**Preanalytical stability of plasma biomarkers for Alzheimer's disease pathology**

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**Abstract**

INTRODUCTION: Plasma tests have demonstrated high diagnostic accuracy for identifying Alzheimer’s disease pathology. To facilitate the transition to clinical utility, we assessed whether plasma storage duration and temperature affect the biomarker concentrations.

METHODS: Plasma samples from 13 participants were stored at +4°C and +18°C. Concentrations of six biomarkers were measured after 2, 4, 6, 8, 10, and 24 hours (h) by Single molecule array assays.

RESULTS: Phosphorylated tau 181 (p-tau181), phosphorylated tau 231 (p-tau231), neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) concentrations were unchanged both when stored at +4°C and +18°C. Amyloid-β 40 (Aβ40) and amyloid-β 42 (Aβ42) concentrations were stable for 24h at +4°C but declined when stored at +18°C for longer than 6h. This decline did not affect the Aβ42/Aβ40 ratio.

DISCUSSION: Plasma samples can be stored for 24h at +4°C or +18°C and result in valid assay results for p-tau181, p-tau231, Aβ42/Aβ40 ratio, GFAP and NfL.

**Key words:** Alzheimer disease, plasma, biomarker, phosphorylated tau, amyloid beta, glial fibrillary acidic protein, neurofilament protein light, pre-analytics

**1. Background**

Alzheimer’s disease (AD) is the most common form of dementia. At present, an *in vivo* diagnosis can be made using molecular biomarkers in cerebrospinal fluid (CSF) and positron emission tomography (PET) [[1]](https://paperpile.com/c/XHKPxR/9HW4); yet, a definitive diagnosis can still only be made post-mortem. CSF and PET diagnostic procedures are highly accurate for the underlying pathology of AD, but the high cost and minimal accessibility hamper their feasibility. The expected rise in dementia prevalence in the coming years [[2]](https://paperpile.com/c/XHKPxR/x9WW) and the increasing need for evidence of underlying AD pathology before entering anti-amyloid therapeutic trials enhance the relevance of an early, more accessible, and cost-effective measure of AD.

Ultrasensitive blood tests predicting AD pathologies, amyloid-β and tau, in the brain have shown potential for both diagnostic and prognostic clinical application [[3–7]](https://paperpile.com/c/XHKPxR/3amO2%2BU9Nq4%2BEHl6X%2B4t0ba%2B0dBmP)). Among the most promising blood-based biomarkers are plasma phosphorylated tau 181 (p-tau181) [[8,9]](https://paperpile.com/c/XHKPxR/ikbkx%2BZnRO) and phosphorylated tau 231 (p-tau231) [[10]](https://paperpile.com/c/XHKPxR/saedq). Other plasma biomarkers that might aid future diagnostics of AD are plasma glial fibrillary acidic protein (GFAP) [[11]](https://paperpile.com/c/XHKPxR/ikYH), plasma amyloid-β 42 to amyloid-β 40 ratio (Aβ42/Aβ40 ratio) [[12]](https://paperpile.com/c/XHKPxR/Vfha) and plasma neurofilament light (NfL) [[13]](https://paperpile.com/c/XHKPxR/eRKb). The combination of different biomarkers has also shown promising results [[14]](https://paperpile.com/c/XHKPxR/Ncq1z).

Due to the prospect that these plasma assays may greatly improve the diagnostic accuracy of AD pathology, a study of the possibility to use these tests in general practice is warranted. It has been shown that patients assessed for cognitive decline in primary healthcare had higher age, poorer cognition, and more limitations in activities of daily living than patients assessed in specialist healthcare [[15]](https://paperpile.com/c/XHKPxR/PlcSU). Reviews suggest that primary care providers experience difficulty recognizing early AD [[16]](https://paperpile.com/c/XHKPxR/rtuiz), lack confidence with diagnosing dementia, and express the need for better screening and diagnostic tools [[17]](https://paperpile.com/c/XHKPxR/B7Cqn). However, there are certain demands a blood test administered in primary care must fulfill to be of practical use. Primary care centers do not have the facilities to analyze high-technology assays and seldom have immediate access to ultra-low temperature freezers. Therefore, the plasma samples have to be stored and transported to a central laboratory for analysis. It is crucial to know if the storage temperature and duration of time from phlebotomy to ultra-low temperature freezing (ULTF) of the plasma affect the result of the assays.

This study aimed to assess the stability of ultrasensitive assays of plasma p-tau181, p-tau231, GFAP, Aβ40, Aβ42 and NfL for sample storage temperature and duration of time from phlebotomy to ULTF.

**2. Methods**

**2.1 Design**

Blinded, controlled experiment

**2.2 Sample**

Four general practitioner (GP) offices in the Stavanger area, with at least three GPs per office, were selected for recruiting patients, based on their proximity to Stavanger University Hospital and willingness to help with recruitment. Additional participants were recruited from the Centre for Age-related medicine in Stavanger. The inclusion period lasted from February 28th to June 23rd 2022. Informed written consent was obtained from all participants.

Inclusion criteria: Either (i) or (ii). (i) Persons aged 40 years or older suspected by their GP to have possible dementia, based on history, clinical examination and/or cognitive screening. These participants were recruited from the GP offices only. (ii) Assumed cognitively unimpaired participants aged 65 years or above. These participants were recruited from the GP offices and from the Centre for Age-related medicine at Stavanger University Hospital.

Exclusion criteria applied to cognitively impaired participants: Either (i) or (ii). (i) Lack of capacity for consent as judged by the GP. (ii) Severe psychiatric disease, use of medication or physical disease that according to the GP may affect participation or likely contribute significantly to the observed cognitive impairment.

A total of 13 participants were included, of whom 5 were female. Ten participants were cognitively unimpaired. Average age was 72,5 years (standard deviation 6,7).

**2.3 Measures**

Biomarker concentrations in plasma aliquots stored at -80°Celsius (°C) within two hours of phlebotomy were compared with aliquots frozen 4 hours, 6 hours, 8 hours, 10 hours, and 24 hours after phlebotomy, respectively stored at +4°C and at room temperature (mean +18.3°C) prior to ULTF.

**2.4 Procedure**

2.4.1 Sample handling

Blood samples were taken by phlebotomy between 8:15 am and 10 am and collected in tubes with K2-ethylenediaminetetraacetic acid (K2-EDTA). Three 6 milliliter tubes (Vacuette G456043) filled to maximum volume were collected per participant. Fasting was not required. The time of phlebotomy was noted (hour and minute). Tubes were mixed by gently being inverted 5 to 10 times, thereafter centrifuged at 2200xg for 10 minutes at room temperature (RT). RT at this stage of the sample handling was defined as the temperature at the laboratories at the GP offices and at the research laboratory at Stavanger University Hospital and was not measured. Plasma was pipetted off using a Low-Density Polyethylene transfer pipette (Sarstedt 86.1172.001), sparing out the buffy coat layer. Plasma from the same patient was pooled in a 15 ml polypropylene tube (Sarstedt 62.554.502). Tubes collected at the GP offices were stored in a refrigerator at +4°C and then transported in a cooling bag (Sarstedt 95.995) directly to the hospital laboratory arriving no later than 10:30 am. At the hospital laboratory the plasma in the 15 ml polypropylene tubes was mixed by gently inverting the tube 10 times, then twelve 0.5 mL aliquots of the plasma sample were pipetted into polyethylene cryotubes (Sarstedt CryoPure tubes, 72.377) by using a Low-Density Polyethylene transfer pipette. Half of the plasma aliquots were thereafter kept at +4°C and half were kept at RT. RT was automatically measured and adjusted, with the mean value being +18.3°C (minimum +16.1°C; maximum +19.5°C). One aliquot from each of these two groups was frozen at -80°C at defined time points: 10:30 to 11am (depending on the time of phlebotomy), 12:30pm, 2:30pm, 4:30pm, 6:30pm and 8am, equivalent to storage duration approximately 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, and 24 hours after phlebotomy. The ultra-low-temperature-frozen cryotubes were stored at -80°C until samples from all participants had been collected, then sent from Stavanger to Gothenburg by temperature-regulated dry ice transport.

Plasma samples were analyzed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. The laboratory was blinded to which aliquot had been frozen at which point of time and whether the aliquot had been stored at RT or at +4°C prior to freezing. Prior to analysis the plasma samples were thawed, vortexed at 2000 revolutions per minute for 30 seconds and centrifuged at 4000xg for 10 minutes at RT. Analyses were conducted no later than 30 minutes after plasma thawing. The twelve aliquots belonging to the same patient were always analyzed in succession in the same analytical run.

2.4.2 Platform and assays

Plasma analysis was performed on the HD-X Analyzer (Quanterix). Plasma p-tau181 and p-tau231 concentrations were measured using in-house assays developed by the University of Gothenburg [[8,10]](https://paperpile.com/c/XHKPxR/saedq%2Bikbkx). Plasma Aβ42, Aβ40, GFAP and NfL were measured using commercially available immunoassay from Quanterix (Neurology 4-Plex E).

**2.5 Statistics**

Descriptive analyses were performed by estimating means and standard deviation (SD) for quantitative variables and percentages for categorical variables at 2h (baseline), where sex and cognitive status were treated as dummy variables. Independent repeated measurements ANOVA were used for the longitudinal assessments for each variable, including two random effects: the variation within subjects as a variance component and the time-variation in each storage method as an unstructured variance component. P-tau181 and p-tau231 were logarithm-transformed to reach the normality assumption. One subject was excluded from the GFAP model since it was detected as an outlier. Also, a second subject was excluded for p-tau231. All models were adjusted for sex, age and cognitive status. Where there was found no significant effect this adjustment was excluded to keep the models as simple as possible. Thus, the Aβ40 model was adjusted by sex, the Aβ42, Aβ40/42, and p-tau181 models by sex and age, the GFAP and p-tau231 models by sex, and the NfL model by sex and cognitive status. The models were fitted by restricted maximum likelihood (REML), and the t-test for the hypothesis testing in the least squared means post-estimations used the Kenward-Roger method to estimate the degrees of freedom. Tukey’s p-values adjustment was used to correct for multiple testing, and the hypotheses were rejected in each model on an alpha level of 0.05. PROC MIXED in SAS 9.4 was used for modeling and R version 4.2.1 for data manipulation and graphics.

**2.6 Ethics**

The Regional committee for medical and health research ethics approved the study on November 4th 2021 (REK Vest ID 206473). Informed written consent was obtained from all participants.

**3. Results**

Descriptive analyses at baseline are summarized in Table 1. Figure 1 shows median concentrations of plasma p-tau181, p-tau231, GFAP, NfL, Aβ40, Aβ42 and Aβ42/Aβ40 when plasma was stored at RT and when stored at +4°C for up to 24 hours. Mean time between phlebotomy and ULT freezing of the first aliquot was 86 minutes (min. 40 minutes, max. 147 minutes, median 93 minutes).

We found no significant changes in concentrations of plasma p-tau181, p-tau231 and GFAP for neither the examined time nor temperature variables. For NfL, there was a significant difference in biomarker concentration comparing aliquots stored at +4°C versus RT after 8- and 10-hours storage but not after 24 hours. For Aβ40 and Aβ42, there were significant differences in biomarker concentrations between storage at +4°C and RT at 8 hours, 10 hours, and 24 hours and between baseline and storage at RT for 24 hours. For Aβ40, there was also a significant difference in biomarker concentrations comparing storage at RT at baseline with storage after 10 hours. For the Aβ42 to Aβ40 ratio, there was a significant difference comparing storage at +4°C and room temperature at 24 hours but not compared with baseline. Significant findings are shown in Table 2. See appendix for a table with all findings.

**Table 1**  Descriptive analysis for Time 2h presented as mean ± standard deviation (SD)

|  |  |  **Room temp** |  |  **Fridge +4C** |
| --- | --- | --- | --- | --- |
| Variable |  | Mean | ± SD |  | Mean | ± SD |
| Glial fibrillary acidic protein (GFAP) |  | 118\*  | 103 |  | 123\*  | 101 |
| Phosphorylated tau 231 (p-tau231) |  | 17.3\*  |  3.4 |  | 16.9\*  |  3.5 |
| Phosphorylated tau 181 (p-tau181) |  | 16.1\*  |  9.4 |  | 16.2\*  | 10.2 |
| Neurofilament light (NfL) |  | 19.7\*  | 10.6 |  | 20.0\*  |  10.8 |
| Amyloid-β 40 (Aβ40) |  | 92.7\*  | 20.5 |  | 92.8\*  | 25.6 |
| Amyloid-β 42 (Aβ42) |  | 5.7\*  |  1.9 |  | 6.1\*  |  2.5 |
| Aβ42/40 ratio  |  | 0.06  | 0.02 |  | 0.07  | 0.02 |

\* Biomarker concentrations are given in picograms per milliliter (pg/ml).

**Table 2** Findings of significant differences in estimated mean biomarker concentrations

| Variable | Comparison | Estimate | Std. Err. | P Value | Adj. P Value |
| --- | --- | --- | --- | --- | --- |
| Aβ40 |  |  |  |  |  |
|  | Fridge +4C vs Room Temp. at Time 8 | 7.0\* | 1.6 | 0.001 | 0.000 |
|  | Fridge +4C vs Room Temp. at Time 10 | 10.7\* | 2.6 | 0.001 | 0.001 |
|  | Fridge +4C vs Room Temp. at Time 24 | 17.8\* | 2.2 | <.001 | <.001 |
|  | Room Temp. at Time 2 vs at Time 10 | -8.5\* | 3.3 | 0.018 | 0.019 |
|  | Room Temp. at Time 2 vs at Time 24 | -16.6\* | 3.1 | <.001 | <.001 |
| Aβ42 |  |  |  |  |  |
|  | Fridge +4C vs Room Temp. at Time 8 | 0.5\* | 0.2 | 0.027 | 0.022 |
|  | Fridge +4C vs Room Temp. at Time 10 | 0.7\* | 0.2 | 0.004 | 0.002 |
|  | Fridge +4C vs Room Temp. at Time 24 | 1.5\* | 0.2 | <.001 | <.001 |
|  | Room Temp. at Time 2 vs at Time 24 | -1.3\* | 0.4 | 0.003 | 0.004 |
| Aβ42/40  |  |  |  |  |  |
|  | Fridge +4C vs Room Temp. at Time 24 | 0.004 | 0.001 | 0.001 | 0.001 |
|  | Fridge +4C at Time 2 vs at Time 4 | -0.004 | 0.002 | 0.020 | 0.021 |
| NFL |  |  |  |  |  |
|  | Fridge +4C vs Room Temp. at Time 8 | 0.8\* | 0.3 | 0.018 | 0.008 |
|  | Fridge +4C vs Room Temp. at Time 10 | 1.4\* | 0.4 | 0.002 | 0.001 |

\* Biomarker concentrations are given in picograms per milliliter (pg/ml).
Abbreviations: Aβ40, Amyloid-β 40; Aβ42, Amyloid-β 42; Aβ42/40, Amyloid-β 42 to Amyloid-β 40 ratio; NFL, neurofilament light; C, Celcius; vs, versus; Temp., temperature; Std. Err., Standard error; Adj., Adjusted.

**4. Discussion**

In a blinded, controlled study, we investigated if plasma sample storage duration and temperature before ultra-low temperature freezing causes changes in the concentration of six plasma biomarkers associated with Alzheimer's disease pathology. For p-tau181, p-tau231 and GFAP, we found no significant changes in biomarker concentrations during a storage time of 24 hours, both when plasma was stored at room temperature and when stored at +4°C prior to ULTF, *e.g.*, these storage factors do not seem to influence these plasma biomarker concentrations. To our knowledge, this study is the first to investigate these preanalytical qualities for plasma p-tau231. Even though calculation showed a significant difference in NfL biomarker concentration comparing storage between +4°C and RT after 8 and 10 hours, we assume that this was due to normal variance as there was no significant difference in biomarker concentration after 24 hours storage compared with baseline. For plasma Aβ40 and Aβ42 concentrations, the study found a clear temperature-dependent effect. Concentrations were stable for 24 hours at +4°C but were reduced when kept at RT for 8 hours or longer. When using the Aβ42 to Aβ40 ratio there was no significant change after 24 hours storage at RT compared with baseline.

Our results are consistent with the findings of Verberk *et al*. [[18]](https://paperpile.com/c/XHKPxR/Ck1yp). In this study, plasma p-tau181, GFAP and NfL concentrations were unaffected by post-centrifugation 24 hours storage at either RT or 2-8°C whereas the concentrations of plasma Aβ40 and Aβ42 showed a decline at RT. Plasma Aβ40 and Aβ42 values were stable at 2-8°C for 24 hours, whereas in RT only for 4 hours. In this study no measurements between 4 and 24 hours were conducted.

Using the Aβ42/Aβ40 ratio mitigated the observed decline during storage in RT in some but not all the immunoassays tested by Verberk *et al*. [[18]](https://paperpile.com/c/XHKPxR/Ck1yp). The assay used in our study was among those found to mitigate the decline in Aβ40 and Aβ42. Similarly, a study [[19]](https://paperpile.com/c/XHKPxR/m41Vo) examined if there was a time- and temperature-dependent difference in plasma Aβ40 and Aβ42 values. This study found that Aβ40 and Aβ42 concentrations were stable up to 6 hours but not 24 hours when fresh EDTA plasma was stored at +4°C. The observed inter-immunoassay variability found by Verberk *et al*. [[18]](https://paperpile.com/c/XHKPxR/Ck1yp) might explain why the results from Rozga *et al*. [[19]](https://paperpile.com/c/XHKPxR/m41Vo) are somewhat different from ours.

We considered several preanalytical variables when planning this study. All our samples were collected in K2-ethylenediaminetetraacetic acid (K2-EDTA) tubes. Previous studies have shown that different blood collection tube types influence the measured amount of plasma biomarkers Aβ42, Aβ40, p‐tau181, GFAP, total tau and NfL [[18–20]](https://paperpile.com/c/XHKPxR/Ck1yp%2Bm41Vo%2BLkMRm). K2-EDTA tubes have been suggested as a standard tube in a recently recommended standardized operating procedure [[18]](https://paperpile.com/c/XHKPxR/Ck1yp). The effect of centrifugation temperature, aliquot volume and number of freeze-thaw-cycles has been tested for plasma p-tau181, GFAP, NfL, Aβ40 and Aβ42 [[18–21]](https://paperpile.com/c/XHKPxR/Ck1yp%2B6XCX4%2Bm41Vo%2BLkMRm). No such studies for p-tau231 could be identified. We also considered if circadian rhythm might affect sample values. [Rózga](http://paperpile.com/b/XHKPxR/m41Vo) *et al*. [[19]](https://paperpile.com/c/XHKPxR/m41Vo) found that plasma Aβ40 and Aβ42 showed a weak circadian rhythmicity. In our study, all blood samples were drawn in the morning, and we therefore do not expect that our results are influenced by circadian rhythmicity variability. The effect of tube material, tube size and the presence of gel separators has not been found to influence the quantification of plasma Aβ40 and Aβ42 [[19]](https://paperpile.com/c/XHKPxR/m41Vo). RT in our study was between +16.1 to 19.5°C. Higher storage temperatures might have a different effect on plasma biomarker levels than found in this study.

We consider it a strength that more than half of the blood samples were drawn in general practitioner’s offices following a standardized procedure. Overall, there were no difficulties related to this setting. Time of phlebotomy at the GP offices had to be adjusted to real-life clinical situations, *e.g.*, when participants had their visit scheduled. Time of phlebotomy varied from 8:13am to 9:59am. In two of the thirteen cases, it took 143 and 137 minutes, respectively, to freeze the first aliquot, whereas the protocol planned for a maximum time of 120 minutes. We have no reason to believe that this affected the overall results. We did not include centrifugation-postponed EDTA-blood samples in our project. Blood samples stored before centrifugation might have worse biomarker stability than plasma stored after centrifugation, although Verberk *et al*. [[18]](https://paperpile.com/c/XHKPxR/Ck1yp) did not find such differences for p-tau181, GFAP and NfL, and for Aβ42/Aβ40 ratio only for some immunoassays. From a primary care perspective, it could be labor- and time-saving to send blood samples uncentrifuged and postpone centrifugation and plasma-pipetting to a centralized laboratory having access to an ultra-low temperature freezer. On the other hand, it could be overall time-saving if the centralized laboratories receive plasma samples that can be frozen immediately without further handling.

A strength in our study is that we have included two phosphorylated tau isoforms, currently seen as some of the most promising plasma biomarkers for diagnosing AD pathology, as well as the Aβ42/Aβ40 ratio, the astrogliosis biomarker GFAP and NfL, a marker for axonal neurodegeneration. This allows for comparing their sensitivities to variation in pre-analytical factors such as sample storage time and temperature. This information is important in the design of future clinical trials that consider decentralized sampling of plasma samples for AD-related biomarkers.

We did not assess the participants’ cognitive status with tests before inclusion, which might be considered a limitation of our study. We included participants whose primary care doctor suspected possible cognitive impairment due to dementia and elderly participants without known cognitive impairment. However, as our aim was not to assess the diagnostic ability of the biomarkers but the effect of sample storage time and temperature on assay results this is considered negligible. There is no reason to believe that cognitive status would influence biomarker stability. Other studies on preanalytical qualities of plasma biomarkers have included healthy volunteers [[19]](https://paperpile.com/c/XHKPxR/m41Vo) or adults who presented for a diagnostic blood draw for any disease [[18]](https://paperpile.com/c/XHKPxR/Ck1yp). The low number of participants in our study might be seen as a limitation. This is outweighed by the high number of aliquots per participant, creating a total of 156 measurements per biomarker.

**Implications for clinical use**

In conclusion, we found that K2-EDTA plasma samples can be stored for 24 hours at +4°C or at a room temperature of +18°C before ULTF and still result in valid assay results for a panel of phosphorylated tau isoforms p-tau181 and p-tau231, Aβ42/Aβ40 ratio, GFAP, and NfL. Therefore, plasma samples for these biomarkers seem suitable for use in a primary care setting where sample storage and transportation to a facility with ultra-low temperature freezing can be achieved within this frame.

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**Author contribution**

Anita L. Sunde contributed to the development of the concept and design of the study, the practical work of including participants, collection and freezing of the samples, analysis and interpretation of the results as well as preparation of the manuscript. Ingvild V. Alsnes, Dag Aarsland, Kaj Blennow, Henrik Zetterberg and Svein Kjosavik had an important role in the establishment of the research group, the application for funds and the overall project plan of which this study is a part of. Nicholas J. Ashton, Giovanni De Santis, Kaj Blennow and Henrik Zetterberg contributed regarding the plasma biomarker analysis. Diego A. Tovar-Rios was mainly involved in the statistical assessment of the data. In addition, all authors contributed to revising the manuscript.

**Conflict of Interest/Disclosure Statement**

A.L. Sunde, I.V. Alsnes, N.J. Ashton, D.A. Tovar-Rios, G. De Santis and S.R. Kjosavik report no conflict of interest. K. Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. H. Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, 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). D. Aarsland has received a research grant and honoraria from Roche Diagnostics (outside submitted work).

**Highlights**

* Plasma samples were stored for 24 hours at +4°C and +18°C, mimicking clinical practice.
* Concentrations for Alzheimer's disease biomarkers were measured at six time-points.
* P-tau181, p-tau231, NfL and GFAP concentrations were unchanged during the experiment.
* Storage at +18°C affected Aβ40 and Aβ42 concentrations while storage at +4°C did not. The Aβ42/Aβ40 ratio was unaffected.
* These plasma tests seem suitable for use in general practice.

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**Figure 1**. Concentrations of plasma p-tau181, p-tau231, GFAP, NfL, Aβ40, Aβ42, and Aβ42/Aβ40 ratio (y-axis) when stored at +4°C and at room temperature for up to 24 hours (x-axis). The horizontal line in each box represents the median, below and above are the second and third quartile. The lower whisker represents the first quartile, the upper whisker the fourth quartile. Outliners are represented as dots. Biomarker concentrations are given in picograms per milliliter (pg/ml).

Abbreviations: *p-tau181*, phosphorylated tau 181; *p-tau231*, phosphorylated tau 231; *GFAP*, glial fibrillary acidic protein;
*NFL,* neurofilament light; *Aβ40*, Amyloid-β 40; *Aβ42*, Amyloid-β 42; *Aβ42/Aβ40*, Amyloid-β 42 to Amyloid-β 40 ratio; *h,* hour; *C,* Celcius