results indicate high intra-assay precision with single digit coefficients of variance (%CVs).

In addition to within-assay precision, reproducibility across users and instruments was evaluated. Independent control preparations were generated by eight different operators and analyzed across at least 23 Ella instruments, 68 cartridges, and three cartridge lots.

The resulting data showed strong consistency across users, cartridges, and instruments, highlighting the robustness of the automated Simple Plex workflow and its ability to generate reproducible results across laboratories (Figure 3).

FIGURE 3

Inter-Assay Precision

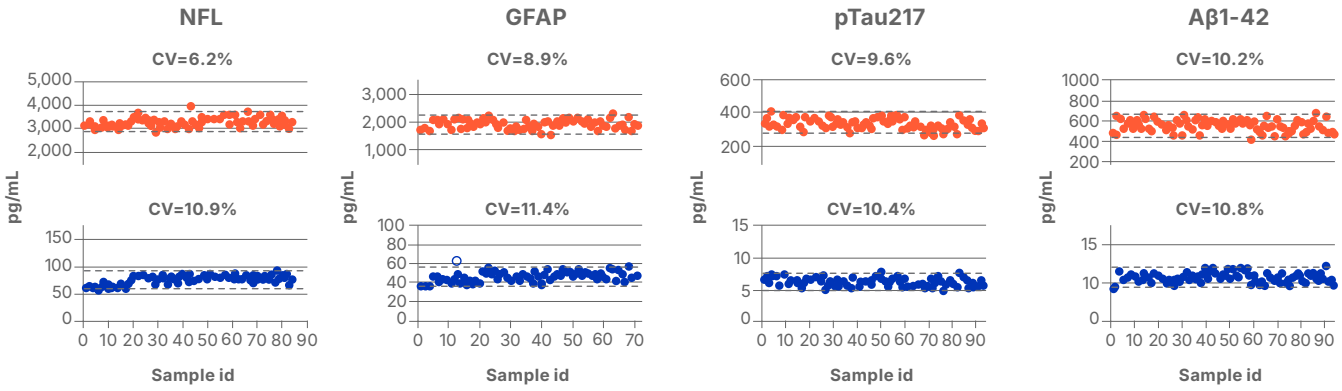


FIGURE 3

Inter-Assay Precision *(continued)*

	Inter-Assay %CV	
	Low	**High**
NFL	10.9%	6.2%
GFAP	11.4%	8.9%
pTau217	10.4%	9.6%
Aβ1-42	10.8%	10.2%

Figure 3. Inter-assay precision. Reproducibility of each assay is shown through evaluation of high and low control samples across multiple operators and system variables, illustrating consistency across instruments, cartridges, and lots.

Parallelism and Accuracy

Accurate analyte quantitation in complex biological matrices such as plasma requires assays to maintain linear response upon dilution and to recover known analyte concentrations with minimal bias. Parallelism, assessed through dilutional linearity, confirms that analytes behave consistently with the assay calibration curve and are not significantly affected by matrix interference. Accuracy, evaluated through spike recovery, demonstrates the ability of the assay to measure known quantities of analyte within an acceptable range.

Parallelism was assessed through dilutional linearity studies, where serial dilution of spiked plasma samples using a two-fold dilution scheme from the minimum required dilution (MRD = 1.25) demonstrated consistent recovery across dilution factors (Figure 4). Recovery values remained within acceptable ranges, supporting both dilutional linearity and accuracy of the assays in plasma.

Across all assays, average parallelism values ranged from 99% to 118%, indicating minimal matrix effects and consistent performance across the dynamic range. Accuracy was further supported by spike recovery results, with values ranging from 73% to 99%, confirming reliable quantitation of target biomarkers in blood-based matrices. (Figure 5)

Together, these results demonstrate that the Ultra-Sensitive Simple Plex Assays on the Ella platform deliver robust analytical performance for accurate and reliable measurement of low-abundance neurological biomarkers in plasma.

FIGURE 4

Evaluating Parallelism in Plasma

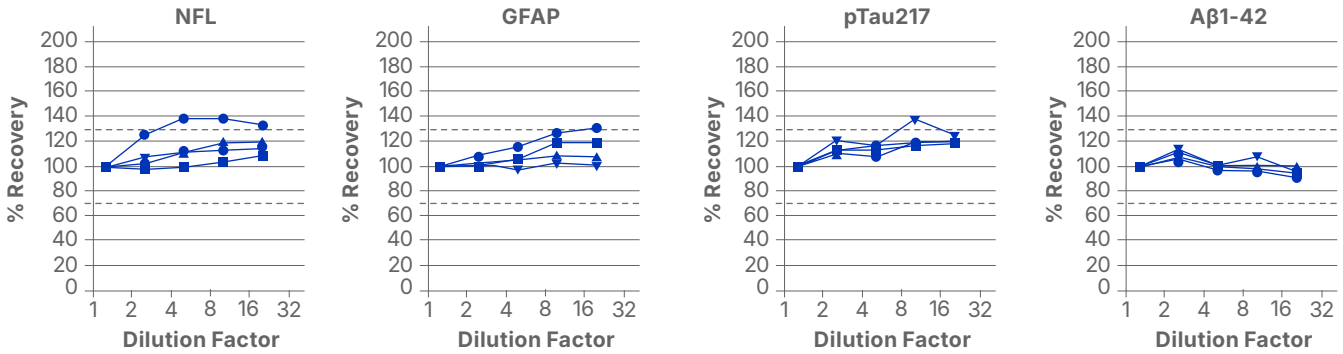


Figure 4. Dilutional linearity was assessed by serial dilutions of spiked plasma/EDTA samples. Shown above are 4 separate spiked plasma samples, each represented by unique symbols (circles, squares, triangles).

FIGURE 5

Mean Linearity & Spike/Recovery Values

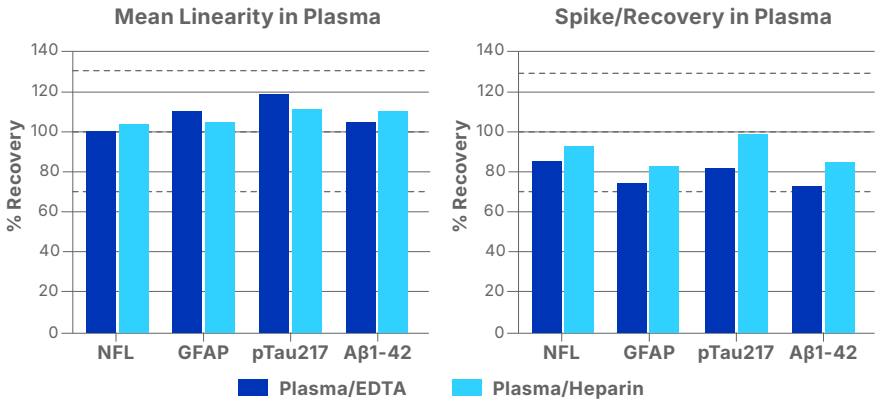


Figure 5. Mean linearity and spike/recovery values across assays demonstrated strong parallelism and accurate quantitation in plasma.

Biomarker Detection in Healthy Plasma Samples

To evaluate assay performance in biological samples, the Ultra-Sensitive assays were applied to plasma samples from a pilot cohort of 24 healthy donors (aged 55+), alongside Alzheimer's disease samples. All four biomarkers were detected in 100% of samples above the limit of detection (LOD), demonstrating robust assay sensitivity in real biological matrices (Figure 6). Additionally:

- NFL, GFAP, and Aβ1-42 were quantifiable above the LLOQ in all samples
- pTau217 was quantifiable in 96% of samples

These results demonstrate that the assays can reliably quantify low-abundance neurological biomarkers in healthy individuals.

FIGURE 6

Detection & Quantification of Neurological Biomarkers in Healthy Plasma

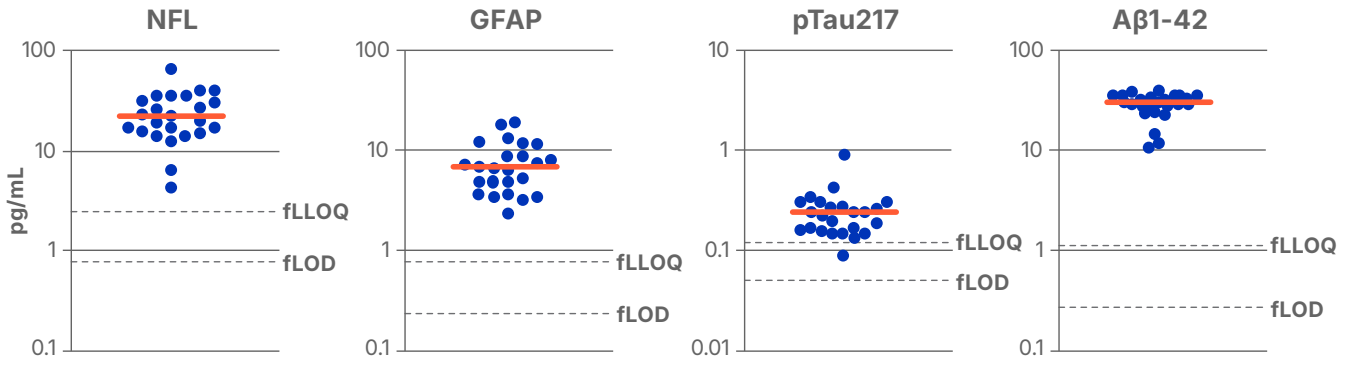


Figure 6. Plasma samples from 24 healthy donors were analyzed using the Ultra-Sensitive Simple Plex Assays. All four biomarkers were detectable above the limit of detection (LOD), with NFL, GFAP, and Aβ1-42 quantifiable above the lower limit of quantitation (LLOQ) in all samples. pTau217 was quantifiable in 96% of donors, with one sample falling below the LLOQ. These results highlight the assays' sensitivity and ability to reliably measure low-abundance biomarkers in normal plasma.

Assessing Neurodegeneration and Neuroinflammation in Alzheimer's Disease

To evaluate biomarker utility, samples from healthy donors were compared with those from Alzheimer's disease (AD) patients.

Significant elevations in blood NFL and GFAP levels were observed in the AD cohort, consistent with increased neurodegeneration and neuroinflammatory activity. Receiver operating characteristic (ROC) analysis demonstrated strong differentiation between healthy and AD samples, with areas under the curve (AUC) of 0.90 for both biomarkers (Figure 7). These results support the utility of NFL and GFAP as indicators of neuronal injury and inflammation in neurodegenerative disease.

In addition to these markers, pTau217 and amyloid beta were evaluated for their ability to differentiate between cohorts. Plasma pTau217 levels and the pTau217/Aβ1-42 ratio showed clear separation between healthy and AD samples, with ROC AUC values of 0.88 and 0.89, respectively (Figure 7). Consistent with the extensive literature on the clinical utility of amyloid beta in biofluids [17], the separation between AD patients and controls was smaller in plasma (AUC 0.71) than in CSF (AUC 0.89). Once again consistent with published literature [18], introduction of amyloid β 1-40 measurements on Ella and deriving amyloid β 1-42/40 ratio values lead to an almost perfect separation between the controls and the AD group (AUC 0.98), supporting strong biomarker utility for amyloid β in CSF using the Ella system (Figure 7).

Together, these findings highlight the complementary and multi-modal value of biomarkers reflecting neurodegeneration, neuroinflammation, and amyloid-related pathology.

FIGURE 7

Evaluation of Neurodegeneration Neuroinflammation & Tau Pathology in Alzheimer's Disease

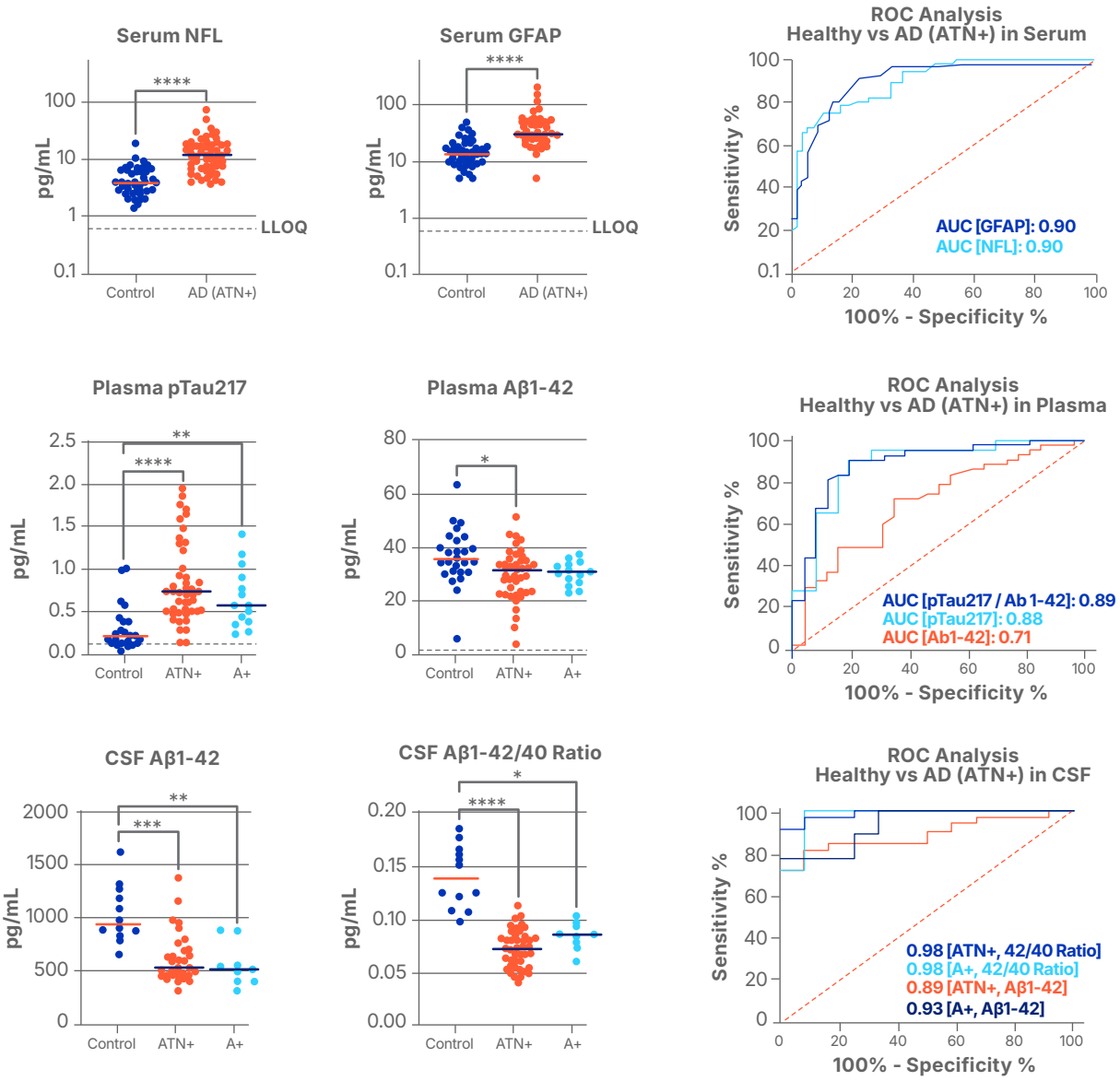


Figure 7. Evaluation of neurodegeneration, neuroinflammation, and Tau amyloid pathology in Alzheimer's disease. Top: Serum samples from healthy donors and ATN-positive Alzheimer's disease (AD) patients were analyzed using the Simple Plex Ultra-Sensitive Assays. NFL and GFAP concentrations were elevated in the AD cohort, reflecting increased neurodegeneration and neuroinflammation. ROC analysis demonstrated strong discrimination between healthy and AD samples, with areas under the curve (AUC) of 0.90 for both biomarkers. Middle: Plasma pTau217 levels and the pTau217/Aβ1-42 ratio demonstrated strong differentiation between healthy and ATN-positive Alzheimer's disease (AD) samples, with ROC AUC values of 0.88–0.89. In concordance with published literature, amyloid β 1-42 possessed weaker diagnostic accuracy in plasma but had strong utility in CSF (bottom), which was even further enhanced by incorporating Aβ1-40 measurements on Ella and deriving Aβ42/40 ratio. Comparison across groups was performed using the Mann-Whitney test (upper panel) or Kruskal-Wallis nonparametric test, followed by Dunn's post-hoc test (*p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001)

Consistent Biomarker Measurements Across Platforms

Previous studies have established good correlation between Ella's standard neurological biomarker assays and other next-generation immunoassay platforms [10–16]. To assess cross-platform performance of the Simple Plex Ultra-Sensitive Assays, biomarker measurements generated on the Ella system were compared with results obtained using a bead-based immunoassay platform.

Ultra-Sensitive measurements of blood pTau217 and GFAP from control and Alzheimer's patients demonstrated strong correlations across platforms, with Spearman correlation coefficients of 0.92 and 0.91, respectively. Moreover, pTau217 measurements on both platforms demonstrated good differentiation between healthy controls and ATN+ Alzheimer's patients, with all samples measured on Ella measuring above the detection limit (Figure 8). These results demonstrate strong agreement between platforms and support the reliability of the Simple Plex Ultra-Sensitive Assays in comparison to other available methods.

Commutability with Standard Simple Plex Assays

In addition to cross-platform comparisons, the new Ultra-Sensitive Assays were evaluated against existing standard Simple Plex assay formats to assess commutability. Both assay versions utilize the same antibody pairs and overall assay configuration but differ in their running protocol to enable enhanced sensitivity.

Strong correlation was observed between standard and Ultra-Sensitive assay formats across the measured range, demonstrating a high degree of agreement between the two approaches (Figure 8). These findings confirm that results generated using Ultra-Sensitive assays are consistent with established Simple Plex Assays, supporting continuity for users transitioning between formats.

FIGURE 8

Cross-Platform Comparison of Biomarker Measurements.

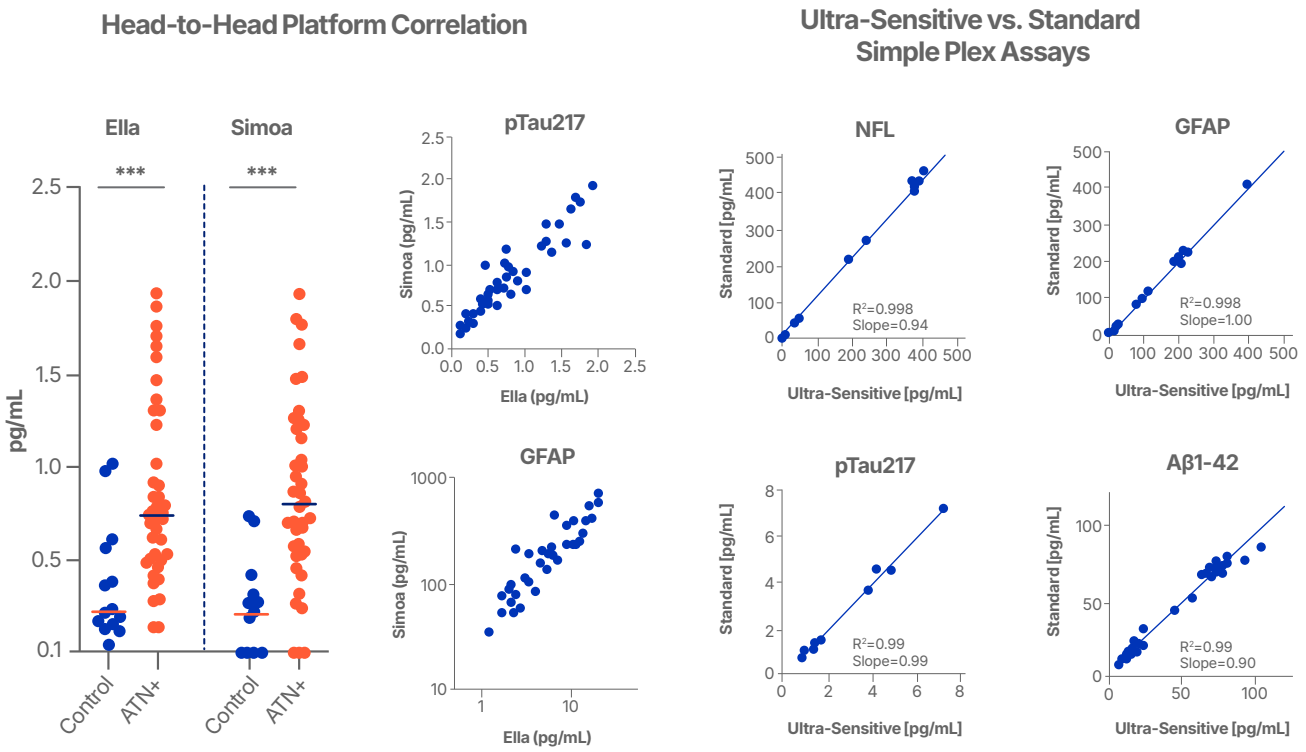


Figure 8. Cross-platform comparison of biomarker measurements. **Left:** Biomarker concentrations measured on the Ella platform were compared with results obtained using a bead-based immunoassay platform (Simoa™) in plasma (pTau217; data shown) and serum (GFAP). Strong correlations were observed between platforms, with Spearman correlation coefficients of 0.92 for pTau217 and 0.91 for GFAP. **Right:** Biomarker concentrations measured using standard Simple Plex Assays were compared with those obtained using Ultra-Sensitive assay formats. Strong linear correlations were observed across all analytes (for the standard Simple Plex assays, only samples above LLOQ were included in the analysis).

These results demonstrate that the Simple Plex Ultra-Sensitive Assays provide reliable measurements consistent with other established analytical methods. Together, these capabilities make the Ella system well-suited for biomarker research applications where both analytical sensitivity and reproducibility are critical.

Conclusion

The Ella system is an established platform offering a broad range of biomarker research solutions for neurology in both CSF and blood. In this work, we demonstrate how optimized Ultra-Sensitive Simple Plex Assays on the Ella platform enable precise and reliable quantification of low-abundance biomarkers in blood, at femtogram-per-milliliter levels.

The novel Ultra-Sensitive Simple Plex Assays running on the Ella platform provide a powerful, automated solution for quantifying low-abundance neurological biomarkers in blood.

Beyond analytical performance, the assays demonstrated clear differentiation between healthy individuals and Alzheimer's disease samples, and receiver operating characteristic (ROC) analysis confirmed the potential utility of these biomarkers for clinical research applications targeting neurodegeneration, neuroinflammation, and amyloid pathology.

Together, these results validate the Ella system as a precise, highly sensitive, and automated benchtop solution for quantifying neurodegenerative biomarkers in blood. By combining femtogram-level sensitivity with a standardized microfluidic workflow, Simple Plex Ultra-Sensitive Assays enable reproducible, scalable biomarker measurements that support the next generation of studies aimed at improving the understanding of onset and progression of neurological diseases.

References

1. Khalil M, et al. Neurofilaments as biomarkers in neurological disorders — towards clinical application. Nat Rev Neurol. 2024;20(5):269–287. **https://doi.org/10.1038/s41582-024-00955-x**

2. Zheng X, et al. Prediction of clinical progression in nervous system diseases: Plasma glial fibrillary acidic protein (GFAP). Eur J Med Res. 2024;29:51. **https://doi.org/10.1186/s40001-023-01631-4**

3. Ashton NJ, et al. Diagnostic accuracy of a plasma phosphorylated tau 217 immunoassay for Alzheimer disease pathology. JAMA Neurol. 2024;81(3):255–263. **https://doi.org/10.1001/jamaneurol.2023.5319**

4. Petersen KK, et al. Predicting onset of symptomatic Alzheimer's disease with plasma p-tau217 clocks. Nat Med. 2026;32(3):1085–1094. **https://doi.org/10.1038/s41591-026-04206-y**

5. Zetterberg H, Bendlin BB. Biofluid biomarkers in Alzheimer's disease and other neurodegenerative dementias. Nature. 2026;650:49–59. https://doi.org/10.1038/s41586-025-10018-w

6. Teunissen CE, et al. Blood-based biomarkers for Alzheimer's disease: Towards clinical implementation. Lancet Neurol. 2022;21(1):66–77. **https://doi.org/10.1016/S1474-4422(21)00361-6**

7. Chitnis T, et al. Blood and CSF biomarkers for multiple sclerosis: Emerging clinical applications. Lancet Neurol. 2025;24(12):1066–1078. **https://doi.org/10.1016/S1474-4422(25)00249-2**

8. Healey N. Blood tests for Alzheimer's disease could reshape research and care. Nature. 2026. **https://doi.org/10.1038/d41591-026-00008-4**

9. Wilson JM, Dage JL, Qian H-R. Dulaglutide and neurodegeneration biomarkers: REWIND post hoc analysis. Alzheimers Dement. 2026;22:e71391. **https://doi.org/10.1002/alz.71391**

10. Baiardi S, et al. Head-to-head comparison of four cerebrospinal fluid and three plasma neurofilament light chain assays in Parkinsonism. npj Parkinsons Dis. 2025;11:98. **https://doi.org/10.1038/s41531-025-00951-y**

11. Witzel S, et al. Population-based evidence for the use of serum neurofilaments as individual diagnostic and prognostic biomarkers in amyotrophic lateral sclerosis. Ann Neurol. 2024;96(6):1040–1057. **https://doi.org/10.1002/ana.27054**

12. Halbgebauer S, et al. Age-specific control and Alzheimer disease reference curves and z-scores for glial fibrillary acidic protein in blood. Clin Chem. 2025;71(12):1234–1242. **https://doi.org/10.1093/clinchem/hvaf120**

13. Furlan R, et al. Granulocyte and astrocyte markers distinguish MOG-antibody disease and neuromyelitis optica from multiple sclerosis. Brain. 2025;awaf345. **https://doi.org/10.1093/brain/awaf345**

14. Andreasson U, et al. Assessing the commutability of candidate reference materials for the harmonization of neurofilament light measurements in blood. Clin Chem Lab Med. 2023;61(7):1245–1254. **https://doi.org/10.1515/cclm-2022-1181**

15. Fazeli B, et al. Quantification of blood glial fibrillary acidic protein using a second-generation microfluidic assay: Validation and comparative analysis with two established assays. Clin Chem Lab Med. 2024;62(8):1591–1601. **https://doi.org/10.1515/cclm-2023-1256**

16. Willis S, et al. An automated assay for precise and sensitive quantification of pTau-217 in plasma and CSF. Poster presentation, Society for Neuroscience Annual Meeting, San Diego, CA. 2024.

17. Olsson B, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673–684. **https://doi.org/10.1016/S1474-4422(16)00070-3**

18. Hansson O, et al. Advantages and disadvantages of the use of the CSF amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's disease. Alzheimers Res Ther. 2019;11:34. **https://doi.org/10.1186/s13195-019-0485-0**

R&D systems by bio-techne

11058.0.0626