ORIGINAL ARTICLE

Short-term and long-term stability of antioxidant species in plasma samples

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ABSTRACT

Introduction: Reactive oxygen species and other oxidants can target various biological macromolecules and the organism responds with internal antioxidant systems. The antioxidant capacity in biological systems can be measured by tests like Plasma Antioxidant Test (PAT test), which evaluates the entire antioxidant capacity rather than just one analyte and is not affected by antioxidant enzymatic activity. Stability of antioxidant species in biological samples is crucial data for reliable measurements since preanalytical degradation of the analyte can affect results significantly. We examined short-term stability of antioxidant species in plasma under different temperature conditions and long-term stability of the analyte in frozen plasma samples.

Methods: Antioxidant species in plasma measured using PAT test, which is designed to assess the antioxidant capacity of plasma by measuring the total iron-reducing capacity in a sample.

Results and Conclusions: Storage of plasma samples for up to 30 hours at 2-8 °C or even at room temperature did not cause substantial bias to the measurement. The influence on results did not exceed the repeatability of the method. Long-term storage of plasma samples up to 12 months at –30 °C did not have a significant impact on results even if the samples were thawed and frozen again.

Keywords: oxidative stress; antioxidant capacity; PAT test; sample stability

INTRODUCTION

Oxidative stress is associated with various diseases, so antioxidant species are widely measured in clinical diagnostics and in research. Oxidative stress is a consequence of imbalance between endogenous or external pro-oxidants and anti-oxidants. Pro-oxidants like reactive oxygen species (ROS) can target proteins, lipids, DNA and other biological macromolecules. The organism responds with internal adaptive antioxidant systems to restore equilibrium. The antioxidant capacity in biological systems can be measured by different tests [1,2], which can evaluate the entire antioxidant capacity or just selected analytes while some tests focus on antioxidant enzymatic activity.

Stability of antioxidant species in biological samples is a crucial data for the reliable measurement since preanalytical errors like degradation of the analyte can affect results significantly. The stability limit for an analyte can be defined as the time-point when the deviation of the result acquires a bias higher than a defined specification [3]. Stability limits should be defined for each analyte and for each sample matrix as a part of method validation in order to guarantee reliable results that are useful in clinical and research context. While the stability of antioxidant species in human plasma at lower temperatures (from -20 °C to -80 °C) has been investigated [4,5], the data regarding the stability at 2-8 and the effect of freeze-thaw cycles is scarce.

The aim of our study was to examine the short-term and long-term stability of antioxidant species in plasma under different temperature conditions and to assess the effect of repeated thawing and freezing.

MATERIALS AND METHODS

Blood samples were collected in tubes containing Li-heparin as the anticoagulant. Plasma was separated after centrifugation (1,500 × g for 10 min). Antioxidant species in plasma were measured using Plasma Antioxidant Test (PAT test; Redox Diagnostics, Philadelphia, PA, USA). PAT test is designed to assess the antioxidant capacity of plasma by measuring its ferric (Fe^{3+}) reducing ability. The reduction of iron ion by reducing substances in a plasma sample causes a color change of chromogenic substrate (thiocyanate), which can be measured photometrically at 505 nm [6]. The antioxidant capacity is measured in Cornelli Units (CU) relative to the tested substrate (1 CU corresponds to the antioxidant capacity of 1.4 µmol/L ascorbic acid). Since ferric ions are naturally occurring in plasma samples, PAT test serves as a measure of the total iron-reducing antioxidant capacity in a biological system. Expected normal values of plasma antioxidant capacity measured with PAT test are between 2000 and 2500 CU [6,7].

To determine the analyte's short-term stability under different temperature conditions, sample aliquots were stored up to 30 hours at room temperature or refrigerated at 2-8 °C. Long-term stability and the impact of freezing and thawing was also evaluated. Frozen sample aliquots were stored up to 12 months at –30 °C to determine the long-term stability of the analyte. At different time-points, one aliquot was analysed in triplicate and discarded after analysis. Another aliquot, which was used for the evaluation of impact of freezing and thawing, was repeatedly thawed and frozen again after each analysis.

In addition to sample stability, the verification of "Within Run" and "Between Run" CV (also named "Repeatability" and "Intermediate precision") in our analytical conditions was performed. Ten samples with antioxidant capacity between 2000 and 2500 CU were analysed in duplicate within the same day to determine within-run CV.

Between-run CV was calculated from repeated measurements over 7 consecutive days.
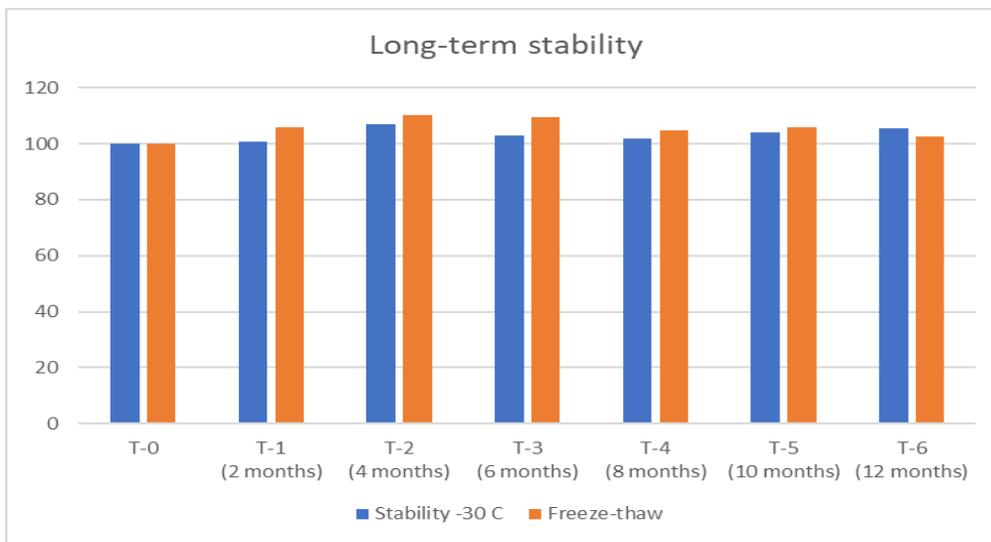
RESULTS AND DISCUSSION

Short-term stability

During the evaluation of short-term stability, there were variations between measurements at different time-points as presented in Figure 1. These variations can be mainly considered as analytical. However, the
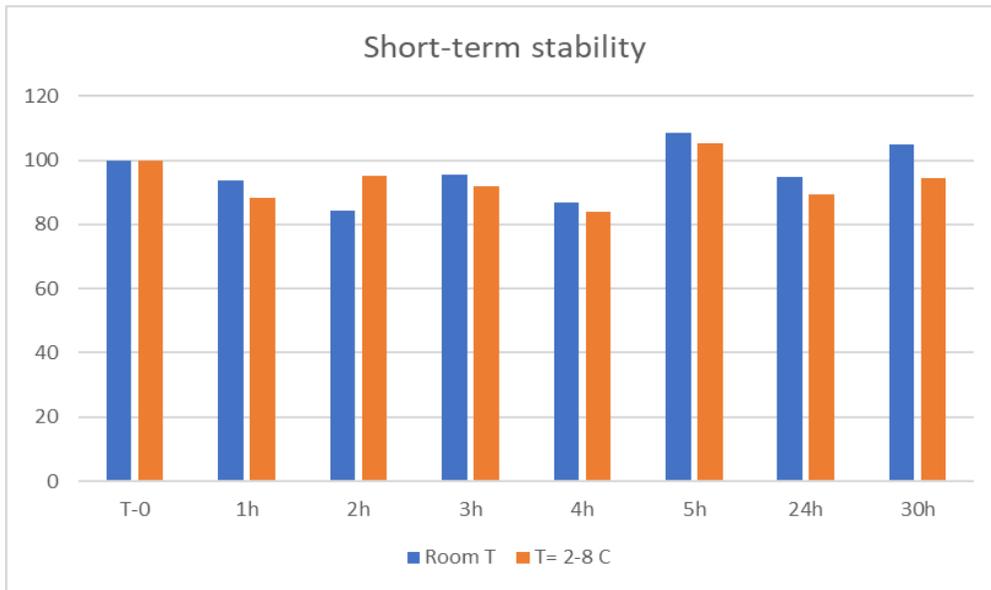
Figure 1. Short-term stability: plasma antioxidant capacity (% of initial value).

Figure 2. Long-term stability: plasma antioxidant capacity (% of initial value).

differences between the measurements of samples stored at room temperature and samples stored at 2-8 °C didn't exceed the variations observed between the time-points.

Long-term stability

Freezing and thawing did not have a significant impact on results compared to samples that were continuously stored at –30 °C (Figure 2). Similar findings regarding long-term stability were published for some other selected components contributing to the total antioxidant capacity [4] measured with various other methods.

"Within Run" and "Between Run" CV

The "Within Run" and "Between Run" CV (also named "Repeatability" and "Intermediate precision") for our analytical conditions was 6.7 % and 8.0 %, consecutively. These results were slightly higher than 5.5 %, which is the CV declared by the producer [6].

CONCLUSIONS

In our research we found that storage of plasma samples up to 30 hours at temperatures 2-8 °C or even at room temperature does not cause substantial bias to the measurement of antioxidant species. The influence of sample storage on scattering of the results in this time-frame does not exceed the repeatability of the method. Long-term storage of plasma samples up to 12 months at –30 °C does not have a significant impact on results even if the samples are thawed and frozen again.

The very good short-term and long-term stability of these antioxidant species could be an important information for any laboratory measuring antioxidant species using this method.

REFERENCES

1. Silvestrini A, Meucci E, Ricerca BM, et al. Total Antioxidant Capacity: Biochemical Aspects and Clinical Significance. Int J Mol Sci. 2023; 24: 10978.

2. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021; 22: 3380.

3. Gómez Rioja R, Martínez Espartosa D, Segovia M, et al. Laboratory Sample Stability. Is It Possible to Define a Consensus Stability Function? An Example of Five Blood Magnitudes. Clin Chem Lab Med. 2018; 56: 1806–1818.

4. Jansen EH, Beekhof PK, Viezeliene D, et al. Long-Term Stability of Oxidative Stress Biomarkers in Human Serum. Free Radic Res. 2017; 51: 970–977.

5. Firuzi O, Mladenka P, Riccieri V, et al. Parameters of Oxidative Stress Status in Healthy Subjects: Their Correlations and Stability after Sample Collection. J Clin Lab Anal. 2006; 20: 139–148.

6. H&D s.r.l.: PAT Test IFU, Italy, 2023.

7. Serena B, Primiterra M, Catalani S, et al. Performance Evaluation of the Innovative PAT Test, Comparison with the Common BAP Test and Influence of Interferences on the Evaluation of the Plasma Antioxidant Capacity. Clin Lab. 2013; 59: 1091–1097.

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