Insights into preservation of blood biomarkers in biobank samples

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INTRODUCTION

A biobank is a collection of human samples with informed consent of the donors and a unique linkage to the donor's health data. Various research fields, e.g. on cancer medicine, benefit from high-quality biobanking, and concomitantly many biobanks have been established worldwide[1]. Overall, donated samples linked to the health data, represent a highly valuable research resource enabling the development of advanced personalized diagnostics and treatment. Furthermore, the obligation to return the raw analysis data derived from the samples back to the biobanks makes this body of data even more valuable over time.[2]

The quality of collected biospecimens and material are crucial to biobanks and researchers utilizing them. Great care is needed when handling blood samples starting from sample collection to long-term storage[3]. The quality of samples is often hampered by preanalytical variability and differences in short- and long-term storage, which are known to affect analyte stability[4]. We examined the stability of commonly measured analyte levels and overall protein degradation in whole blood and plasma samples after various storage conditions. The results provide valuable information both for the biobanks and researchers utilizing them.

MATERIALS AND METHODS

Collection and handling of blood samples

Whole blood samples (4 x 9 ml) were drawn from female volunteers (n = 9) into BD Vacutainer® Li-Heparin tubes (BD Biosciences). After collection, two tubes were centrifuged (2000-2500 x g for 10 min at +4°C) to obtain the plasma fraction. The delay before centrifugation was between 30-60 minutes. The fractionated plasma and whole blood were divided into subgroups stored differently (RT or +4°C) for various lengths (0, 3 and 6 days) as

illustrated in **Figure 1A**. After the incubations, the samples were centrifuged as described above, and the collected plasma was stored at -20°C. Plasma samples frozen immediately served as reference samples. For biomarker analysis, only immediately frozen plasma and plasma stored as whole blood at +4°C for 3d were used. The actual delay between phlebotomy and storage in the Oulu University Hospital catchment area was obtained from the Biobank Borealis of Northern Finland, based on the data of November during years 2017 - 2019.

Measurement of overall protein fragmentation and biomarkers in blood samples

Overall protein degradation was determined in samples from four individuals by separating the fragments by gel electrophoresis (SDS-PAGE, 16% gel) at 120V for 1 h. Fragments were visualized with silver staining, as described[5] and measured with ImageStudio Lite (LICOR) software. Active matrix metalloproteinase (aMMP8) levels were determined in samples up to seven individuals with time-resolved immunofluorometric assay (IFMA) as described earlier.[6] Hormones estradiol and progesterone, thyroid markers (TSH, anti-TPO, FT3, FT4 and anti-Tg) and vitamins active B12 and D 25-OH were measured in samples from five individuals on Architect i2000SR (Abbott) equipment according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed with SPSS Statistics for Windows version 25.0. (IBM Corp.). The real-world biobank delay data were compared with Kruskal-Wallis and levels of aMMP8 and blood biomarkers with Wilcoxon signed-rank test. Values of $p < 0.05$ were considered statistically significant.

Ethical issues

Blood samples were collected as part of the internal quality control procedures of the Biobank Borealis of Northern Finland. No personal data was collected or used for this study. The study was evaluated and approved by the Borealis Biobank scientific committee (BB_2019_3012).

RESULTS AND DISCUSSION

Blood samples arrival to the Biobank Borealis of Northern Finland

In optimal conditions, blood samples are processed and stored immediately after phlebotomy. Yet this is not possible especially in biobanks with large catchment areas, such as Biobank Borealis, which covers 173 000 km^2 and approximately 740 000 persons. As depicted in the **Figure 1B**, the majority (95%) of blood samples collected for Biobank Borealis were stored after ~3 – 80h (average 17-22 h) of sample withdrawal. This time frame is likely to be typical when compared with other biobanks that receive their samples from laboratories scattered within a large geographical area.

The effect of sample handling and storage conditions on overall protein degradation and aMMP8 (neutrophil collagenase) levels

Detection of total protein showed that plasma samples did not have any noticeable degradation, yet the amount of a small fragment (~12-13 kDa, **Figure 2A**) is highest in samples stored for 6d as whole blood, especially RT (**Figure 2B**). Assays using higher sensitivity methods have shown that delay before centrifugation, and storage temperature, affect the intracellular protein levels in plasma samples the most[7]. The degradation might be explained by the breakage of leukocytes during storage, as decreased viability of leukocytes have been reported after 24h storage at RT[8]. As a marker for selective neutrophil degranulation, we evaluated the levels of a proteolytic enzyme from neutrophil granules, aMMP8, in the samples. We observed a

significant increase in aMMP8 levels in most samples stored as whole blood compared to controls (**Figure 2C**), whereas in plasma fractions (free of cells), the levels remained more stable. Thus, in whole blood samples, significant cell lysis might occur, in which released proteinases may participate. The levels of various MMPs have been previously studied in plasma and serum[9], but to our knowledge, our study is the first to examine the stability of aMMP8 over varying storage conditions.

The effect of sample handling and storage conditions on selected analytes

We studied the stability of selected markers after various handling conditions in which the samples currently arrive to the Biobank Borealis, i.e. stored as whole blood up to 3 days at +4°C (near the maximum observed freezing delay time). The levels of all the selected analytes determined remained stable during the storage (**Figure 3**), in line with what has been previously published for majority of these analytes[10]. Yet some analytes, such as estradiol, are more sensitive to delays in processing[11]. The variation detected during storage in analytes, except anti-TPO, is less than the within-subject variation (VC_I) reported before[12].

CONCLUSION

We provide novel information on the possible factors affecting the whole blood and plasma sample stability under varying storage conditions. Although our study analyzed a relatively low number of samples, we believe that our data is valid in emphasizing the importance of constant high quality of the biobanked specimens to obtain reliable results. Moreover, quality management of sample handling by biobanks is crucial to enable scientists to consider the suitability of the samples for their purposes.

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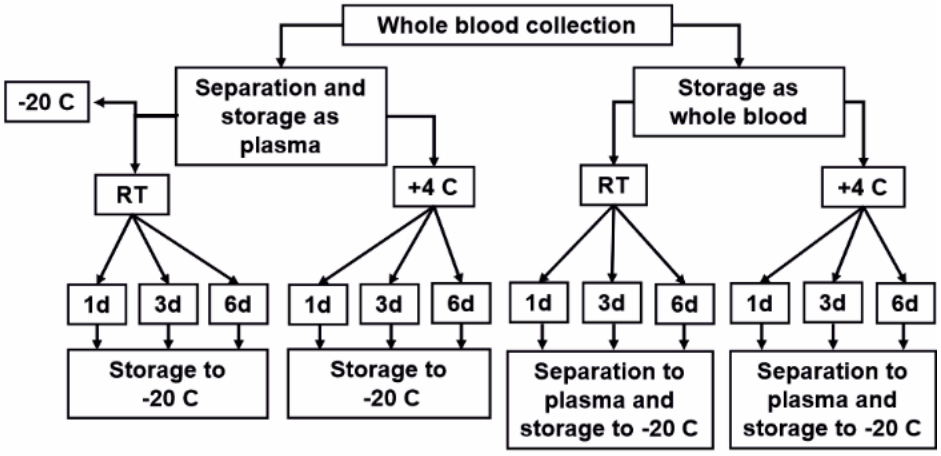
A



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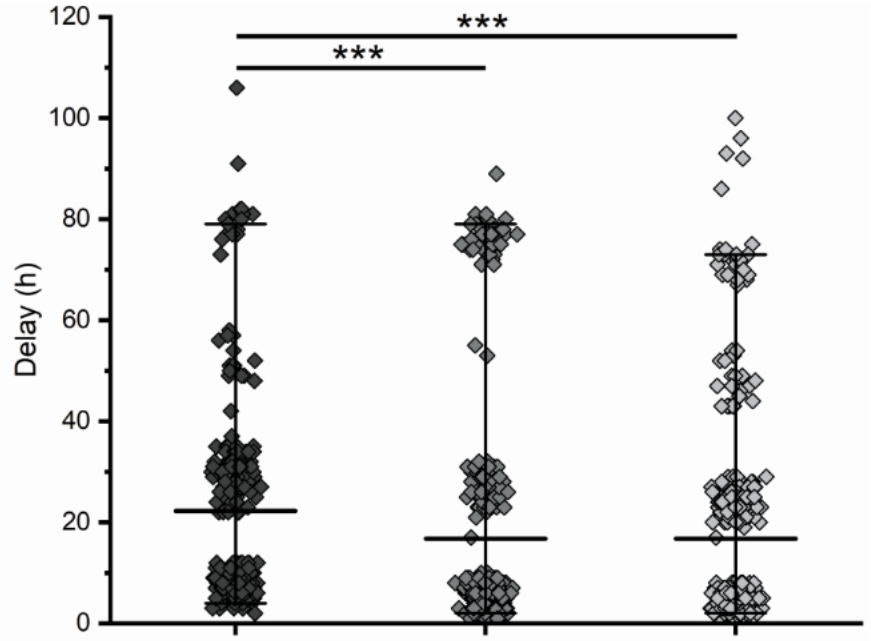

Month, year	November 2017	November 2018	November 2019
n	375	460	451
n (0-6h)	52 (13,9%)	215 (46,7%)	219 (48,6%)
n (6-24h)	143 (38,1%)	133 (28,9%)	120 (26,6%)
n (>24h)	180 (48,0%)	112 (24,3%)	112 (24,8%)
Mean (h)	22,24	16,73	16,73
Median (h)	22,00	7,00	7,00
Min-Max (h)	2-106	0-89	0-100

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158 **Figure 1.** The collection of blood samples for this study and in Northern Finland Biobank

159 Borealis. **A**) Blood sample storage conditions prior to freezing depicted as a flow chart. **B**)

160 Sample collection delays in hours in Northern Finland Biobank Borealis in November 2017-

2019. Black line depicts average delay, the whiskers depict 95% of sample population and *** depicts p <0.001.

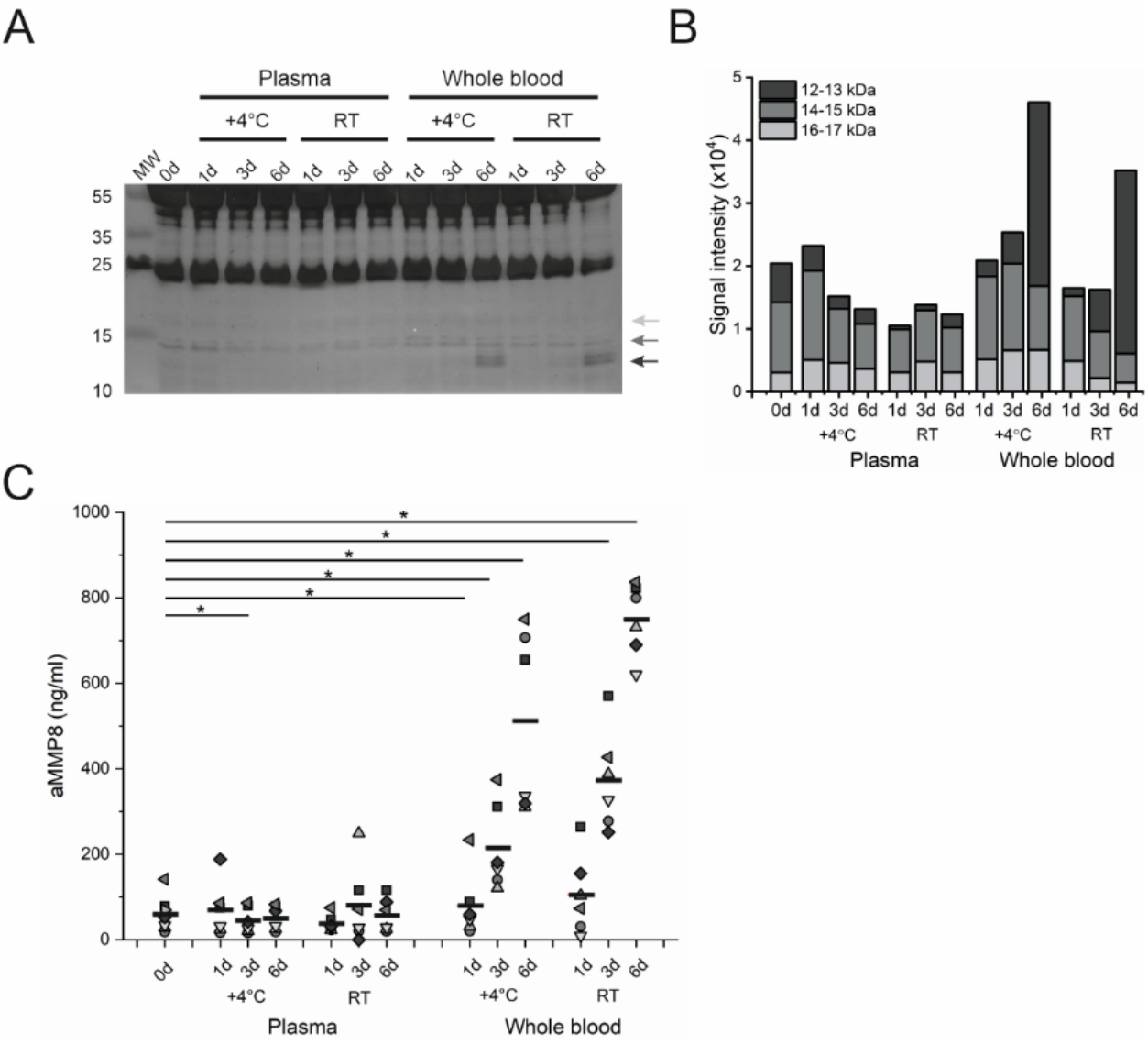

Figure 2. Analysis of **A-B**) overall degradation (SDS-PAGE, representative image and quantification (of quadruplicate experiments)) and **C**) active MMP8 (aMMP8) levels as measured by IFMA in blood samples (n=7 for 0d, n=6 for other timepoints). Shape and color in the symbols depicts related samples. Black line depicts average (**C**) and grey arrows (**A**) represent measured fragments (**B**). * depicts p < 0.05.

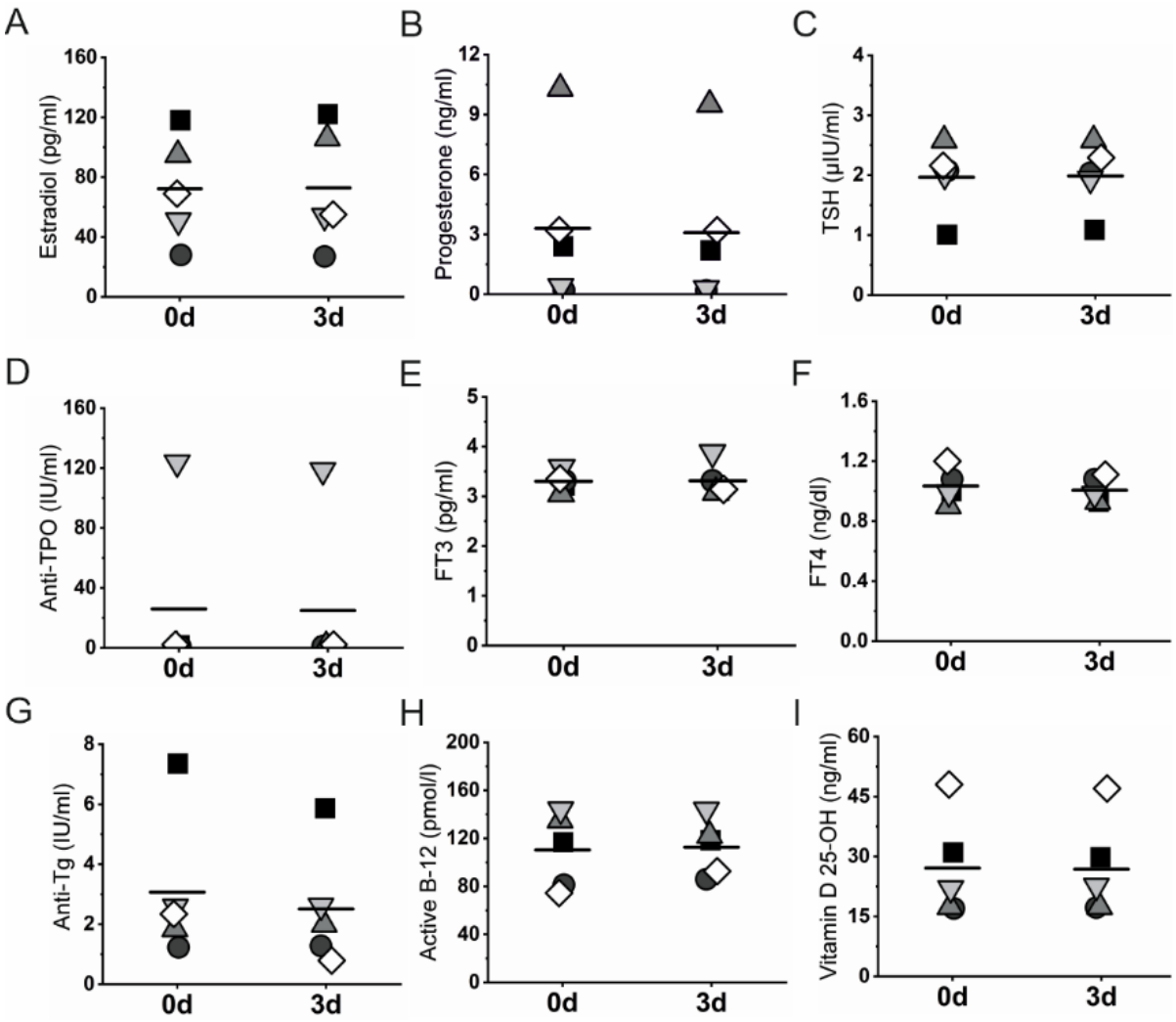

Figure 3. Analysis of levels of **A**) estradiol, **B**) progesterone, **C**) thyroid stimulating hormone (TSH), **D**) Anti-TPO, **E**) free triiodothyronine (FT3), **F**) free thyroxine (FT4), **G**) Anti-Tg, **H**) active B12 and **I**) vitamin D 25-OH in (Abbott ARCHITECT i2000SR, n=5) in plasma samples frozen immediately or after 3d storage as whole blood at +4°C. Shape and color in the symbols depicts related samples and black line depicts average.