Effect of Times to Blood Processing on the Stability of Blood Proteins Associated with Dementia

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Abstract

Background: The stability of proteins in the collecting tubes after blood draw is critical to the measured concentrations of the proteins. Although the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) suggest centrifugation should take place within 2 h of drawing blood, it is very difficult to follow these guidelines in hospitals or clinics. It is necessary to study the effect of times to blood processing on the stability of the proteins of interest. Methods: In this work, the plasma proteins of interest were those relevant to dementia, such as amyloid β 1–40 (Aβ\textsubscript{1–40}), Aβ\textsubscript{1–42}, Tau protein (Tau), and α-synuclein. The times to blood processing after blood draw ranged from 0.5 to 8 h. The storage temperatures of blood were room temperature (approx. 25 °C) and 30 °C. After storage, blood samples were centrifuged at room temperature to obtain plasma samples. Ultra-sensitive immunomagnetic reduction was applied to assay these proteins in the plasma. Results: The levels of plasma Aβ\textsubscript{1–40}, Tau, and α-synuclein did not significantly change until 8 h after blood draw when stored at room temperature. Plasma Aβ\textsubscript{1–42} levels did not change significantly after 8 h of storage at room temperature before blood processing. Higher storage temperatures, such as 30 °C, for blood samples accelerated the significant variations in the measured concentrations of Aβ\textsubscript{1–40}, Tau, and α-synuclein in plasma. Conclusion: According to these results, for clinical practice, it is suggested that blood samples be stored at room temperature for no longer than 4.5 h after blood draw until centrifugation for the assay of dementia biomarkers in plasma.

Introduction

Pathological markers of Alzheimer’s disease (AD) or Parkinson’s disease (PD) are amyloid β (Aβ) plaques, neurofibrillary tangles, and Lewy bodies in the brain [1–5]. These plaques are peptides of 36–43 amino acids that result from the cleavage of the amyloid precursor protein (APP) by β and γ secretases [6]. The 2 critical Aβ plaques associated with AD are Aβ\textsubscript{1–40} and Aβ\textsubscript{1–42}. Neurofibrillary
tangles are dead neurons caused by the hyperphosphorylation of Tau proteins in dementia. Tau protein is a microtubule-associated protein [7]. Tau proteins bind microtubules through their microtubule-binding domains. Once the Tau protein is hyperphosphorylated, the assembly function of the Tau protein is disabled, and neurons are damaged. Tau protein is expressed from dead neurons so that neurofibrillary Tau tangles are usually observed in biopsies [8]. Lewy bodies are mainly composed of α-synuclein [9]. The formation of Lewy bodies predominantly results from the β-sheet of α-synuclein. Hence, from the proteinopathic point of view, the biomarkers of AD and PD are Aβ1–40, Aβ1–42, Tau, and α-synuclein.

In the clinic, neuroimaging such as positron emission tomography (PET), magnetic resonance imaging, or dopamine scan is applied to find Aβ plaques, neurofibrillary tangles, or Lewy bodies in the brain [10–15]. However, such neuroimaging is very expensive or is not easily available. The detection of these biomarkers in the cerebrospinal fluid (CSF) is an alternative means to assess the pathological hallmarks of AD or PD for diagnosis [16–20]. Unfortunately, lumbar puncture is necessary to obtain CSF samples. Several drawbacks of lumbar puncture seriously limit the clinical applications of assaying these biomarkers in CSF.

In the last decade, several groups have demonstrated the feasibility of precisely detecting these biomarkers in human plasma by utilizing ultrasensitive assay technologies [21–26]. The authors applied the so-called SQUID-based immunomagnetic reduction (IMR) to quantify the levels of Aβ1–40, Aβ1–42, and Tau in AD, as well as α-synuclein in PD [27–29]. The reported results show high discrimination (i.e., an area under the receiver-operating characteristics (ROC) curve of >80) between individuals with dementia and healthy controls (HCs) by using concentrations of these plasma biomarkers in independent cohorts [30, 31].

It is well known that preclinical factors might affect the measured concentrations of blood proteins. For example, the prolonged contact of plasma or serum with cells could lead to spurious results. Typical analytes such as glucose, phosphate, potassium, and creatinine cannot be accurately assayed once centrifugation of the blood samples is delayed by several hours [32–34]. Hence, the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) suggest the separation of plasma and serum from cells within 2 h after blood draw [35]. However, it is impractical to centrifuge blood samples within this time frame at urban hospitals or clinics. The CLSI guidelines suggest that the times to process blood samples depend on the analytes and the temperature at which the samples are stored. Unfortunately, the times to blood processing for dementia biomarkers have been poorly investigated. In this work, the times to blood processing varied from 0.5 to 8 h. The storage temperatures of blood samples were room temperature (approx. 25 °C) and 30 °C. The recovery rates of the measured concentrations at different times to blood processing, with respect to the concentrations at 0.5 h to blood processing, are calculated to determine the stability of dementia biomarkers in the blood.

### Materials and Methods

#### Preparation of Human Plasma

Ten-milliliter K3 EDTA tubes (455036, Greiner) were used for the blood draw. Each blood tube was gently inverted 10 times immediately after blood collection. Blood tubes were stored at room temperature (approx. 25 °C) or 30 °C for 0.5, 3.5, 5.5, and 8 h, followed by centrifugation at room temperature at 2,500 g for 15 min. A swing-out (basket) rotor was used for centrifugation. Every 1 mL of plasma (supernatant) was transferred to a fresh 1.5-mL Eppendorf tube using a disposable 1-mL micropipette tip. All plasma samples were frozen at –80 °C before being measured.

#### IMR Measurements

The frozen plasma was moved from a refrigerator and placed at room temperature for 20 min. For assaying Aβ1–40, Tau, or α-synuclein, 40 µL of plasma was mixed with 80 µL of reagent (MF-AB0–0060, MF-TAU–0060, and MF-ASC–0060, MagQu). For assays of Aβ1–42, 60 µL of plasma was mixed with 60 µL of reagent (MF-AB2–0060). For each batch of measurements, calibrators (CA-DEX–0060 and CA-DEX–0080, MagQu) and control solutions (CL-AB0–000T, CL-AB0–005T, CL-AB2–000T, CL-AB2–005T, CL-TAU–000T, and CL-TAU–005T, MagQu) were used. The IMR analyzer (XacPro-S361, MagQu) was utilized for the precise and quantitative detection of the concentrations of biomarkers of interest. For each biomarker per sample, duplicated measurements were performed. The averaged concentration of the duplicated measurements was reported.

### Table 1. Sex and age of the 8 enrolled healthy controls

| Subject | Sex | Age, years |
|---------|-----|------------|
| 1       | female | 50         |
| 2       | male   | 25         |
| 3       | female | 52         |
| 4       | female | 52         |
| 5       | female | 31         |
| 6       | female | 40         |
| 7       | male   | 32         |
| 8       | male   | 42         |

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Results

Eight subjects without any clinical signs of dementia were enrolled for blood collection in this work. The sex and age of each subject are listed in Table 1. The measured concentrations of plasma Aβ1–40 of the 8 subjects at times to blood processing of 0.5–8 h are listed in Table 2. The measured concentrations at 0.5 h to blood processing were used as baseline concentrations for each individual. The recovery rate of the measured concentration at a given time for blood processing was calculated as follows:

\[
\text{Recovery rate} \% = \left( \frac{\text{Concentration at a given time to blood processing}}{\text{Baseline concentration}} \right) \times 100\% \tag{1}
\]

The recovery rate at each time to blood processing is listed in Table 2. Recovery rates between 90 and 100% correspond to nonsignificant changes in the measured concentrations at a given time to blood processing compared to the baseline concentration. When the collected blood samples were stored at room temperature for 3.5 h, the recovery rates of all subjects were within the range of 93.0–105.2%. For 5.5 h of storage before centrifugation, the recovery rates of all subjects were within the range of 93.0–100.0%. For blood samples that were stored at room temperature for 8 h, some subjects showed recovery rates of <90%. These results reveal that there were no significant changes in plasma Aβ1–40 concentrations when the storage time before centrifugation was <8 h. However, in 6 of the 8 subjects (75%), there were significant changes in the Aβ1–40 concentrations in the plasma when centrifugation was delayed until 8 h after blood draw. When the collected blood samples were stored at 30 °C, significant changes in the measured plasma Aβ1–40 concentrations were observed at 8 h. Seven of 8 subjects (87.5%) showed significant changes in measured Aβ1–40 concentrations in plasma when centrifugation was delayed until 8 h after blood draw.

In the case of plasma Aβ1–42, regardless of whether the blood samples were stored at room temperature or 30 °C, the recovery rates at different times to blood processing for all subjects ranged from 93.2 to 101.4% (Table 3). This implies that Aβ1–42 is stable in whole blood at room temperature or at 30 °C for 8 h before centrifugation.

| Subject No. | Baseline concentration, pg/mL | Blood sample storage temperature | Concentration, pg/mL (recovery rate at different times to blood processing, %) |
|-------------|--------------------------------|---------------------------------|--------------------------------------------------------------------------------|
|             |                                |                                 | at 3.5 h | at 5.5 h | at 8 h   |
| 1           | 63.83                          | RT 30°C                         | 62.28 (97.6) | 63.10 (98.9) | 58.20 (91.2) |
|             |                                |                                 | 61.56 (96.4) | 58.63 (91.9) | 54.97 (86.1) |
| 2           | 63.40                          | RT 30°C                         | 61.43 (96.9) | 63.38 (100) | 48.34 (76.2) |
|             |                                |                                 | 62.39 (98.4) | 57.74 (91.1) | 49.34 (77.8) |
| 3           | 57.62                          | RT 30°C                         | 55.74 (96.7) | 53.56 (93.0) | 48.12 (83.5) |
|             |                                |                                 | 54.88 (95.2) | 56.38 (97.8) | 48.39 (84.0) |
| 4           | 54.67                          | RT 30°C                         | 50.87 (93.0) | 52.29 (95.6) | 48.72 (89.1) |
|             |                                |                                 | 54.68 (100) | 51.37 (94.0) | 47.49 (86.9) |
| 5           | 54.99                          | RT 30°C                         | 57.84 (105.2) | 53.81 (97.9) | 52.91 (96.2) |
|             |                                |                                 | 55.26 (100.5) | 52.69 (95.8) | 45.47 (82.7) |
| 6           | 58.39                          | RT 30°C                         | 56.28 (96.4) | 56.25 (96.3) | 51.93 (88.9) |
|             |                                |                                 | 55.24 (94.6) | 55.94 (95.8) | 46.30 (79.3) |
| 7           | 56.25                          | RT 30°C                         | 56.22 (99.9) | 54.92 (97.6) | 48.13 (85.6) |
|             |                                |                                 | 57.77 (102.7) | 53.79 (95.6) | 49.89 (88.7) |
| 8           | 63.75                          | RT 30°C                         | 62.12 (97.4) | 60.32 (94.6) | 59.87 (93.9) |
|             |                                |                                 | 61.72 (96.8) | 61.34 (96.2) | 58.59 (91.9) |

RT, room temperature (approx. 25°C).

a Recovery rate is outside the range of 90–110%.
Table 3. Measured Aβ<sub>1-42</sub> concentrations in plasma and recovery rates at different times

| Subject No. | Baseline concentration, pg/mL | Blood sample storage temperature | Concentration, pg/mL (recovery rate at different times to blood processing, %) |
|-------------|-------------------------------|----------------------------------|--------------------------------------------------------------------------------|
|             |                               |                                  | at 3.5 h                     | at 5.5 h                     | at 8 h                      |
| 1           | 16.63                         | RT 30°C                          | 16.67 (100.2)                | 16.64 (100.1)                | 15.99 (96.2)                |
|             |                               |                                  | 16.61 (99.9)                 | 16.59 (99.8)                 | 15.88 (95.5)                |
| 2           | 16.37                         | RT 30°C                          | 16.48 (100.7)                | 16.33 (99.8)                 | 15.25 (93.2)                |
|             |                               |                                  | 16.61 (99.9)                 | 16.59 (99.8)                 | 15.88 (95.5)                |
| 3           | 16.44                         | RT 30°C                          | 16.40 (99.8)                 | 16.23 (98.7)                 | 16.17 (98.4)                |
|             |                               |                                  | 16.44 (100)                  | 16.26 (98.9)                 | 16.21 (98.6)                |
| 4           | 15.93                         | RT 30°C                          | 15.92 (99.9)                 | 15.59 (97.9)                 | 15.56 (97.7)                |
|             |                               |                                  | 16.00 (100.4)                | 15.63 (98.1)                 | 15.30 (96.0)                |
| 5           | 16.33                         | RT 30°C                          | 16.34 (100.1)                | 16.49 (101.0)                | 16.12 (98.7)                |
|             |                               |                                  | 16.56 (101.4)                | 16.33 (100)                  | 15.72 (96.3)                |
| 6           | 16.68                         | RT 30°C                          | 16.45 (98.6)                 | 16.54 (99.2)                 | 16.09 (96.5)                |
|             |                               |                                  | 16.32 (97.8)                 | 16.42 (98.4)                 | 16.15 (96.8)                |
| 7           | 16.66                         | RT 30°C                          | 16.57 (99.5)                 | 16.44 (98.7)                 | 16.07 (96.5)                |
|             |                               |                                  | 16.66 (100)                  | 16.09 (96.6)                 | 16.31 (97.9)                |
| 8           | 16.54                         | RT 30°C                          | 16.42 (99.3)                 | 16.38 (99.0)                 | 15.86 (95.9)                |
|             |                               |                                  | 16.42 (99.3)                 | 16.16 (97.7)                 | 15.99 (96.7)                |

RT, room temperature (approx. 25°C in this work).

Table 4. Measured Tau concentrations in plasma and recovery rates at different times

| Subject No. | Baseline concentration, pg/mL | Blood sample storage temperature | Concentration, pg/mL (recovery rate at different times to blood processing, %) |
|-------------|-------------------------------|----------------------------------|--------------------------------------------------------------------------------|
|             |                               |                                  | at 3.5 h                     | at 5.5 h                     | at 8 h                      |
| 1           | 19.90                         | RT 30°C                          | 19.32 (97.1)                 | 18.46 (92.8)                 | 17.63 (88.6)<sup>a</sup> |
|             |                               |                                  | 19.49 (97.9)                 | 17.75 (89.2)<sup>a</sup>     | 17.14 (86.1)<sup>a</sup>   |
| 2           | 19.67                         | RT 30°C                          | 19.55 (99.4)                 | 19.43 (98.8)                 | 17.03 (86.6)<sup>a</sup>   |
|             |                               |                                  | 19.48 (99.0)                 | 18.98 (96.5)                 | 16.43 (83.5)<sup>a</sup>   |
| 3           | 20.86                         | RT 30°C                          | 19.42 (93.1)                 | 19.58 (93.9)                 | 17.04 (81.7)<sup>a</sup>   |
|             |                               |                                  | 19.43 (93.1)                 | 17.57 (84.2)<sup>a</sup>     | 16.16 (77.5)<sup>a</sup>   |
| 4           | 19.60                         | RT 30°C                          | 19.48 (99.4)                 | 19.03 (97.1)                 | 18.75 (95.7)                |
|             |                               |                                  | 19.57 (99.8)                 | 18.87 (96.3)                 | 17.55 (89.5)<sup>a</sup>   |
| 5           | 21.26                         | RT 30°C                          | 2.186 (102.8)                | 20.04 (94.3)                 | 17.44 (82.0)<sup>a</sup>   |
|             |                               |                                  | 19.80 (93.1)                 | 18.18 (85.5)<sup>a</sup>     | 17.48 (82.2)<sup>a</sup>   |
| 6           | 19.53                         | RT 30°C                          | 20.49 (104.9)                | 19.41 (99.4)                 | 17.98 (92.1)                |
|             |                               |                                  | 20.40 (104.5)                | 18.22 (93.3)                 | 18.39 (94.2)                |
| 7           | 20.08                         | RT 30°C                          | 20.87 (103.9)                | 18.38 (91.5)                 | 17.24 (85.9)<sup>a</sup>   |
|             |                               |                                  | 19.88 (99.0)                 | 17.76 (88.4)<sup>a</sup>     | 16.90 (84.2)<sup>a</sup>   |
| 8           | 20.30                         | RT 30°C                          | 19.43 (95.7)                 | 20.03 (98.7)                 | 17.24 (84.9)<sup>a</sup>   |
|             |                               |                                  | 19.35 (95.3)                 | 19.26 (94.9)                 | 16.51 (81.3)<sup>a</sup>   |

RT, room temperature (approx. 25°C in this work). * Recovery rate is outside the range of 90–110%.
Regarding Tau, when the blood samples were stored at room temperature, the recovery rates of measured concentrations when centrifugation was delayed until 3.5 or 5 h after blood draw were observed to be in the range of 90.5–104.9% (Table 4). When centrifugation was delayed by 8 h, 6 of 8 subjects (75%) showed recovery rates < 90%. Thus, whole blood should not be stored at room temperature for longer than 5.5 h before centrifugation. When the blood samples were stored at 30 °C, 4 of 8 subjects (50%) showed recovery rates < 90% when centrifugation was delayed by 5.5 h. Furthermore, 7 of 8 subjects (87.5%) had significant changes, i.e., recovery rates < 90%, in plasma Tau concentrations when centrifugation was delayed by 8 h.

As listed in Table 5, the recovery rates of measured plasma α-synuclein concentrations when stored at room temperature for 3.5 and 5.5 h ranged from 90.4 to 108.6%. Once the time to blood processing reached 8 h, the recovery rates ranged from 75.6 to 97.9%. Five of 8 subjects (62.5%) showed significant changes in the concentration of plasma α-synuclein. When the blood samples were stored at 30°C before centrifugation, significant changes in the concentrations of plasma α-synuclein were observed when the time to blood processing reached 8 h. All subjects showed recovery rates of measured α-synuclein concentrations in plasma of <90%.

**Table 5. Measured α-synuclein concentrations in plasma and recovery rates at different times**

| Subject No. | Baseline concentration, fg/mL | Blood sample storage temperature | Concentration, fg/mL (recovery rate at different times to blood processing, %) |
|-------------|-------------------------------|---------------------------------|---------------------------------------------------------------------|
|             |                               | RT 30°C                         | at 3.5 h | at 5.5 h | at 8 h |
| 1           | 83.97                         | RT 30°C                         | 85.91 (102.3) | 91.19 (108.6) | 82.20 (97.9) |
|             |                               |                                 | 82.84 (98.7) | 81.05 (96.5)  | 63.97 (76.2)^
| 2           | 95.59                         | RT 30°C                         | 93.57 (97.9) | 94.28 (98.6)  | 79.18 (82.8) |
|             |                               |                                 | 89.80 (93.9) | 98.68 (103.2) | 65.74 (68.8) |
| 3           | 86.81                         | RT 30°C                         | 82.52 (95.1) | 80.76 (93.0)  | 71.37 (82.2)^
|             |                               |                                 | 85.51 (98.5) | 82.79 (95.4)  | 67.56 (77.8)^
| 4           | 89.45                         | RT 30°C                         | 80.90 (90.4) | 80.94 (90.4)  | 67.61 (75.6)^
|             |                               |                                 | 87.29 (97.6) | 84.82 (94.8)  | 62.45 (69.8)^
| 5           | 96.67                         | RT 30°C                         | 100.2 (103.6) | 96.42 (99.7)  | 79.52 (82.3)^
|             |                               |                                 | 94.65 (97.9) | 92.71 (95.9)  | 80.55 (83.3)^
| 6           | 80.54                         | RT 30°C                         | 83.97 (104.3) | 80.73 (100.2) | 73.37 (91.1) |
|             |                               |                                 | 82.91 (102.9) | 84.33 (104.7) | 70.24 (87.2)^
| 7           | 82.79                         | RT 30°C                         | 81.24 (98.1) | 76.63 (92.6)  | 73.92 (89.3)^
|             |                               |                                 | 87.67 (105.9) | 79.83 (96.2)  | 67.16 (81.1)^
| 8           | 82.71                         | RT 30°C                         | 81.79 (98.9) | 77.57 (95.7)  | 74.15 (89.8)^
|             |                               |                                 | 86.96 (105.1) | 77.57 (93.8)  | 74.15 (89.6)^

RT, room temperature (approx. 25°C in this work).
* Recovery rate is outside the range of 90–110%.

In the case of storage at room temperature, the measured concentrations of biomarkers such as Aβ1–40, Aβ1–42, Tau and α-synuclein remained almost unchanged in all individuals for the times to blood processing of 3.5 and 5.5 h. For a time to blood processing of 8 h, the measured concentrations of these biomarkers, except in the case of Aβ1–42 in some individuals, are significantly reduced compared to baseline concentrations. These results reveal that the measured Aβ1–42 concentrations are the most consistent for times to processing from 0.5 to 8 h. We could obtain spurious results of measured concentrations for the other 3 biomarkers with times to blood processing of 8 h. Thus, the time to centrifugation after blood draw should not be longer than 5.5 h, to ensure accuracy in the measured concentrations of these biomarkers.

**Discussion**

In the case of storage at room temperature, the measured concentrations of biomarkers such as Aβ1–40, Aβ1–42, Tau and α-synuclein remained almost unchanged in all individuals for the times to blood processing of 3.5 and 5.5 h. For a time to blood processing of 8 h, the measured concentrations of these biomarkers, except in the case of Aβ1–42 in some individuals, are significantly reduced compared to baseline concentrations. These results reveal that the measured Aβ1–42 concentrations are the most consistent for times to processing from 0.5 to 8 h. We could obtain spurious results of measured concentrations for the other 3 biomarkers with times to blood processing of 8 h. Thus, the time to centrifugation after blood draw should not be longer than 5.5 h, to ensure accuracy in the measured concentrations of these biomarkers.
In the case of storage at 30 °C, the measured Tau levels in the plasma were significantly reduced at the time to blood processing of 5.5 h for some individuals. Notably, none of the biomarkers showed significant reductions in the measured concentrations at a time to blood processing of 5.5 h after storing the blood samples at room temperature. At a time to blood processing of 8 h, significant reductions in the measured concentrations, i.e., recovery rates < 90%, of Aβ 1–40, Tau, and α-synuclein occurred more frequently in all 8 subjects when the blood samples were stored at 30 °C storage (vs. at room temperature). The breakdown of the accuracy of assays of these dementia biomarkers happened more frequently when blood samples were stored at higher temperatures after blood draw.

Notably, according to the results listed in Tables 2–4, no subject showed a recovery rate <90% or >110%, regardless of whether the blood samples were stored at room temperature or 30 °C in the case of Aβ1–42. This implies that Aβ1–42 could remain stable in whole-blood samples. This would be possible because soluble Aβ1–42 in the blood sticks easily to proteins such as albumin, tissue transglutaminase, and apolipoprotein. Several papers have demonstrated that the stability of peptides could be enhanced once they bind to proteins [36, 37].

At a time to blood processing of 8 h at room temperature, 6 of 8 subjects (75%) showed recovery rates <90% for Tau. For Aβ1–40 and α-synuclein, 5 of 8 subjects (62.5%) showed recovery rates <90%. There were no significant changes in Aβ1–42. At a time to blood processing of 8 h at 30 °C, all 8 subjects (100%) showed recovery rates < 90% for Tau. For Aβ1–40 and α-synuclein, 7 of 8 subjects (87.5%) showed recovery rates < 90%. Again, there were no significant changes in Aβ1–42. Although there is only a small number of subjects, the results seem to imply that Tau is the least stable biomarker in the blood. Lower stability of Tau was also reported in our previous study, in which the recovery rate of the Tau concentration in plasma stored at –80 °C remained at 90–100% for 1.5 years [38]. However, the stable storage period at –80 °C for Aβ1–40 and Aβ1–42 can be up to 5 years. The lower stability of Tau can probably be attributed to the truncation of Tau [39, 40].

In clinics, it is rare to assay only one of these biomarkers. For example, to assess the request for an amyloid PET scan, plasma Aβ1–40 and Aβ1–42 are assayed [14]. The eval-

### Table 6. Measured Aβ1–42/Aβ1–40 in plasma and recovery rates at different times

| Subject No. | Baseline concentration | Blood sample storage temperature | Aβ1–42/Aβ1–40 (recovery rate at different times to blood processing, %) |
|-------------|------------------------|----------------------------------|--------------------------------------------------------------------------------|
|             |                        |                                  | at 3.5 h  | at 5.5 h  | at 8 h   |
| 1           | 0.261                  | RT 30°C                          | 0.268 102.7 | 0.264 101.2 | 0.275 105.5 |
|             |                        |                                  | 0.270 103.6 | 0.283 108.6 | 0.289 110.9 |
| 2           | 0.258                  | RT 30°C                          | 0.268 103.9 | 0.258 99.8  | 0.316 122.2 |
|             |                        |                                  | 0.263 101.8 | 0.274 106.0 | 0.305 118.2 |
| 3           | 0.285                  | RT 30°C                          | 0.294 103.1 | 0.303 106.2 | 0.336 117.8 |
|             |                        |                                  | 0.300 105.0 | 0.288 101.1 | 0.335 117.4 |
| 4           | 0.291                  | RT 30°C                          | 0.313 107.4 | 0.298 102.3 | 0.319 109.6 |
|             |                        |                                  | 0.293 100.4 | 0.304 104.4 | 0.322 110.6 |
| 5           | 0.297                  | RT 30°C                          | 0.283 95.1  | 0.306 103.2 | 0.305 102.6 |
|             |                        |                                  | 0.300 100.9 | 0.310 104.4 | 0.346 116.4 |
| 6           | 0.286                  | RT 30°C                          | 0.292 102.3 | 0.294 102.9 | 0.310 108.5 |
|             |                        |                                  | 0.295 103.4 | 0.294 102.8 | 0.349 122.1 |
| 7           | 0.296                  | RT 30°C                          | 0.295 99.5  | 0.299 101.1 | 0.334 112.7 |
|             |                        |                                  | 0.288 97.4  | 0.299 101.0 | 0.327 110.4 |
| 8           | 0.260                  | RT 30°C                          | 0.264 101.9 | 0.272 104.7 | 0.265 102.1 |
|             |                        |                                  | 0.266 102.5 | 0.263 101.5 | 0.273 105.2 |

RT, room temperature (approx. 25°C in this work).

*Recovery rate is outside the range of 90–110%.*
Stability of Proteins in Blood with Variations in Time to Centrifugation

The ratio of plasma Aβ1–42 and Aβ1–40 concentrations, i.e., Aβ1–42/Aβ1–40, of the 8 subjects at times to blood processing of 0.5–8 h are listed in Table 6. When the collected blood samples were stored at room temperature for 5.5 h, the recovery rates of all subjects were within the range of 95.1–107.4%. For blood samples that were stored at room temperature for 8 h, some subjects showed recovery rates >110%. These results reveal that there were no significant changes in plasma Aβ1–42/Aβ1–40 until the storage time before centrifugation reached 5.5 h at room temperature for the 8 subjects.

When the storage temperature of the collected blood samples was 30 °C, significant changes in measured plasma Aβ1–42/Aβ1–40 were observed at 8 h. Seven of 8 subjects (87.5%) showed significant changes in measured plasma Aβ1–42/Aβ1–40 when centrifugation was delayed until 8 h after blood draw. 

The product of plasma Aβ1–42 and Tau concentrations, i.e., Aβ1–42 × Tau, of the 8 subjects at times to blood processing from 0.5 to 8 h are listed in Table 7. The recovery rates of all subjects were within the range of 90–110% when the collected blood samples were stored at room temperature for 5.5 h. For blood samples that were stored at room temperature for 8 h, 7 of 8 subjects (87.5%) showed recovery rates <90%. These results reveal that there were no significant changes in plasma Aβ1–42 × Tau when the storage time before centrifugation reached 5.5 h at room temperature for the 8 subjects.

Notably, the subjects enrolled in this study were normal controls, not demented patients. The stability of plasma biomarkers of demented patients might be weaker than that of normal controls. Thus, conservatively speaking, it is suggested to centrifuge blood samples no later than 4.5 h after collection and stored at room temperature.

When the collected blood samples were stored at 30 °C before centrifugation, significant changes in the plasma Aβ1–42 × Tau were observed at 5.5 h. Four of 8 subjects (50.0%) showed significant changes in measured plasma Aβ1–42 × Tau when centrifugation was delayed until 5.5 h after blood draw.

### Table 7. Measured Aβ1–42 × Tau in plasma and recovery rates at different times

| Subject No. | Baseline concentration | Blood sample storage temperature | Aβ1–42 × Tau (recovery rate at different times to blood processing, %) |
|-------------|-------------------------|-----------------------------------|---------------------------------------------------------------------|
|             |                         | at 3.5 h                          | at 5.5 h                         | at 8 h                         |
| 1           | 330.9                   | RT 30°C                           | 322.1 (97.3)                    | 307.2 (92.8)                    | 281.9 (85.2)*                   |
|             |                         |                                   | 323.7 (97.8)                    | 294.5 (89.0)*                   | 272.2 (82.2)*                   |
| 2           | 322.0                   | RT 30°C                           | 322.2 (100.1)                   | 317.3 (98.5)                    | 259.7 (80.7)*                   |
|             |                         |                                   | 319.5 (99.2)                    | 299.9 (93.1)                    | 247.4 (76.8)*                   |
| 3           | 342.9                   | RT 30°C                           | 318.5 (92.9)                    | 317.8 (92.7)                    | 275.5 (80.3)*                   |
|             |                         |                                   | 319.4 (93.1)                    | 285.7 (83.3)*                   | 262.0 (76.4)*                   |
| 4           | 312.2                   | RT 30°C                           | 310.1 (99.3)                    | 296.7 (95.0)                    | 291.8 (93.4)                    |
|             |                         |                                   | 313.1 (100.3)                   | 294.9 (94.5)                    | 268.5 (86.0)*                   |
| 5           | 347.2                   | RT 30 °C                           | 357.2 (102.9)                   | 330.5 (95.2)                    | 281.1 (81.0)*                   |
|             |                         |                                   | 327.9 (94.4)                    | 296.9 (85.5)*                   | 274.8 (79.1)*                   |
| 6           | 325.8                   | RT 30°C                           | 337.1 (103.5)                   | 321.0 (98.6)                    | 289.3 (88.8)*                   |
|             |                         |                                   | 332.9 (102.2)                   | 299.2 (91.8)                    | 287.0 (91.2)*                   |
| 7           | 334.5                   | RT 30 °C                           | 345.8 (103.4)                   | 302.2 (90.3)                    | 277.0 (82.8)*                   |
|             |                         |                                   | 331.2 (99.0)                    | 285.8 (83.4)*                   | 275.6 (82.4)*                   |
| 8           | 335.8                   | RT 30°C                           | 319.0 (95.0)                    | 328.1 (97.7)                    | 273.4 (81.4)*                   |
|             |                         |                                   | 317.7 (94.6)                    | 311.2 (92.7)                    | 264.0 (78.6)*                   |

RT, room temperature (approx. 25°C in this work).

*Recovery rate is outside the range of 90–110%.
Conclusion

To avoid spurious results for assays of plasma Aβ\textsubscript{1–40}, Aβ\textsubscript{1–42}, Tau, and α-synuclein using IMR, blood samples should be centrifuged within 4.5 h after collection and stored at room temperature. When the collected blood samples are stored at 30 °C, centrifugation should be performed within 3.5 h after blood draw.

Statement of Ethics

All subjects were recruited at Taipei Veterans General Hospital, Taiwan. This study was approved by the Hospital Ethics Committee. All research was performed in accordance with relevant guidelines/regulations. All participants provided written informed consent prior to study enrollment.

References

1 Mackenzie IR. The pathology of Parkinson’s disease. BCMJ. 2001;43:142–7.
2 Binder LI, Guillotet-Bongaarts AL, Garcia-Sierra F, Berry RW. Tau, tangles, and Alzheimer’s disease. Biochim Biophys Acta. 2005 Jan;1739(2-3):216–23.
3 Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011 Sep;1(1):a006189.
4 Dickson DW. Parkinson’s disease and parkinsonism: neuropathology. Cold Spring Harb Perspect Med. 2012 Aug 24;4(8):a009258.
5 Vickers JC, Mitev S, Woodhouse A, Fernandez-Martos CM, Kirkcalde MT, Canty AJ, et al. Defining the earliest pathological changes of Alzheimer’s disease. Curr Alzheimer Res. 2016;13(3):281–7.
6 Sipe JD, Benson MD, Buxton JN, Ikeda SI, Merlino G, Saravia MJ, et al. Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines. Amyloid. 2016 Dec;23(4):209–13.
7 Kadavath H, Holele RV, Birnart J, Kumar S, Tepper K, Ural H, et al. Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. Proc Natl Acad Sci USA. 2015 Jun;112(24):7501–6.
8 Schönheit B, Zarski R, Ohm TG. Spatial and temporal relationships between plaques and tangles in Alzheimer-pathology. Neurol Aging. 2004 Jul;25(6):697–711.
9 Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jake S, Goedert M. α-synuclein in Lewy bodies. Nature. 1997 Aug;388(6645):839–40.
10 Nordberg A, Rinne JO, Kadir A, Långström B. The use of PET in Alzheimer disease. Nat Rev Neurol. 2010 Feb;6(2):78–87.
11 Rabinovici GD, Gorton C, Avgar C, Chaudhary K, Gareen I, Hanna L, et al. Association of amyloid positron emission tomography with subsequent change in clinical management among Medicare beneficiaries with mild cognitive impairment or dementia. JAMA. 2019 Apr;321(13):1286–94.
12 Gordon BA, Blazyk TM, Christensen J, Dincer A, Flores S, Keefe S, et al. Tau PET in autosomal dominant Alzheimer’s disease: relationship with cognition, dementia and other biomarkers. Brain. 2019 Apr;142(4):1063–76.
13 Pantano P, Caramia F, Piallini A. The role of MRI in dementia. Ital J Neurol Sci. 1999; 20(5 Suppl):S250–3.
14 Fan LY, Tzen KY, Chen YF, Chen TF, Lai YM, Yen RF, et al. The relation between brain amyloid deposition, cortical atrophy, and plasma biomarkers in amnesic mild cognitive impairment and Alzheimer’s disease. Front Aging Neurosci. 2018;10:175.
15 Peng S, Doudet DJ, Dhawan V, Ma Y. Dopaamine: PET Imaging and Parkinson Disease. PET Clin. 2013 Oct;8(4):469–85.
16 Aono A, Singh PK, Jacob RS, Maji SK. CSF biomarkers for Alzheimer’s disease diagnosis. Int J Alzheimers Dis. 2010 Jun;2010:606802–1–12.
17 Kim D, Paik JH, Shin DW, Kim HS, Park CS, Kang JH. What is the clinical significance of cerebrospinal fluid biomarkers in Parkinson’s disease? Is the significance diagnostic or Prognostic? Exp Neurobiol. 2014 Dec;23(4):352–64.
18 Stav AL, Johansen KK, Auning E, Kelheim LF, Søhns P, Bjørnerud A, et al. Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson’s disease: a cross-sectional study. npj Parkinsons Dis. 2016;2:150301–17.
19 Doecke JD, Rembach A, Villemagne VL, Varghese S, Rainey-Smith SM, Sarros S, et al.; AIBL Research Group. Concordance between cerebrospinal fluid biomarkers with Alzheimer’s disease pathology between three independent assay platforms. J Alzheimer’s Dis. 2018;61(1):169–83.
20 Alcolea D, Pegueroles J, Muñoz L, Camacho V, López-Mora D, Fernández-León A, et al. Agreement of amyloid PET and CSF biomarkers for Alzheimer’s disease on Lumipulse. Ann Clin Transl Neurol. 2019 Sep;6(9):1815–24.
21 Oliver KG, Kettman JR, Fulton RJ. Multiplexed analysis of human cytokines by use of the FlowMetrix system. Clin Chem. 1998 Sep;44(9):2057–60.
22 Birkmann E, Henke F, Weinmann N, Dumptak C, Groschup M, Funke A, et al. Counting of single prion particles bound to a capture-antibody surface (surface-FIDA). Vet Microbiol. 2007 Aug;123(4):294–304.
23 Xia W, Yang T, Shankar G, Smith IM, Shen Y, Walsh DM, et al. A specific enzyme-linked immunosorbent assay for measuring beta-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease. Arch Neurol. 2009 Feb;66(2):190–9.
24 Oh ES, Mielke MM, Rosenberg PB, Jain A, Fedarko NS, Lyketsos CG, et al. Comparison of conventional ELISA with electrochemiluminescence technology for detection of amyloid-β in plasma. J Alzheimers Dis. 2010; 21(3):769–73.
25 Kim JS, Auh HS, Cho SM, Lee JE, Kim Y, Lee C. Detection and quantification of plasma amyloid-β by selected reaction monitoring mass spectrometry. Anal Chim Acta. 2014 Aug;840:1–9.

Conflict of Interest

J.-F.C., H.-C.L., H.-H.C., and S.-Y.Y. are employees of MagQu Co., Ltd. Shieh-Yueh Yang is a shareholder of MagQu Co., Ltd. S.-Y.Y. and W.-P.C. are employees of MagQu LLC. J.-L.J. is an employee of Bio-Check Laboratories, Ltd. P.-N.W. has nothing to disclose.

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Author Contributions

J.-F.C. performed the blood draw and plasma preparation. H.-C.L. conducted statistics. H.-H.C. performed the IMR measurements. W.-P.C., J.-L.J., and S.-Y.Y. prepared the manuscript. P.-N.W. enrolled subjects.
31 Chang CW, Yang SY, Yang CC, Chang CW, Wu YR. Plasma and serum alpha-synuclein as a biomarker of diagnosis in patients With Parkinson’s disease. Front Neurol. 2020 Jan; 10:1388.

32 Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem. 1998 Jun;44(6 Pt 1):1325–33.

33 Clark S, Youngman LD, Palmer A, Parish S, Peto R, Collins R. Stability of plasma analytes after delayed separation of whole blood: implications for epidemiological studies. Int J Epidemiol. 2003 Feb;32(1):125–30.

34 Tanner M, Kent N, Smith B, Fletcher S, Lewer M. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Ann Clin Biochem. 2008 Jul;45(Pt 4):375–9.

35 CLSI document GP44-A4. Procedures for the Handling and Processing of Blood Specimens. 4th ed. Approved Guideline; 2010.