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RESEARCH ARTICLE

Increasing the sensitivity of Simoa via bead count reduction facilitates the quantification of pTau-181 in dried plasma spots

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Abstract

Introduction: The exclusion of affected populations from Alzheimer's disease (AD) clinical research limits our understanding of disease heterogeneity and its impact on clinical care. While micro sampling with dried plasma spots (DPS) can promote inclusivity by enabling sample collection in remote areas, current techniques lack the sensitivity required for the quantification of phosphorylated tau at Thr181 (pTau-181) in DPS extracts.

Methods: We developed an assay for pTau-181 with reduced bead count and improved bead read efficiency (BRE) using a prototype Simoa instrument. This novel assay's performance was evaluated against standard pTau-181 assays on two Simoa platforms, and DPS extracts were tested for pTau-181 quantification feasibility.

Results: The novel assay quantifies pTau-181 at concentrations up to 16x lower than traditional pTau-181 assays on HD-X and SR-X platforms. DPS extracts tested with our low-bead assay were quantified considerably above the lower limit of quantification (LLOQ), indicating the suitability of this assay for future DPS extract measurements.

Discussion: Implementing DPS sampling and pTau-181 quantification could increase participation from underrepresented groups in AD research. However, additional assay optimization and an in-depth study of preanalytical sample stability are essential for the transition to clinical applicability.

KEYWORDS

Alzheimer's, biomarkers, dementia, dried blood spots, dried plasma spots, phosphorylated tau, pTau-181, scientific inclusion, Simoa, tau

1 | INTRODUCTION

The prospect of identifying blood-based biomarkers indicative of neurodegenerative dementias has for a long period seemed inconceivable. The recent epoch, however, has witnessed an evolution in technology, bolstering the sensitive quantification of proteins and propelling forward research efforts centered around identifying blood biomarkers

for neurological disorders such as Alzheimer's disease (AD). Highlighted biomarkers include tau protein with phosphorylated threonine occupying the 181st or 217th position in the amino acid sequence (pTau-181 and pTau-217, respectively), amyloid- β 42/40, and neurofilament light (NFL) which demonstrate conspicuous aberrations in both cerebrospinal fluid (CSF) and blood during the progression of AD pathology. These markers collectively present a compelling case for

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prospective clinical application.^{1–4} Of particular note, the early detection of pTau irregularities in blood precedes positivity in tau positron emission tomography (tau-PET) and incrementally increases as the disease advances.^{5,6} This paradigm furnishes vital information pertaining to early diagnosis, disease stage monitoring, and overseeing disease progression in clinical trials.

The emergence of blood biomarkers for neurodegenerative dementias offers a promising pathway for improved diagnosis and disease monitoring in developing countries. While advanced diagnostic technologies are prevalent and well-managed in developed nations, their adoption in economically challenged regions remains limited, thus curtailing access to sophisticated diagnostic instruments and comprehensive AD research.⁷ In AD research, inclusivity is essential, as demonstrated by differing genetic risk profiles between populations.⁸ For instance, ABCA7 has a stronger effect size in African Americans than the apolipoprotein E (APOE) ϵ 4 allele, the most prominent risk factor in Caucasians.^{9,10} These populations also exhibit distinct protein aberration patterns in AD, highlighting the need for inclusive research cohorts.¹¹ Recognizing the importance of making patient sample collection achievable even in remote and underprivileged environments, alternative blood sampling protocols should be a key consideration. Overcoming these hurdles requires cost-efficient, swift methodologies that circumvent the constraints of strict cold-chain logistics. Additionally, the implementation of in-home blood sample collection strategies could substantially enhance clinical outreach and trial participation rates. This transformation in protocols could yield extensive impacts on the inclusion of underrepresented demographics in research, fostering a more globally diverse and representative scientific study. Achieving these goals may be facilitated through sample collection techniques such as dried plasma spots (DPS) with plasma separation cards.¹² These cards, acting as micro sampling tools, allow for non-volumetric whole blood deposition, followed by cell fraction separation, yielding a 3 μ L plasma sample. The plasma, once collected on a disc, can be dried and stored. This approach has proven effective in quantifying NFL.¹³ However, current methods lack the necessary sensitivity for pTau biomarkers quantification from DPS extracts.

The Single Molecule Array (Simoa) technology has pioneered advancements in blood-based neurological clinical research.^{14–16} Various diagnostic assays for pTau-181 are provided by the Simoa platform and have undergone extensive comparison.^{17–19} In the majority of these assays, a fraction of plasma samples from controls often yield readings that fall below the Lower Limit of Quantification (LLOQ). Therefore, an assay with improved sensitivity could potentially enable the quantification of pTau-181 from DPS and improve the quantifiability of control samples. Recent findings suggest that assays with a reduced bead count, in conjunction with an optimized platform designed for efficient bead reading, could significantly bolster the analytical performance of several Simoa-based assays.²⁰ In this study, we designed an assay for pTau-181 with reduced bead count and with improved bead read efficiency (BRE) using a prototype Simoa instrument. The performance of the assay was then compared with Quanterix' pTau-181 assay (V2.1), carried out on both the fully automated HD-X platform and through manual sample preparation on

RESEARCH IN CONTEXT

- 1. Systematic review:** In a comprehensive survey of extant literature sourced primarily from traditional repositories like PubMed using specified keywords, the authors discerned a notable trend. A conspicuous absence was observed in the quantification of phosphorylated tau in either dried blood spots, or DPS.
- 2. Interpretation:** Based on the insights derived, we engineered an advanced Simoa assay with heightened sensitivity, which successfully enables the quantification of pTau-181 from dried plasma spot extracts. These findings provide compelling evidence that pTau-181 quantification might feasibly be accomplished using blood samples drawn onto plasma separation cards.
- 3. Future directions:** The subsequent phase of this research warrants assay refinement and rigorous exploration into the preanalytical stability of samples, prerequisites for its transition into clinical contexts. Such innovative micro sampling methodologies hold promise in revolutionizing the diagnosis and monitoring of Alzheimer's Disease, especially in geographically challenged regions.

the SR-X benchtop instrument. Additionally, we evaluated extracts obtained from DPS with the objective of ascertaining the viability of pTau-181 quantification via the newly developed assay.

2 | METHODS

2.1 | Low-bead assay development and analytical validation

The experiments were carried out in the laboratories of Quanterix Corporation (Billerica, MA, USA) using two prototype instruments, as previously detailed.²⁰ All assays employed an identical antibody pair and calibrator antigen. pTau-181 assay bead reagent stock was provided by Quanterix, and calibrator diluent reagent, control samples, and panels were also sourced from the same organization. Detector reagent, streptavidin- β -galactosidase (SBG) reagent, calibrators, and sample diluent reagent were obtained from pTau-181 V2.1 assay kits (Quanterix Corporation, Billerica, MA, USA). The number of assay beads per sample was set at 10,000 beads without helper beads. In order to extend the calibration curve at lower concentrations, the calibrators for the calibration curve were prepared by serially diluting the lowest calibrator (calibrator B) using calibrator diluent. The protocol for the assay is provided in [Supplementary material S1a](#). The LLOQ was established through interpolating triplicate measurements of serially diluted calibrator on the calibration curve over five runs across two instruments. The lowest concentration that yielded measurements

within the range of 80%–120% of the expected value, with a coefficient of variation (%CV) less than 20%, was defined as the analytical LLOQ. To account for the dilution factor used for plasma and serum samples, the functional LLOQ, which represents the analytical LLOQ multiplied by the dilution factor, was set at four times the analytical LLOQ for plasma and serum, in accordance with the pTau-181 (V2.1) assay kits. The limit of detection (LOD) was determined by interpolating the background signal with 2.5 times the standard deviation from the mean background on each calibration curve over five runs across two instruments. To determine the precision, one endogenous serum panel, one endogenous EDTA plasma panel, one spiked EDTA plasma panel, one CSF panel, and two controls based on the calibrator were measured in triplicate over five runs across two different instruments. Serum samples were prepared using dedicated serum tubes and plasma samples were prepared with EDTA-treated tubes. To assess the linearity of the sample readings in plasma and serum, admixtures with 12 different ratios were prepared using a low concentration sample and a spiked sample with a high concentration of pTau-181.

2.2 | pTau-181 assay comparison in endogenous samples from cognitively unimpaired individuals

The efficacy of three distinct immunoassays for the quantification of pTau-181 in endogenous plasma and serum samples were compared. Matched plasma and serum samples ($n = 20$) were procured from a cohort of cognitively unimpaired individuals obtained from BioIVT (Westbury NY, USA). The low-bead assay, was compared with two commercially available pTau-181 (V2.1) assays from Quanterix (Billerica, MA, USA). One of the assays was operated on the fully automated HD-X platform while the other employed the SR-X instrument with manual sample preparation. The procedural guidelines provided by the supplier were strictly adhered to while deploying the pTau-181 (V2.1) assay on both the HD-X and the SR-X platforms. Preparatory to the analytical phase, plasma and serum samples underwent brief thawing, followed by vortex mixing and subsequent centrifugation at $8000 \times g$ for a period of 6 minutes. Samples were then diluted 4 \times using sample diluent. A total of 20 plasma and 20 serum samples were analyzed in triplicate with each respective assay.

2.3 | pTau-181 assay comparison in patient samples

To assess the correlation between platforms within patient samples, this study incorporated matched plasma and serum samples obtained from the Montpellier Memory Resources Center (CMRR). Participants included pathology-confirmed AD ($n = 10$), and a control group composed of cognitively unimpaired participants or participants with subjective cognitive decline ($n = 10$). Cognitive performance of the participants was evaluated with the Mini-Mental State Examination (MMSE). Patients' CSF was assayed for A β 42 or A β 42-40, phosphory-

lated tau at Thr181 (p-tau181), and total tau (t-tau). The demographic characteristics are displayed in Table 1.

2.4 | DPS preparation, extraction, and analysis

To ascertain the feasibility of pTau-181 detection from DPS, we employed whole blood specimens ($n = 10$) obtained from the CMRR, Montpellier, France. An amount of 80 μ L was deposited onto a Telimmune (formerly Noviplex) DUO Plasma Separation Card. After a duration of 3 minutes, the upper layer of the card was separated, followed by an air-drying process for a span of 15 minutes. For the purpose of ensuring analytical replication and facilitating inter-platform comparison, six distinct cards were prepared per each sample. Subsequently, the discs were gathered and placed within 1.5 mL Eppendorf tubes, which were then preserved at a temperature of -80°C . For the extraction procedure, the discs were first brought to room temperature by thawing for 30 minutes, succeeded by the addition of 700 μ L sample diluent and incubation at 37°C , 1000 rpm, for a period of 1 hour. The processed samples were subsequently harvested and maintained at -80°C . The extracts were analyzed neat in triplicate with both the low-bead assay and the pTau-181 assay on the HD-X platform. Hence, the functional LLOQ for DPS extracts is equal to the analytical LLOQ of the assay. For correlation purposes, the matching plasma samples were analyzed on the HD-X platform.

2.5 | Data analysis

The method validation was conducted in adherence to the acceptance criteria delineated in the section dedicated to method development and analytical validation. A suite of graphical representations such as precision plots, scatterplots, and raincloud plots were constructed employing R programming language (version 4.1.1). The production of precision plots was facilitated by the incorporation of the ggplot2 package (version 3.4.1), along with the dplyr package for data filtration and the gridExtra package for the organization of grids. The construction of scatterplots, solely employed the ggplot2 package. Raincloud plots were produced using the ggdist, tidyquant, and tidyverse packages. For assay comparison, the degree of correlation between measurements was evaluated through the Spearman's rank correlation. For any pTau-181 measurements that were found to fall below LLOQ, an interpolated concentration was employed.

3 | RESULTS

3.1 | Analytical performance of the low-bead assay for pTau-181

The low-bead assays analytical LLOQ was established at 0.13 pg/mL, with a coefficient of variation (CV) of 19% and an average recovery

TABLE 1 Demographic characteristics of patient samples.

| Demographic characteristics | AD | Controls | p-Value |
|-----------------------------|------------------------|-------------------|---------|
| N | 10 | 10 | |
| Age at CSF (years) | 72 [67–77] | 66 [61–77] | 0.55 |
| Sex (male/not male) | 2/8 | 5/5 | 0.35 |
| A+/A– | 10/0 | 0/10 | <0.001 |
| CSF t-tau (pg/mL) | 853.5 [683–1007.25] | 313 [267–390.25] | <0.001 |
| CSF p-tau181 (pg/mL) | 143.5 [103.75–150.75] | 34.5 [25.25–41.5] | <0.001 |
| MMSE | 18 [16.5–22.75] | 28 [26.25–28.75] | <0.001 |

Note: demographic and pathological variants are presented using median and interquartile range values. To assess the differences across the two groups for continuous variables, a Mann-Whitney *U* test was performed. For the analysis of categorical variables, which in this context include sex and amyloid positivity, the chi-squared goodness of fit test was employed. The *p*-values resulting from these statistical analyses are provided in the accompanying table for comprehensive reference and interpretation.

Abbreviations: CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination.

rate of 93%, yielding a functional LLOQ of 0.52 pg/mL for plasma and serum samples (Supplementary material S1b). Precision assessments were carried out utilizing a control sample and three distinct panels, barring the spiked plasma panel and an additional control as these measurements fell outside the established calibration range. The calculated average within-run precision was recorded at 5.7%, and the between-run precision at 11.2% (Table S1c). Cross-validation was executed on two separate instruments, delivering a between-instrument precision of 5.1%. The dilution linearity for both serum and plasma specimens was ascertained via the creation of admixture preparations, yielding an average bias of 16% and 13% for serum and plasma, respectively (Supplementary material S1d). The characteristics of the designed assay and the characteristics of the pTau-181 (V2.1) assays, as furnished by the supplier are displayed in Table 2.

3.2 | Quantifiability of pTau-181 in endogenous samples of cognitively unimpaired individuals with three different Simoa platforms

The pTau-181 (V2.1) assay, when executed on the SR-X platform, quantified 15 out of 20 serum samples (75%) and 3 out of 20 plasma samples (15%) beneath the functional LLOQ. Furthermore, 55% of serum samples ($n = 11$) and 25% of plasma samples ($n = 5$) were noted with a %CV exceeding 20%. When performed on the HD-X platform, the same assay registered 60% of serum samples ($n = 12$) and 5% of plasma samples ($n = 1$) below the LLOQ, whereas a %CV greater than 20% was observed in 25% of the serum samples ($n = 5$) and 5% of the plasma samples ($n = 1$). Notably, the low-bead assay successfully measured all serum and plasma samples above the LLOQ. The serum samples were quantified with a %CV under 20% and 10% of the plasma samples ($n = 2$) exhibited a %CV above 20%. The data are represented in Supplementary Material S1e and is graphically represented in precision plots in Figure 1.

3.3 | Correlation between assays

A strong correlation was observed across the pTau-181 concentrations in cognitively unimpaired individuals, as derived from three disparate assays, as evidenced by the Spearman's rank correlation coefficient (Figure 2). The pTau-181 (V2.1) assay executed on the HD-X and SR-X instruments showed a strong correlation, with Spearman's rho values of 0.79 in serum, 0.93 in plasma, and a composite correlation of 0.92. Similarly, a high degree of correlation was seen with the low-bead assay and the pTau-181 (V2.1) assay on the SR-X, yielding a Spearman's rho of 0.79 in serum, 0.80 in plasma, and an overall correlation of 0.92. The low-bead assay also showed a strong correlation with the pTau-181 (V2.1) assay on the HD-X instrument, as indicated by a Spearman's rho of 0.87 in serum, 0.77 in plasma, and a composite correlation of 0.90.

3.4 | Comparison of bead read efficiency

In the examination of BRE across the three assays, our findings were based on a consistent quantity of 200,000 assay beads for evaluations performed using HD-X and SR-X platforms and 10,000 assay beads for the low-bead assay. The efficiency, calculated as the proportion of successfully analyzed beads, varied between 2.2% and 6.9% on the HD-X platform, breaking down to 4.6%–6.9% in calibrators, 5%–6.5% in serum samples, and 2.2%–6.4% in plasma samples. The SR-X platform demonstrated a slightly wider range of BRE, from 0.7% to 6.5%, consisting of 3.3%–6.5% in calibrators, 1.9%–5.3% in serum samples, and 0.7%–5.5% in plasma samples. For the low-bead assay, we observed a greater yet notably more variable efficiency in terms of the fraction of assay beads successfully analyzed, fluctuating between 4.2% and 42.8%. This discrepancy was further characterized as 13.0%–42.8% in calibrators, 15.5%–40.8% in serum, and 4.2%–33.7% in plasma samples. The bifurcation per sample type is displayed in Figure 3.

TABLE 2 Analytical characteristics of pTau-181 low-bead assay and pTau-181 (V2.1) commercial assays of Quanterix Corporation.

| Parameter | pTau-181 (V2.1) SR-X | pTau-181 (V2.1) HD-X | Low-bead assay prototype |
|---|---------------------------|-------------------------------------|---|
| Platform | SR-X | HD-X | Prototype instrument |
| Status | Commercial | Commercial | Prototype |
| Handling | Manual | Fully automated | Manual |
| Capture antibody | Kitted | Kitted | Assay beads without helper beads were provided by Quanterix Corporation |
| Detector antibody | Kitted | Kitted | Kitted |
| Calibrator | Kitted | Kitted | Kitted |
| Calibrator range pg/mL | 2.07–423 | 2.07–423 | 0.26–50 |
| Assay type | 2-step | 2-step | 2-step |
| No. of assay beads (Quanterix, pTau-181 V2.1 assay beads) | 200,000 | 200,000 | 10,000 |
| No. of helper beads | 300,000 | 300,000 | 0 |
| Incubation time (bead-detector incubation – SBG incubation), min | 20–10 | 35–5 (47–7 cadences) | 240–10 |
| Incubation temperature, °C | 30 (800 rpm) | 30 onboard | 30 (800 rpm) |
| Calibrator volume, μ L | 100 | 100 | 100 |
| Sample volume (serum/plasma – sample diluent), μ L | 25–75 | 25–75 | 25–75 Or 16.7–83.3 |
| Bead volume, μ L | 25 | 25 | 25 |
| Detector volume, μ L | 20 | 20 | 20 |
| Detector concentration | Kitted | kitted | kitted |
| Washing protocol | 2-step washing proocol | Onboard (2-step washing proocol) | Dedicated washing protocol for low-bead assays (2-step) |
| SBG volume, μ L | 100 | 100 | 100 |
| SBG concentration, pM | Kitted | kitted | kitted |
| RGP volume, μ L | 25 | 50 | 25 |
| Analytical LLOQ, pg/mL | 2.23 | 2 | 0.13 |
| Functional LLOQ in plasma and serum, pg/mL | 8.9 | 8 | 0.52 |
| Functional LOD pg/mL | 4.16 | 2.48 | 0.28 |
| Curve fitting | 4PL | 4PL | Cubic |
| Weighting | $1/y^2$ | $1/y^2$ | $1/y$ |

Abbreviations: LOD, limit of detection; LLOQ, lower limit of quantification; RGP, resorufin β -D-galactopyranoside.

3.5 | Comparison in patient samples

We conducted a comparative analysis with patient samples between the novel assay on the prototype platform and the established pTau-181 (V2.1) assay utilizing the automated HD-X platform. Both assays demonstrate a high degree of correlation in patient-derived samples of plasma and serum (Figure 4). This was substantiated by Spearman's rank correlation coefficients, which yielded values of $\rho = 0.93$ and $\rho = 0.94$ in plasma and serum, respectively, attesting to the robust-

ness and reliability of the novel assay in comparison to the pTau-181 standard.

3.6 | pTau-181 quantification in DPS

DPS extracts were subjected to analysis using both the low-bead assay and the HD-X platform. The measurements obtained on the HD-X platform were consistently above LLOQ, with the majority of samples

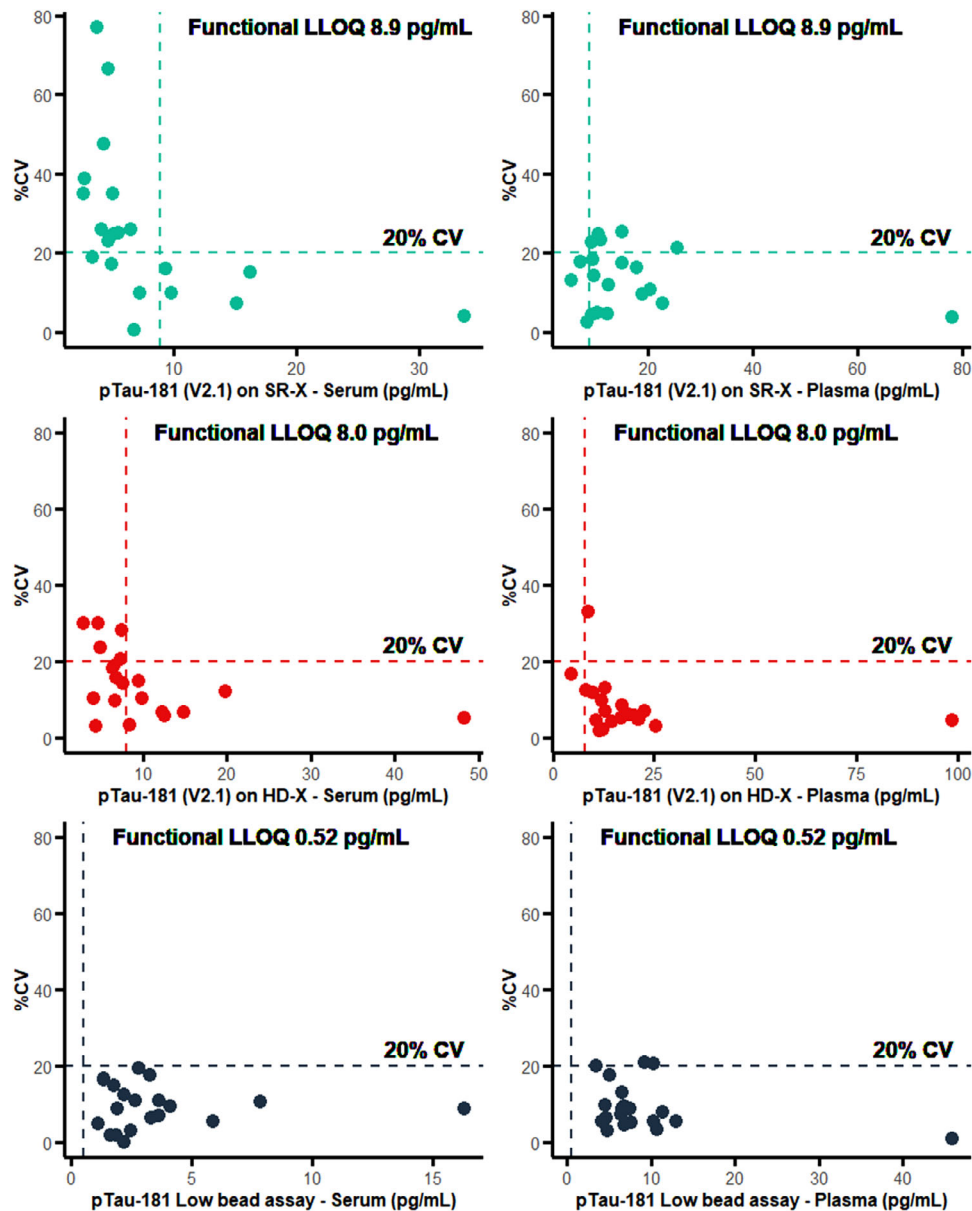


FIGURE 1 Precision plots for the low-bead assay (illustrated in blue) and the pTau-181 (V2.1) assay on both the manual SR-X platform (represented in turquoise) and the fully automated HD-X platform (depicted in orange). Each pTau-181 concentration was measured in triplicate and set against the %CV on the graph. A horizontal dashed line signifies the 20% CV, whereas the lower limit of quantification (LLOQ) for each respective assay is indicated by a vertical dashed line. %CV, coefficient of variation.

recording slightly higher values than the analytical LLOQ of 2.0 pg/mL. All measurements acquired with the low-bead assay considerably surpassed the analytical LLOQ. When comparing DPS readings on the HD-X platform with corresponding plasma measurements, a strong correlation was observed, as indicated by a Spearman's rho of 0.77. Conversely, the measurements obtained with the low-bead assay exhibited a slightly lower but commendable correlation with the plasma readings, with a Spearman's rho of 0.61. Despite the fact that the DPS extracts demonstrate readings considerably surpassing the LLOQ when using the low-bead assay, it was observed that 40% of the samples ($n = 4$) exhibited notable variability in AEB, characterized by a %CV exceeding 20%. On the HD-X platform 30% ($n = 3$) of sample

readings surpassed a %CV of 20%. The correlations are displayed in scatterplots in Figure 5.

4 | DISCUSSION

In this study, we performed a comparative analysis of three assays, each one executed on a separate Simoa platform. Despite employing the identical antibody pair and equivalent reagents, these assays varied in terms of the quantity of assay beads deployed and the Simoa platform used. The "low-bead assay" employed merely 5% of the assay beads relative to traditional pTau-181 assays and did not incorporate helper

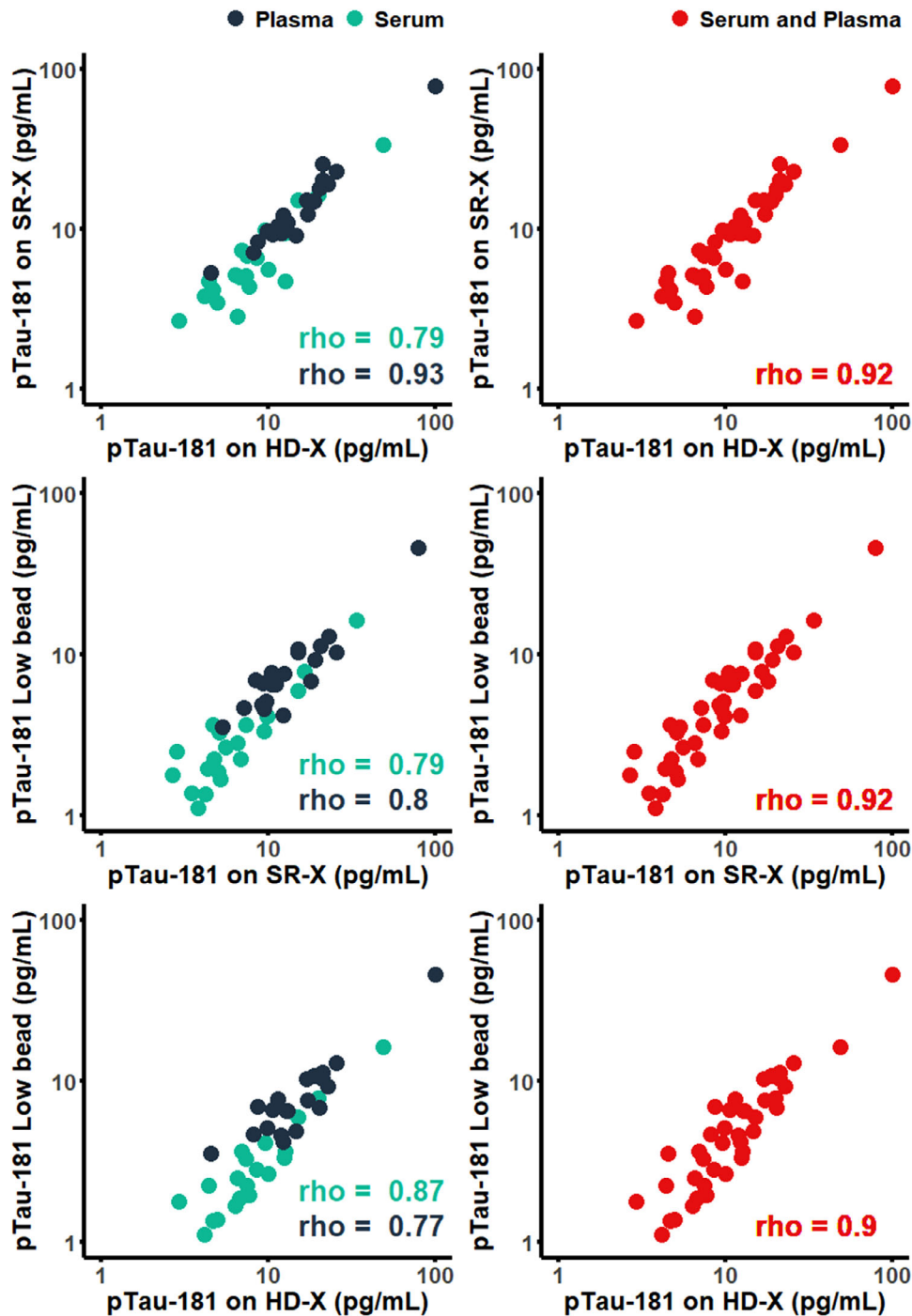


FIGURE 2 Scatterplots representing the outcomes of three distinct assays, distinguished by color based on the sample type: serum (turquoise), plasma (blue), or combined (orange). The data points are presented using logarithmic scales for both the x- and y-axes. The correlation coefficients for these relationships are derived using Spearman's rank correlation method.

beads. The signal in AEB in Simoa is calculated via $-\ln(1-f(\text{on}))$, where $f(\text{on})$ denotes the proportion of beads presenting with one or more immunocomplex. By decreasing the bead count, each "on" bead exerts a greater influence on the AEB, as it constitutes a larger fraction of the overall assay beads. Consequently, this research paradigm generates linearity at lower concentrations, thereby demonstrating enhanced sensitivity in comparison to conventional Simoa assays. Additionally,

loading a greater fraction of the assay beads into the array reduces the Poisson noise, given its proportional relationship with $\sqrt{n/n}$, wherein n represents the quantity of "on" beads. Notwithstanding, the reduction in assay bead count could theoretically introduce an increase in Poisson noise as per the aforementioned formula, potentially impacting the assays precision should the bead count decrease further. However, our results demonstrate the pTau-181 assay, conducted with 10,000 assay

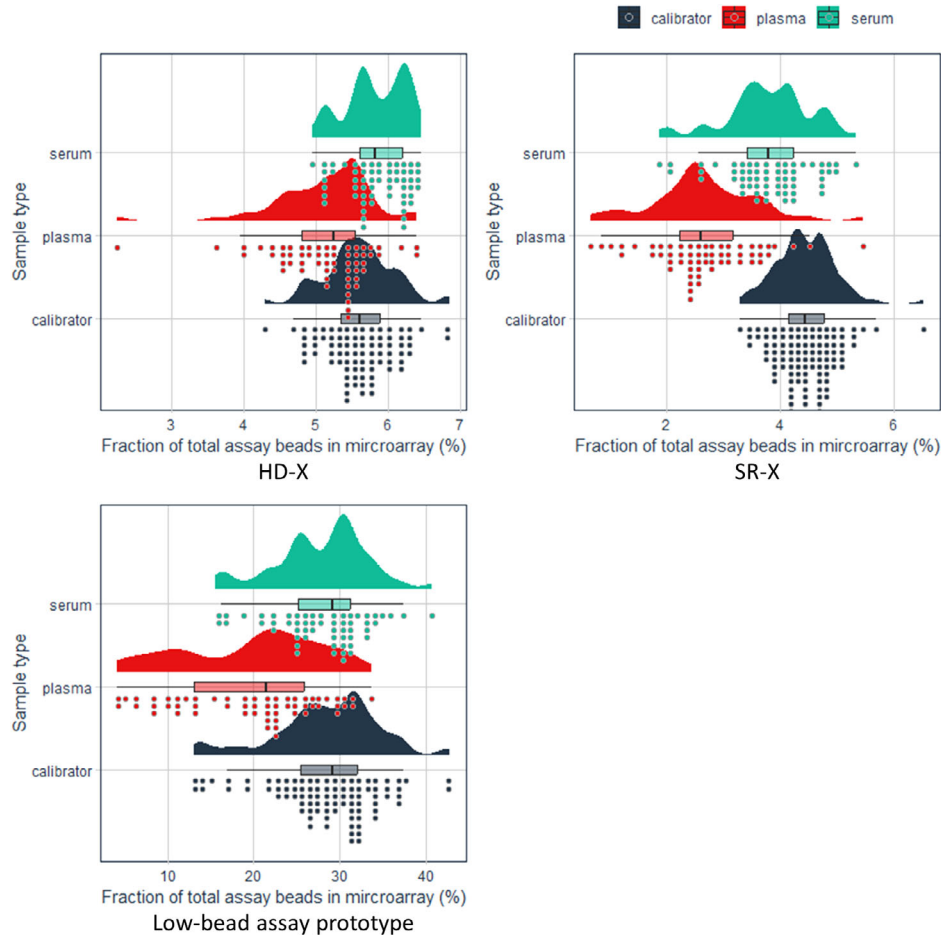


FIGURE 3 BRE across varying samples – including serum, plasma, and calibrators, represented via raincloud plots. This efficiency measure is determined upon a predefined quantity of 200,000 assay beads for tests executed on HD-X and SR-X platforms, in contrast to a smaller quantity of 10,000 assay beads in the low-bead assay. Each individual data point corresponds to a pTau-181 reading from a singular sample. The box plot displays the median, interquartile range, and 1.5 times interquartile range using a vertical line, a box, and whiskers, respectively. The violin plot conveys the distribution of the data by depicting the proportion of data located within a specific shaded area. BRE, bead read efficiency.

beads, exhibits a high degree of precision as indicated by the %CVs. Notably, the variance in plasma samples was higher than in serum samples in the low-bead assay. This discrepancy could potentially be ascribed to decreased bead loading efficiency in plasma samples in the low-bead assay. In the context of the low-bead assay, bead loading in plasma samples follows a multimodal distribution, as opposed to the unimodal distribution observed in serum and calibrators.

The accuracy of the assay remains unaffected by variations in BRE, as it leverages the bead fraction to compute the AEB. However, in low-bead assays conducted on samples with a diminished fraction of “on” beads, the precision may potentially be compromised due to a surge in Poisson noise. Therefore, it is advised that any analysis of plasma samples should involve no less than 10,000 assay beads, in order to prevent potential precision compromises. Despite the use of identical antibody pairs, it was observed that the readings obtained from plasma and serum samples with the low-bead assay were on average two times lower in comparison to the readings obtained with the HD-X and 1.8 times lower compared to the readings obtained with the SR-X. Former studies with low bead Simoa assays yielded corroborative findings with lower concentrations than with conventional Simoa approaches,

aligning with outcomes derived from our low-bead approach.²⁰ However, all three assays are highly correlated, as demonstrated with the Spearman’s rank correlation. It is noteworthy that the low-bead assay successfully quantified pTau-181 in every single endogenous serum and plasma sample, while readings of some samples from other assays fell below the LLOQ.

While all DPS extracts were assessed above LLOQ on the HD-X instrument, a detailed analysis reveals the measurements predominantly hovered around the LLOQ. It is significant to recognize that the DPS extraction procedure has yet to be fully optimized, and this points towards the potential for enhanced quantifiability of DPS extracts on the HD-X instrument through further refinement. Strikingly, all DPS extracts evaluated through the low-bead assay were quantified considerably above the LLOQ, thus underlining the suitability of this assay for future DPS extract measurements. Despite these promising results, the high variability observed in the measurements calls for additional refinement of both the assay and the DPS extraction process. Moreover, as elaborated in the limitations subsection, comprehensive investigation of preanalytical sample stability is imperative to facilitate the transition to clinical applicability.

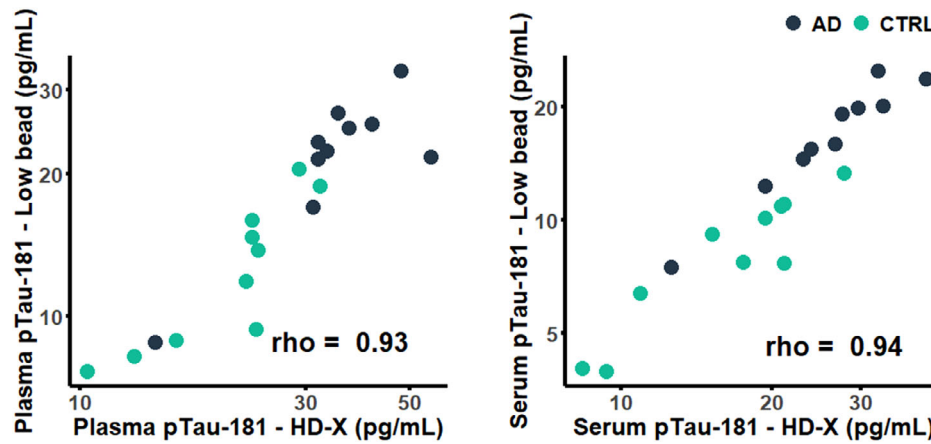


FIGURE 4 Scatterplots representing the outcomes of two distinct assays and two different patient groups, distinguished by color based on the patient group: controls (turquoise) or AD (blue). The correlation coefficients for these relationships are derived using Spearman's rank correlation method.

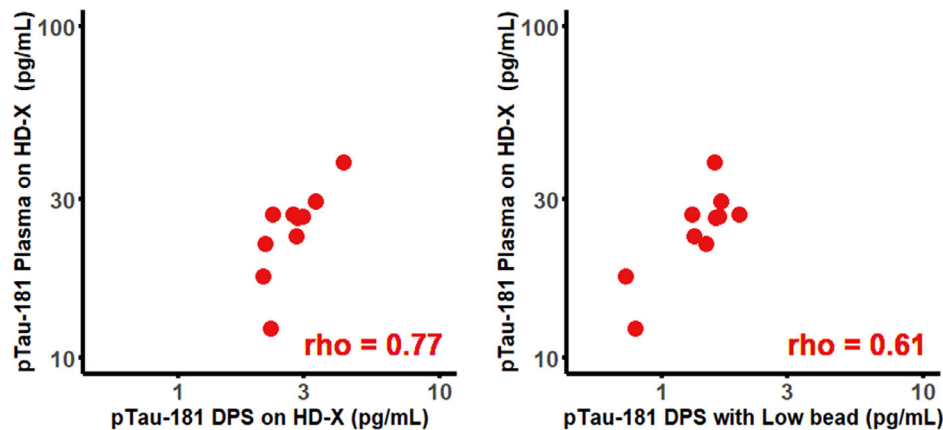


FIGURE 5 Scatterplots representing the outcomes of three distinct measurements; the measurement of pTau-181 in DPS extracts with the pTau-181 (V2.1) assay on the automated HD-X platform, measurement of these same DPS extracts with the novel low-bead assay, and measurements of pTau-181 in matched plasma samples on the HD-X platform. The data points are presented using logarithmic scales for both the x- and y-axes. The correlation coefficients for these relationships are derived using Spearman's rank correlation method. DPS, dried plasma spot.

Further optimization and implementation of sample collection via DPS and quantification with the novel assay for pTau-181 could have far-reaching effects on the participation of underrepresented populations in research studies, thereby promoting inclusive and globally representative AD research. The persistent exclusion of disproportionately impacted groups in clinical research on AD restricts our comprehension of disease heterogeneity, inherent biological mechanisms, and the effects on clinical care and outcomes. This persistent gap emphasizes the importance of inclusivity in clinical research to achieve therapeutic strategies that are broadly applicable and effective for diverse populations.

4.1 | Limitations

The limitations of this exploratory study are the low number of samples and not determining the preanalytical stability of pTau-181 in DPS. Our study primarily focused on examining the practicality of quantifying

pTau-181 using DPS. However, the broader clinical application of this method is significantly influenced by the stability of the preanalytical sample stability. In previous studies, pTau-181 demonstrated considerable stability in solution, maintaining its properties for at least 24 h as reported by two distinct research studies.^{21,22} However, for sample collection via DPS in remote locations, it is essential to evaluate the stability of the dried sample on disc under room temperature conditions, eliminating the need for cold-chain transport.

5 | CONCLUSIONS

The newly developed method introduces a promising avenue for bolstering inclusivity in clinical AD research, with the objective of developing therapeutic strategies that are universally applicable and efficacious for a heterogeneous patient population. To advance this method toward clinical integration, refinement of the assay and comprehensive evaluation of preanalytical sample stability remain imperative.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the [supporting information](#).

CONSENT STATEMENT

All study activities were approved according to regional Ethics Committee of the Montpellier University Hospital and Montpellier CSF-Neurobank #DC-2008-417 at the certified ISO 20387 CHU resource center BB-0033-00031. All human subjects provided written informed consent.

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REFERENCES

- Teunissen CE, Verberk IMW, Thijssen EH, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *Lancet Neurol*. 2022;21:66-77.
- Verberk IMW, Thijssen E, Koelewijn J, et al. Combination of plasma amyloid beta(1-42/1-40) and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. *Alzheimers Res Ther*. 2020;12:118.
- Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau-PET and amyloid-PET. *Alzheimers Dement*. 2018;14:989-997.
- Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry*. 2019;90:870-881.
- Brier MR, Gordon B, Friedrichsen K, et al. Tau and A β imaging, CSF measures, and cognition in Alzheimer's disease. *Sci Transl Med*. 2016;8:338ra66.
- Guo Y, Huang YY, Shen XN, et al. Characterization of Alzheimer's tau biomarker discordance using plasma, CSF, and PET. *Alzheimer's Res Ther*. 2021;13:93.
- Chávez-Fumagalli MA, Shrivastava P, Aguilar-Pineda JA, et al. Diagnosis of Alzheimer's disease in developed and developing countries: systematic review and meta-analysis of diagnostic test accuracy. *J Alzheimers Dis Rep*. 5:15-30.
- Logue MW, Schu M, Vardarajan BN, et al. A comprehensive genetic association study of Alzheimer disease in African Americans. *AMA Arch Neurol*. 2011;68:1569-1579.
- Reitz C, Jun G, Naj A, et al. Variants in the ATP-Binding Cassette Transporter (ABCA7), Apolipoprotein E ϵ 4, and the risk of late-onset Alzheimer disease in African Americans. *JAMA*. 2013;309:1483-1492.

- Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *JAMA*. 1997;278:1349-1356.
- Modeste ES, Ping L, Watson CM, et al. Quantitative proteomics of cerebrospinal fluid from African Americans and Caucasians reveals shared and divergent changes in Alzheimer's disease. *Molecular Neurodegeneration*. 2023;18:48.
- Johnson A, Braga C, de Magalhães Padilha P, Adamec, J. Collection of plasma samples in areas with limited healthcare access. In: Greening DW, Simpson RJ, eds. *Serum/Plasma Proteomics: Methods and Protocols*. Springer; 2023:19-32. doi:10.1007/978-1-0716-2978-9_2
- Lombardi V, Carassiti D, Giovannoni G, Lu CH, Adiatori R, Malaspina A. The potential of neurofilaments analysis using dry-blood and plasma spots. *Sci Rep*. 2020;10:97.
- Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med*. 2016;54:1655-1661.
- Rojas JC, Karydas A, Bang J, et al. Plasma neurofilament light chain predicts progression in progressive supranuclear palsy. *Ann Clin Transl Neurol*. 2016;3:216-225.
- Rissin DM, Kan CW, Campbell TG, et al. Single-Molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol*. 2010;28:595-599.
- Bayoumy S, Verberk IMW, Den Dulk B, et al. Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231. *Alzheimer's Res Ther*. 2021;13:198.
- Janelidze S, Bali D, Ashton NJ, et al. Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. *Brain*. 2022;awac333 doi:10.1093/brain/awac333
- Ashton NJ, Puig-Pijoan A, Milà-Alomà M, et al. Plasma and CSF biomarkers in a memory clinic: head-to-head comparison of phosphorylated tau immunoassays. *Alzheimers Dement*. 2022. doi:10.1002/alz.12841
- Kan, C. W., Tobos CI, Rissin DM, et al. Digital enzyme-linked immunosorbent assays with sub-attomolar detection limits based on low numbers of capture beads combined with high efficiency bead analysis. *Lab Chip*. 2020;20, 2122-2135.
- Verberk IMW, Misdorp EO, Koelewijn J, et al. Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease-related blood-based biomarkers: results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. *Alzheimers Dement*. 2022;18, 1484-1497.
- Sunde AL, Alsnes IV, Aarsland D, et al. Preanalytical stability of plasma biomarkers for Alzheimer's disease pathology. *Alzheimers Dement*. 2023;15:e12439.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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