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1 **Insights into preservation of blood biomarkers in biobank**

2 **samples**

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21 Search terms: blood biomarker, preservation, degradation, quality control, biobanking

22 INTRODUCTION

23 A biobank is a collection of human samples with informed consent of the donors and a unique
24 linkage to the donor's health data. Various research fields, e.g. on cancer medicine, benefit
25 from high-quality biobanking, and concomitantly many biobanks have been established
26 worldwide¹. Overall, donated samples linked to the health data, represent a highly valuable
27 research resource enabling the development of advanced personalized diagnostics and
28 treatment. Furthermore, the obligation to return the raw analysis data derived from the samples
29 back to the biobanks makes this body of data even more valuable over time.²

30 The quality of collected biospecimens and material are crucial to biobanks and
31 researchers utilizing them. Great care is needed when handling blood samples starting from
32 sample collection to long-term storage³. The quality of samples is often hampered by
33 preanalytical variability and differences in short- and long-term storage, which are known to
34 affect analyte stability⁴. We examined the stability of commonly measured analyte levels and
35 overall protein degradation in whole blood and plasma samples after various storage
36 conditions. The results provide valuable information both for the biobanks and researchers
37 utilizing them.

38

39 MATERIALS AND METHODS

40

41 *Collection and handling of blood samples*

42 Whole blood samples (4 x 9 ml) were drawn from female volunteers (n = 9) into BD
43 Vacutainer® Li-Heparin tubes (BD Biosciences). After collection, two tubes were centrifuged
44 (2000-2500 x g for 10 min at +4°C) to obtain the plasma fraction. The delay before
45 centrifugation was between 30-60 minutes. The fractionated plasma and whole blood were
46 divided into subgroups stored differently (RT or +4°C) for various lengths (0, 3 and 6 days) as

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

53

54 *Measurement of overall protein fragmentation and biomarkers in blood samples*

55 Overall protein degradation was determined in samples from four individuals by separating the
56 fragments by gel electrophoresis (SDS-PAGE, 16% gel) at 120V for 1 h. Fragments were
57 visualized with silver staining, as described⁵ and measured with ImageStudio Lite (LICOR)
58 software. Active matrix metalloproteinase (aMMP8) levels were determined in samples up to
59 seven individuals with time-resolved immunofluorometric assay (IFMA) as described earlier.⁶
60 Hormones estradiol and progesterone, thyroid markers (TSH, anti-TPO, FT3, FT4 and anti-Tg)
61 and vitamins active B12 and D 25-OH were measured in samples from five individuals on
62 Architect i2000SR (Abbott) equipment according to the manufacturer's instructions.

63

64 *Statistical analysis*

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

69

70 *Ethical issues*

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

74

75 **RESULTS AND DISCUSSION**

76

77 *Blood samples arrival to the Biobank Borealis of Northern Finland*

78 In optimal conditions, blood samples are processed and stored immediately after phlebotomy.
79 Yet this is not possible especially in biobanks with large catchment areas, such as Biobank
80 Borealis, which covers 173 000 km² and approximately 740 000 persons. As depicted in the
81 **Figure 1B**, the majority (95%) of blood samples collected for Biobank Borealis were stored
82 after ~3 – 80h (average 17-22 h) of sample withdrawal. This time frame is likely to be typical
83 when compared with other biobanks that receive their samples from laboratories scattered
84 within a large geographical area.

85

86 *The effect of sample handling and storage conditions on overall protein degradation and* 87 *aMMP8 (neutrophil collagenase) levels*

88 Detection of total protein showed that plasma samples did not have any noticeable degradation,
89 yet the amount of a small fragment (~12-13 kDa, **Figure 2A**) is highest in samples stored for
90 6d as whole blood, especially RT (**Figure 2B**). Assays using higher sensitivity methods have
91 shown that delay before centrifugation, and storage temperature, affect the intracellular protein
92 levels in plasma samples the most⁷. The degradation might be explained by the breakage of
93 leukocytes during storage, as decreased viability of leukocytes have been reported after 24h
94 storage at RT⁸. As a marker for selective neutrophil degranulation, we evaluated the levels of
95 a proteolytic enzyme from neutrophil granules, aMMP8, in the samples. We observed a

96 significant increase in aMMP8 levels in most samples stored as whole blood compared to
97 controls (**Figure 2C**), whereas in plasma fractions (free of cells), the levels remained more
98 stable. Thus, in whole blood samples, significant cell lysis might occur, in which released
99 proteinases may participate. The levels of various MMPs have been previously studied in
100 plasma and serum⁹, but to our knowledge, our study is the first to examine the stability of
101 aMMP8 over varying storage conditions.

102

103 *The effect of sample handling and storage conditions on selected analytes*

104 We studied the stability of selected markers after various handling conditions in which the
105 samples currently arrive to the Biobank Borealis, i.e. stored as whole blood up to 3 days at
106 +4°C (near the maximum observed freezing delay time). The levels of all the selected analytes
107 determined remained stable during the storage (**Figure 3**), in line with what has been previously
108 published for majority of these analytes¹⁰. Yet some analytes, such as estradiol, are more
109 sensitive to delays in processing¹¹. The variation detected during storage in analytes, except
110 anti-TPO, is less than the within-subject variation (VC_I) reported before¹².

111

112 **CONCLUSION**

113

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

120

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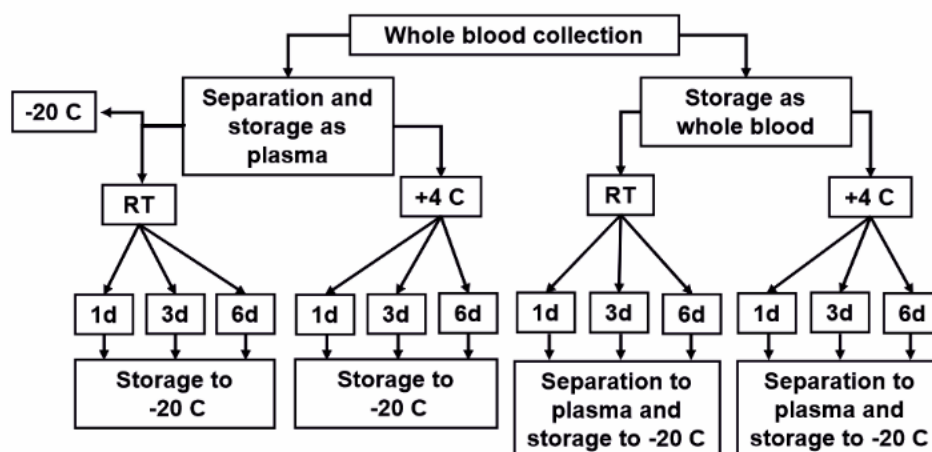
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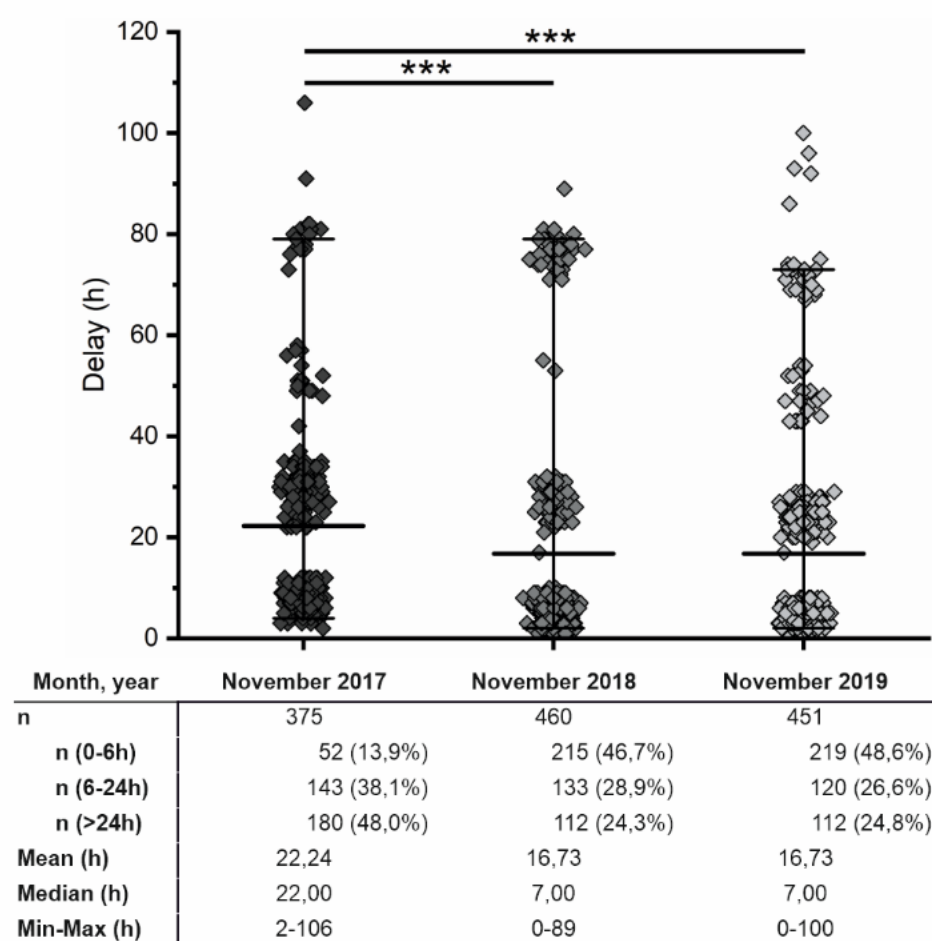
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A



B



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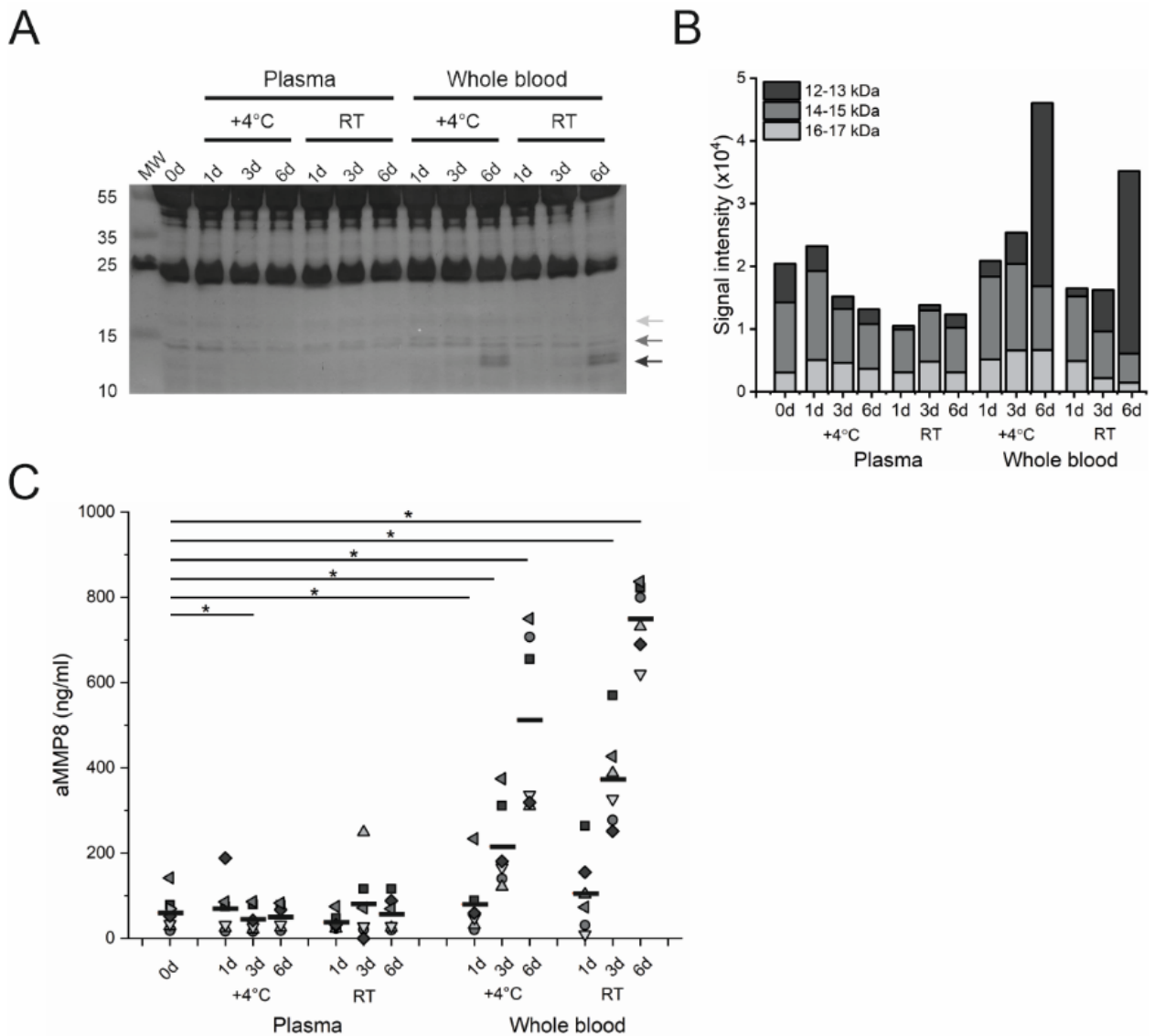
158 **Figure 1.** The collection of blood samples for this study and in Northern Finland Biobank
 159 Borealis. **A)** Blood sample storage conditions prior to freezing depicted as a flow chart. **B)**

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

161 2019. Black line depicts average delay, the whiskers depict 95% of sample population and

162 *** depicts $p < 0.001$.

163



164

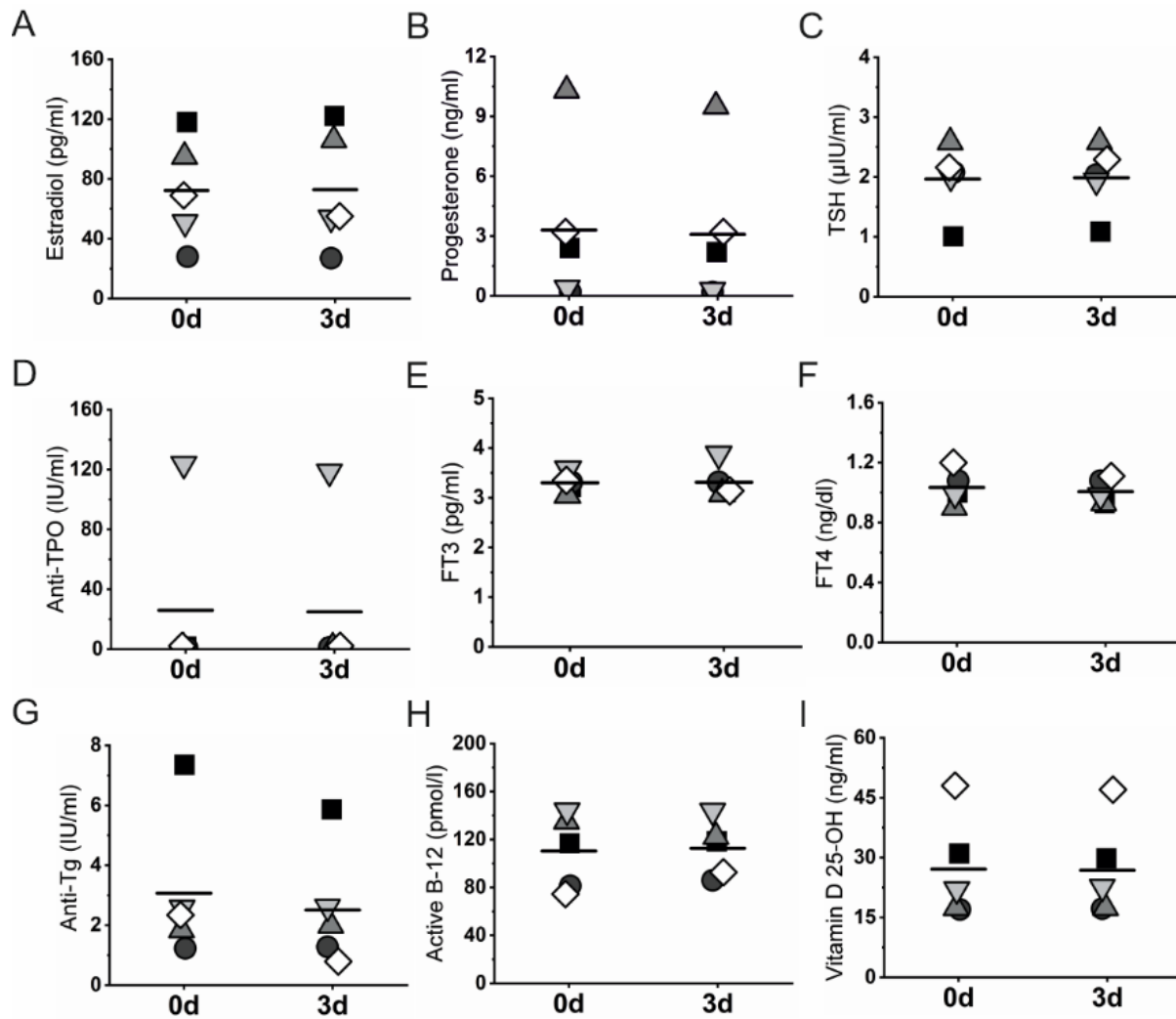
165 **Figure 2.** Analysis of **A-B**) overall degradation (SDS-PAGE, representative image and

166 quantification (of quadruplicate experiments)) and **C**) active MMP8 (aMMP8) levels as

167 measured by IFMA in blood samples ($n=7$ for 0d, $n=6$ for other timepoints). Shape and color

168 in the symbols depicts related samples. Black line depicts average (**C**) and grey arrows (**A**)

169 represent measured fragments (**B**). * depicts $p < 0.05$.



170

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

176