A microfluidic immunoassay method for analyzing blood glial fibrillary acidic protein (GFAP) across neurodegenerative disorders

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Summary

The study of neurodegenerative disease requires the establishment of precise, specific, and sensitive biomarker assays. Elevated circulating levels of the astrocytic protein GFAP in CSF and blood have shown promise as a potential diagnostic and prognostic biomarker in various neurodegenerative conditions. However, while detection of GFAP in blood offers the advantage of minimally invasive biomarker analysis, low levels of GFAP products in plasma and serum pose a challenge for the development of an assay that is both robust, sensitive, and easy to implement in a laboratory setting.

Here, we report a novel assay for analyzing GFAP in CSF and plasma/ serum samples. Utilizing the Ella™ platform, we employed an automated, microfluidic strategy by forming a sandwich immunoassay within glass nanoreactors, allowing synchronized application of assay reagents within a closed microfluidic circuit. Assay performance was evaluated by applying 25 ul of sample to 72-well microfluidic cartridges, with each well yielding triplicate results (leading to 216 readings in <90 minutes). The Limit of Detection of the novel GFAP assay was determined at 0.64 pg/mL, with a Limit of Quantitation range of 2.52 – 9,600 pg/mL. Intra- and Inter-assay precision testing yielded coefficient of variance (CV) of <10%, and <12% respectively, indicating good precision within and across runs. Supporting the overall accuracy of the assay in blood and CSF, spike/recovery and spike/linearity experiments in these human matrices yielded satisfactory recovery across sample types and individual samples, with mean recovery values between 80 and 120%.

We next evaluated the biomarker utility of the assay using a cohort of serum samples (n=220) which included clinically characterized patients of various neurodegenerative and neuroinflammatory disorders. Elevated blood GFAP levels were observed in Alzheimer's disease (AD; p<0.001) upon comparison with controls of the same age range (Kruskal-Wallis with Dunn's post-hoc comparison). In contrast, no significant increase was measured in frontotemporal dementia, multiple sclerosis, encephalitis, and meningitis patients. Notably, analyzing the same patient cohort using a commercially available bead-based assay yielded equivalent results and demonstrated good correlation between the two assays (Spearman *r* = 0.92, 95% CI = 0.89-0.94, p<0.0001).

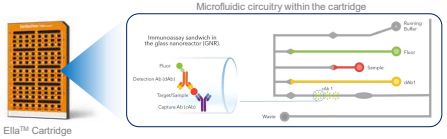
Taken together, these results further establish the utility of GFAP as a blood-based, astrocytic biomarker for neurodegeneration and support the applicability of a microfluidic GFAP immunoassay as a sensitive and easy-to-use benchtop strategy for measuring GFAP protein in biofluids.

The Ella™ workflow

Precision biomarker analysis using an automated microfluidic system

Ella™ instrument

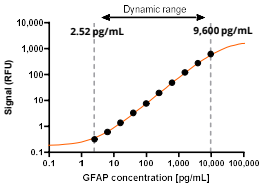
- Sandwich immunoassay occurs within a microfluidic circuit
- Hands-free, automated workflow
- Ultrasensitive, with 4-log dynamic range
- Sample preparation and loading in 15 minutes
- Results in less than 90 minutes
- Triplicate results per sample
- Automatic fitting to a built-in standard curve



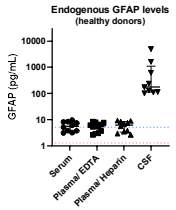
Ella™ Cartridge

A novel immunoassay for GFAP analysis in blood and CSF

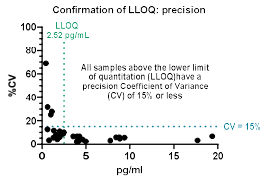
Dynamic range and assay sensitivity



Standard curve, limits of quantitation, and dynamic range of the GFAP 2nd gen assay on Ella (catalog # SPCKB-PS-00913d). Upon running multiple curves on multiple cartridges and cartridge lots, all points within the dynamic range recovered 80-120% to the target and had a coefficient of variance (CV) lower than 20%. Curve was derived using a 4PL fitting.

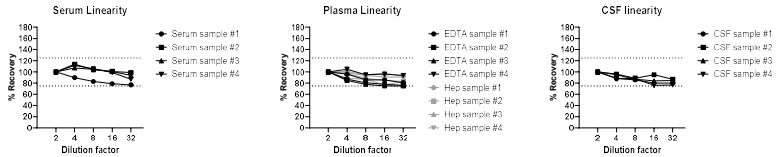


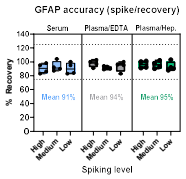
Samples from healthy donors were tested were run to evaluate concentration values. All CSF samples were detectable above the lowe limit of quantitation (LLOQ, blue dotted line). All serum and plasma samples were above the limit detection (LOD, red dotted line), with 63% of healthy serum/plasma samples above LLOQ.



The lower limit of quantitation (LLOQ) was confirmed by running multiple samples at different concentrations, each sample in quadruplicate. All samples above the LLOQ had a coefficient of variance (CV) well below 15%, indicating high precision within the dynamic range.

Assay performance: dilutional linearity and accuracy

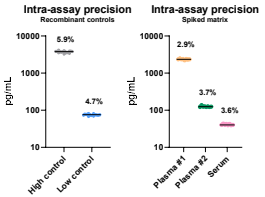




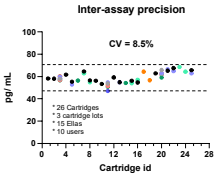
Top: dilutional linearity was assessed by serially diluting samples at a 2-fold dilution scheme. All serum, plasma, and CSF samples exhibited good parallelism properties with mean recovery rates within an acceptable range of 80-120%. The mean recovery rates were 97% for serum, 89% for plasma, and 86% for CSF.

Bottom, left: Spike/ Recovery experiments were carried out by spiking samples of various matrices (serum, plasma/EDTA, plasma/Heparin) with known concentrations of recombinant GFAP at high, medium, and low levels. 4 individual samples from healthy donors were used per sample type. All samples exhibited good recovery (80-120%) at all spiking levels, indicating good assay accuracy.

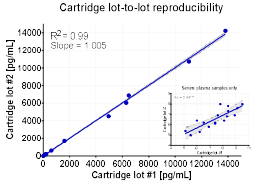
Assay performance: precision and reproducibility



Intra-assay precision was assessed by loading multiple replicates of the same sample on Ella cartridge. Both recombinant controls (left, n=16 per condition) and natural matrix controls (right, n=8 per condition) were assessed at different analyte levels. All controls yielded CV<10%, indicating high intra-assay precision.



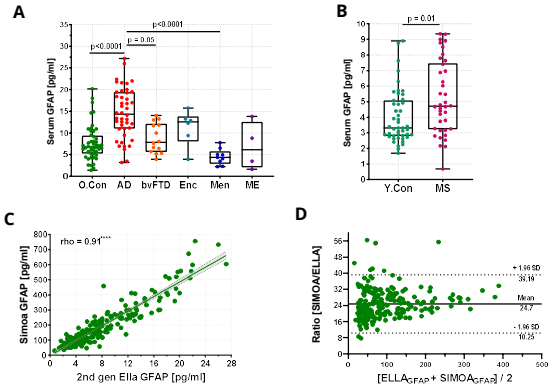
Inter-assay precision was assessed by running a serum matrix control across multiple cartridges (n=26), cartridge lots (n=3), Ella analyzers (n=15), and users (n=10, colored dots). The resulted coefficient of variance (8.5%) indicates high inter-assay precision.



Cartridge lot-to-lot reproducibility was assessed by running multiple CSF, Serum, plasma, and CCS samples at different GFAP levels across 2 independent cartridge lots. The high correlation and the slope (1.005) indicates highly equivalent and reproducible results regardless of cartridge lot used.

GFAP biomarker utility in a clinical patient cohort

Serum GFAP levels in neurodegeneration



(A) Serum GFAP levels in clinical diagnostic groups (AD, n=44; MS, n=38; bvFTD, n=14, Encephalitis, n=6; Meningitis, n=9; Meningoencephalitis, n=4) were compared to age-matched control cohort (O. Con) (n = 95). **(B)** Measurement of serum GFAP in MS patients compared to age-matched controls. **(C)** Linear correlation between the novel Ella GFAP assay and a bead-based assay (Simoa) in 210 samples yielded a correlation coefficient of r = 0.91 (95% CI: 0.88 - 0.93, P < 0.0001). **(D)** Bland-Altman analysis to assess the level of agreement between the novel GFAP assay and a bead-based assay (Simoa).

Boxes in panels A, B represent the median and interquartile range, with whiskers for minimum and maximum. Statistical analysis in panel A was performed using the Kruskal-Wallis test, followed by Dunn's post-hoc test. Comparison of MS patients vs controls in panel B was done using the Mann-Whitney U-test. *Abbreviations:* AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; Enc, Encephalitis; Men, Meningitis; ME, Meningoencephalitis; MS, multiple sclerosis; O. Con, Old control; Y. Con, Young control.

Conclusions

- We describe the validation of a novel microfluidic GFAP (2nd gen) assay on a microfluidic automated platform, enabling the detection of GFAP in serum, plasma, and CSF.
- The novel GFAP assay possesses high degree of precision (single digit %CV), robust performance, and good correlation with a predicate commercial assay.
- Elevated GFAP levels were found in sera of Alzheimer's disease and MS patients, further establishing the utility of GFAP as a blood-based, astrocytic biomarker for neurodegeneration.
- Taken together, these results support the applicability of a microfluidic GFAP immunoassay as a sensitive and easy-to-use benchtop strategy for measuring GFAP protein in biofluids.

References

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