Letter to the Editor

Burak Arslan*, Johan Gobom, Ulf Andreasson, Fernando Gonzalez-Ortiz, Nicholas J. Ashton, Henrik Zetterberg and Hlin Kvartsberg

Comparative analysis of plasma p-tau217 immunoassays: challenges for standardization and harmonization

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To the Editor,

The definitive diagnosis of Alzheimer's disease (AD) still relies on postmortem neuropathological examination, which reveals its two hallmark features: extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau (p-tau) [1]. In living persons, the determination of suspected AD is largely still based on a clinical evaluation despite several methods enabling the detection of AD pathology *in vivo*, including positron emission tomography (PET) and cerebrospinal fluid (CSF) biomarkers. More recently, blood-based biomarkers such as tau phosphorylated at threonine 217 (p-tau217) [2, 3] are set to drastically transform clinical practice by enabling the integration of clinical and biological assessments across far larger populations. While PET and CSF biomarkers remain the current reference standards for supporting the clinical diagnosis of AD *in vivo*, blood-based assays – most notably plasma p-tau217 – have also demonstrated high diagnostic accuracy in detecting AD pathology [2]. The recent FDA approval of Fujirebio's plasma p-tau217/Aβ42 ratio marks the first blood biomarker for AD approved for the early detection of brain amyloid pathology in symptomatic adult patients aged 55 years and older [4], underscoring its potential for widespread clinical use in clinical laboratories. Compared with PET and CSF testing, plasma biomarkers are also easier to implement on fully automated platforms, minimally invasive, enable repeated testing, cost-effective, and more accessible for routine practice [3]. Furthermore, with the advent of first disease-modifying therapies of AD such as lecanemab and donanemab, with more effective and practical solutions on the way, blood-based biomarkers are likely to play a crucial role not only in supporting clinical diagnosis but also in monitoring treatment efficacy [5, 6]. Nevertheless, important challenges – such as the lack of certified reference materials (CRM) and the need for assay harmonization – must still be addressed before plasma p-tau217 can be broadly implemented in clinical practice.

***Corresponding author: Burak Arslan**, MD, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal Hospital, Hus V3, 43180 Mölndal, Sweden, E-mail: burak.arslan@gu.se
Johan Gobom, Ulf Andreasson and Hlin Kvartsberg, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; and Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. https://orcid.org/0000-0001-6193-6193 (J. Gobom)
Fernando Gonzalez-Ortiz, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; and Neurocode USA Inc. Bellingham, WA, USA
Nicholas J. Ashton, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; Banner Alzheimer's Institute and University of Arizona, Phoenix, AZ, USA; and Banner Sun Health Research Institute, Sun City, AZ, USA
Henrik Zetterberg, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA; Wisconsin Alzheimer's Institute, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA; Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK; UK Dementia Research Institute, University College London, London, UK; Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China; and Centre for Brain Research, Indian Institute of Science, Bangalore, India

Despite several head-to-head studies [7, 8] and a recent Round Robin study [9] on plasma p-tau species that evaluated their diagnostic performance (e.g., area under the curve (AUC), positive predictive value (PPV), negative predictive value (NPV), and fold-changes using amyloid-negative individuals as a reference), direct analytical comparisons between commercial and in-house p-tau217 assays remain limited. Moreover, most of the assay comparisons available rely on well-defined research cohorts, which may influence results by having clearly positive and negative groups which do not fully reflect the high heterogeneity observed in clinical practice. Consequently, the use of unselected clinical samples processed by diagnostic laboratories using different immunoassay-based platforms could provide important insights into platform and assay-dependent variability. To address this, we conducted a direct analytical comparison of three available plasma p-tau217 immunoassays (i.e., Lumipulse G (Fujirebio Diagnostics, Japan), MSD S-Plex (Meso Scale Discovery, Rockville, MD, United States), and in-house Simoa assay (UGOT, Sweden) developed at the University of Gothenburg) and platforms in our laboratory, with the aim of evaluating both their analytical performance and the degree of agreement between methods.

For this method comparison, anonymized, consecutively collected leftover plasma samples (n=122) were used. Plasma samples were derived from leftover clinical specimens collected in VACUETTE® K_2EDTA tubes (6 mL; REF:456243, LOT:A24023Q7) and centrifuged at 2,000×*g* for 10 min according to our laboratory's standard operating procedure (SOP) for plasma separation. Plasma was then transferred to secondary polypropylene (PP) tubes (Sarstedt, 2 mL; REF 72.694.007). For other required clinical tests, the necessary volumes (0.5 mL) were aliquoted from the secondary PP tubes into smaller PP tubes (Sarstedt, 0.5 mL; REF 72.730.003). The remaining plasma in the secondary tubes was retained, kept at room temperature for no longer than 30 min, and then immediately stored at −80 °C until analysis. On the day of measurement, study and quality control (QC) samples were thawed at room temperature (30 min), vortexed, and centrifuged according to the respective assay manuals and the protocol for the in-house developed assay. For the Lumipulse plasma p-tau217 assay, unpublished data (Arslan et al.) from our laboratory confirmed that storage at −20 °C for up to three days, long-term storage at −80 °C, and up to three freeze–thaw cycles did not affect analyte stability. Therefore, we considered that one freeze-thaw cycle was unlikely to have a significant impact on analyte concentration. For the plate-based p-tau217 immunoassays (in-house Simoa and MSD S-Plex), a freshly prepared calibration curve was used for each plate. Duplicate measurements of the same sample were performed within the same plate. For the Lumipulse G immunoassay, a single calibration curve was used for all measurements. Duplicate measurements were averaged before method comparison analyses.

Centrifugation protocols varied across assay platforms and were applied as follows:

- Lumipulse p-tau217 assay: 2,000×*g* for 5 min
- MSD S-Plex p-tau217 assay: 2,000×*g* for 3 min
- In-house p-tau217 assay: 4,000×*g* for 10 min

An overview of assay characteristics and analytical platforms is presented in Supplementary Table 1, with additional details provided in the Supplementary Methods. A detailed description of the statistical analyses is provided in the Supplementary Methods.

In brief, the MSD S-Plex assay showed moderate correlations with both the Lumipulse G (ρ=0.45) and the in-house UGOT Simoa (ρ=0.42) assays, while a strong correlation was observed between Lumipulse G and UGOT Simoa (ρ=0.73). Absolute concentration differed across all three assays. MSD S-Plex exhibited both fixed and proportional bias compared with the other two, whereas only proportional bias was observed between Lumipulse and UGOT Simoa. Full results are presented in Table 1, Supplementary Results, and Supplementary Figures 1, 2, 3.

Next, to assess analytical variation, aliquoted plasma samples from 100 individuals – covering the range of p-tau217 – were analyzed on two occasions. Lumipulse G replicates were measured one week apart, while UGOT Simoa replicates were measured on consecutive days. Although statistically significant differences between runs were observed ($p<0.001$), correlations remained strong for both Lumipulse ($\rho=0.98$) and UGOT Simoa ($\rho=0.95$). Intra-assay precision was also evaluated, with mean CV% values as follows: Lumipulse=6.42 %, UGOT Simoa=7.06 %, and MSD S-Plex=8.75 %. These sample-level duplicate CV% values do not represent formal precision experiments as outlined in CLSI EP15-A3 (e.g., 5 × 5 design: at least two concentration pools, one run per day, five replicates per run, for five days, totaling 25 replicates per sample). Instead, they were intended to illustrate the overall distribution of sample-level CV% for plasma p-tau217 across each assay. This approach highlights that variable CV% may influence the classification thresholds in the dual cut-point model for plasma p-tau217 (low, intermediate, and high probability of brain amyloid pathology), where high imprecision could alter risk classification and potentially lead to false-negative or false-positive results in longitudinal follow-up. Before clinical implementation of any candidate plasma p-tau217 assay, full analytical validation – including formal precision experiments – should be performed.

Table 1: Overview of method comparison results.

Comparison	Passing-Bablok regression equation	Systematic bias (intercept, 95 % CI)	Proportional bias (slope, 95 % CI)	Spearman correlation (p-value)	Linear model validity (Cusum test)	Bland-Altman bias (% difference, 95 % CI)	Bland-Altman limits of agreement (LoA)	Interpretation
Lumipulse vs. in-house Simoa	y=−0.0303 + 0.0641x	−0.0303 (−0.0623 to 0.0020) → No significant bias	0.0641 (0.0491 to 0.0782) → Significant bias	0.729 (p<0.0001) → Strong correlation	Significant deviation from linearity (p=0.05)	−176.1 % (−180.7 % to −171.5 %) → Strong negative bias	−226.0 % to −126.2 % → Very wide	Not interchangeable, strong proportional bias (Lumipulse underestimates)
Lumipulse vs. MSD S-Plex	y=0.0630 + 0.0179x	0.0630 (0.0509 to 0.0753) → Significant bias	0.0179 (0.0128 to 0.0219) → Significant bias	0.451 (p<0.0001) → Moderate correlation	No significant deviation from linearity (p=0.37)	−177.3 % (−182.7 % to −171.9 %) → Strong negative bias	−236.6 % to −118.0 % → Very wide	Not interchangeable, strong proportional bias (Lumipulse underestimates) and systematic bias
MSD S-Plex vs. in-house Simoa	y=−4.7361 + 3.3398x	−4.7361 (−7.1702 to −3.1127) → Significant bias	3.3398 (2.6013–4.4323) → Significant bias	0.417 (p<0.0001) → Moderate correlation	No significant deviation from linearity (p=0.37)	18.87 % (8.85 % to 28.88 %) → Positive bias	−90.7–128.4 % → Extremely wide	Not interchangeable, strong proportional bias (MSD S-Plex overestimates) and systematic bias

CI, confidence interval; LoA, limits of agreement; MSD, Meso scale discovery; →, indicates interpretation or conclusion. Method comparison of immunoassays. The "Comparison" column shows the pairwise assay comparisons. Passing–Bablok regression is reported with the regression equation, intercept, and slope (with 95 % CI). Systematic bias is inferred from the intercept, and proportional bias from the slope. Spearman correlation coefficients (ρ) with p-values are provided. Linear model validity was assessed using the Cusum test; p<0.05 indicates a significant deviation from linearity, suggesting that the Passing–Bablok model is not applicable. Bland–Altman analysis shows the mean % bias and limits of agreement (LoA, 95 % CI). % Bias was calculated as ((MethodA–MethodB)/mean of both methods) * 100. Negative values indicate that the first method reports lower concentrations than the second. The 95 % CI of the mean difference reflects the magnitude of the systematic difference.

Our results showed consistent method-dependent (and more likely assay-dependent) proportional bias and, in most pairwise comparisons, systematic bias – factors that limit direct interchangeability across assays. We also observed different analytical variations for each assay, reflecting not formal precision experiments but rather sample-to-sample variability under real-world clinical conditions. These differences may partly arise from manual steps required before quantification or from non-standardized pre-analytical procedures across platforms, potentially blurring the true assay effect. MSD S-Plex, in particular, showed both systematic and proportional biases when compared with Lumipulse G and UGOT Simoa, whereas the Lumipulse G vs. UGOT Simoa comparison revealed only proportional bias. These findings reinforce that plasma p-tau217 assays yield different absolute concentrations of p-tau217 and are not directly interchangeable without calibration or harmonization strategies. This conclusion is also supported by prior studies. Pilotto et al. [10] recently reported that, although Lumipulse and ALZpath Simoa p-tau217 assays showed high correlation, systematic and proportional biases were still present, with the ALZpath assay consistently overestimating p-tau217. Similarly, another comparison of Lumipulse with multiple Simoa assays revealed varying degrees of systematic and proportional bias [8]. Such evidence highlights the need for platform-specific cut-offs or conversion algorithms, particularly in the absence of harmonized calibration. In addition to assay-dependent analytical differences, the modest correlations observed across the three immunoassays may be the result of the following factors. Of the 122 samples analyzed, 100 samples were obtained from individuals across multiple age groups and had p-tau217 concentrations in the range of control samples, suggesting that these individuals were unlikely to have AD pathology; the remaining 22 were deliberately chosen from older individuals where the prevalence of AD pathology is higher (Supplementary Methods). While different assays target p-tau217 using a primary capture antibody, the selection of detector antibodies varies across assays. With Lumipulse and UGOT p-tau217 using N-terminal antibodies, which could explain the higher agreement between these two assays, and MSD S-Plex using a mid-region detector antibody [9]. Overall, the differences observed across the three assays may reflect both the low prevalence of AD in our sample set and the technical specifications unique to each platform. Our findings underscore the importance of considering assay and platform differences, particularly in populations where AD prevalence is low. Further investigations, including large-

scale comparisons of platforms and assay configurations in population-based studies, are needed to validate these results.

While our analysis was performed in line with the CLSI EP09-A3 framework for measurement procedure comparison, commutability studies of reference materials were not included, as this was beyond the scope and space constraints of the present letter. Encouragingly, steps are already being taken to address these challenges. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on Biomarkers of Neurodegenerative Diseases (WG-BND) with the support of Alzheimer's Association and Alzheimer's Drug Discovery Foundation has initiated efforts to establish a reference method and conduct commutability studies for plasma p-tau217. The goal is to develop a CRM to recalibrate existing assays, enabling global harmonization and facilitating broader clinical implementation of plasma p-tau217. We hope these efforts will ultimately enable its confident use in routine practice across diverse clinical laboratories.

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Conflict of interest: HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). NJA has served at scientific advisory boards and/or as a consultant for Abbvie, Alamar Biosciences, Athria, Beckman Coulter, Biogen, Bristol Myers Squibb Eli-Lilly, MapLight Therapeutics, NewAmsterdam AD, Roche, SpearBio, TauRx and has given lectures in symposia sponsored by Biogen, and Roche. The other authors report no disclosures.

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Data availability: The data supporting the findings of this study may be shared with qualified academic investigators for the purpose of result replication, upon reasonable request to the corresponding author and under a material transfer agreement.

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