Article ID: WMC002275 2046-1690

Does Long-term Storage Alter the Haemostatic Potential and Balance of Transfusion Plasma After Thawing?

Corresponding Author:
Dr. Andrea Heger,
Octapharma PPGmbH, Research & Development - Austria

Submitting Author:
Dr. Andrea Heger,
Octapharma PPGmbH, Research & Development - Austria

Article ID: WMC002275

Article Type: Original Articles

Submitted on:28-Sep-2011, 09:43:07 AM GMT **Published on:** 29-Sep-2011, 10:43:11 AM GMT

Article URL: http://www.webmedcentral.com/article_view/2275

Subject Categories:BLOOD TRANSFUSION

Keywords:FFP, Stability, Thawed plasma, Liquid plasma bank, Thrombin generation potential, Haemostatic potency

How to cite the article: Heger A , Roemisch J . Does Long-term Storage Alter the Haemostatic Potential and Balance of Transfusion Plasma After Thawing? . WebmedCentral BLOOD TRANSFUSION 2011;2(9):WMC002275

Competing Interests:

The authors declair no competing interests.

Does Long-term Storage Alter the Haemostatic Potential and Balance of Transfusion Plasma After Thawing?

Author(s): Heger A , Roemisch J

Abstract

Recently, storage of thawed plasma for up to 7 days was discussed as a potential solution to eliminate time loss for thawing in the supply of fresh-frozen plasma (FFP) in bleeding emergencies.

The objective of our study was to assess the quality and haemostatic potency of thawed FFP units during long-term storage at either +4°C or room temperature. A substantial reduction of activities of several coagulation factors and inhibitors, especially of FV, FVIII and protein S, occurred during storage at both temperatures. Activation markers of coagulation and fibrinolysis, such as FVIIa, TAT, F1+2 or D-dimer, increased to different extents. Furthermore, thrombin generation potential substantially decreased, accompanied with a prolongation of the lag times. Single FFP units became extremely turbid during long-term storage of thawed plasma and shifted to acidic pH levels.

Based on these data, thawed plasma should be administered in compliance with current transfusion guidelines.

Introduction

Guidelines have been established specifying the thawing and storage conditions for single-donor FFP [1-4]. Once thawed, FFP should not be refrozen and should be administered as soon as possible. If there is any delay in transfusion, FFP should be stored at +1-6°C in an approved blood storage refrigerator. Different regulatory authorities recommend transfusion within 4 [1] to 6 hours [2], while other transfusion guidelines allow maximum storage times at +4°C for up to 24 hours [3,4]. If FFP is stored longer than 24 hours, the words “fresh frozen” must be removed [4]. The extended post-thaw storage results in activity losses of labile coagulation factors and inhibitors, especially of FVIII and protein S [5-9]. However, it was reported that all activities remained within the reference range required by quality assurance regulations even after 7 days storage at +4°C of thawed FFP. Based on these data, the establishment of a +4°C-liquid plasma bank was recommended by some working groups [8,9], with the aim to improve the rapid availability of plasma in emergency situations and to optimize plasma use and costs for transfusion.

Before modifying clinical practices by establishment of liquid plasma banks, it would be prudent to investigate the effectiveness of thawed stored plasma compared to fresh thawed FFP in clinical studies. Multiple studies have already shown an association between poor outcome and the age of transfused blood [10-12]. However, these studies did not include detailed investigation of storage times and conditions. In addition to single factor/inhibitor quantification, cold activation of haemostatic factors [13-17], contact activation [14-16,18] and/or activation of the complement system [19,20], as well as the possible impact of storage on the haemostatic potency and the clot forming ability of thawed plasma [21,22] must be taken into consideration. All these additional quality parameters were not investigated in the studies recommending the long-term storage of thawed FFP.

The objective of this study was to assess the quality and haemostatic integrity of single-donor FFP units during 7 days storage at +4°C and room temperature (RT). Beside the important coagulation factors [i.e. factors V (FV), VII (FVII), VIII (FVIII), IX (FIX) and XI (FXI)] and protease inhibitors [protein C, protein S and plasmin inhibitor (also known as α2-antiplasmin), C1-inhibitor], thrombin generation assay (TGA) was used to study the overall haemostatic potential in vitro. Markers of activated coagulation and fibrinolysis [activated factor VII (FVIIa), thrombin-antithrombin complex (TAT), prothrombin fragments 1+2 (F1+2), D-dimer)] were quantified in addition. Moreover, pH values, lipid and lipoprotein levels and plasma turbidity were measured as quality control attributes.

Methods

Stability studies

Eight single-donor FFP units of different plasma sources (recovered and source plasma) and different blood groups (blood groups A and O) were included. For the stability studies, plasma containers or plasma bags were thawed in a water bath at +37°C according

to standard operating procedures [1,3,4]. Hundred milliliters of thawed plasma were transferred under sterile conditions into plastic plasma containers, aliquots of which were stored at +4°C ± 2°C in a temperature-controlled cooling room and in parallel at RT (+20-25°C). Samples were collected immediately after thawing (time 0 or baseline levels), as well as after 1, 3 and 7 days storage at +4°C and RT. Each sample was aliquoted (10 aliquots of 1 ml), frozen and stored at -30°C until testing. Prior to analysis, the plasma aliquots were thawed at timed intervals and were tested immediately in the same setting. Residual thawed aliquots were discarded.

Analytical assays

Analytical testing was performed at Octapharma's Research & Development Plasma department using commercially available test kits and standard operation procedures, as described previously [17,23]. aPTT was assessed by the respective clotting assay (APTT lyophilised silica, Instrumentation Laboratories, IL, Milan, Italy). The activities of FV, FVII, FIX and FXI were determined by one-stage coagulation assays, using human deficient plasmas (IL). Protein C and protein S activities were tested by aPTT-based clotting assays (HemosIL ProClot, and HemosIL Protein S, IL), while FVIII and plasmin inhibitor activities were quantified using chromogenic substrate assays (Coamatic FVIII test kit, Chromogenix, Milan, Italy; HemosIL Plasmin Inhibitor, IL). FVIIa was tested using a prothrombin time-based clotting assay (Staclot VIIa-rTF clotting assay, Diagnostica Stago, Asnières, France). TGA was performed using the Technothrombin TGA test kit from Technoclone GmbH (Vienna, Austria) [24]. ELISA test kits were used for the quantitative determination of the prothrombin split product F1+2 (Enzygnost F1+2, monoclonal, Siemens Healthcare Diagnostics), TAT (Enzygnost TAT micro, Siemens Healthcare Diagnostics, Vienna, Austria) and D-dimer levels (Asserachrom D-Dimer, Diagnostica Stago). Cholesterol, triglycerides, phospholipids B and apolipoprotein (A) were quantified by enzymatic colorimetric methods (Cholesterol liquicolor and Triglycerides liquicolor, Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany; Phospholipids B, WAKO Chemicals GmbH, Neuss, Germany; Immunozym Lp(a), Progen Biotechnik GmbH, Heidelberg, Germany). In addition, pH values were measured and plasma turbidity was monitored as optical density (OD) at 660 nm using a photometer (Spectra Max Plus; Molecular Devices Corporation, Sunnyvale, CA, USA).

Results are expressed as mean values ± standard deviation (std. dev.). The student paired t-test was used to identify differences in the quality of single-donor FFP units after 7 days storage relative to baseline levels. P-value of < 0.05 was considered significant.

Results

Biochemical characteristics of FFP units during storage at +4°C and RT for 7 days are presented in Illustrations 1-3.

Coagulation factors

Single-donor FFP units showed high variations in coagulation factor activities directly after thawing. Activities below (0.4 IU/ml for FVIII) and above (1.7 IU/ml for FVII and 1.6 IU/ml for FIX) the reference ranges [25] were observed. FIX and FXI levels remained stable during 7 days storage at both temperatures. FV and FVIII activities substantially decreased over time. During storage at RT, FV activity losses were more pronounced, reaching statistical significance after 3 days (FV losses by 45-50%, $p < 0.01$), while statistically significant reduction of FVIII levels (by 40-45%, $p < 0.05$) was observed during storage at both temperatures. FVII levels increased significantly in a single FFP unit (from 1.26 IU/ml to 2.62-5.80 IU/ml and 1.91-2.66 IU/ml after storage at +4°C and RT, respectively). Differently, in all other FFP units FVII activities decreased by 10-40% during storage at both temperatures.

Protease inhibitors

A substantial variation in activities of protease inhibitors was observed among different FFP units at baseline (0.72-1.22 IU/ml for protein C, 0.60-1.78 IU/ml for protein S, 0.81-1.19 IU/ml for plasmin inhibitor and 0.72-1.22 IU/ml for C1-inhibitor, respectively). At +4°C, protein S levels declined significantly (by approx. 45% after 7 days storage, $p < 0.05$), whereas protein C, plasmin inhibitor and C1-inhibitor activities remained stable throughout the 7 days storage period. After storage at RT for 7 days, all protease inhibitors decreased to different extents; i.e. protein C by 29%, protein S by 76% ($p < 0.005$), plasmin inhibitor by 28% ($p < 0.05$) and C1-inhibitor by 38% ($p < 0.05$), respectively.

Thrombin Generation Assay (TGA)

TGA results are presented in Illustrations 3-4. Peak thrombin (i.e. maximum thrombin concentration) decreased over time during storage at both temperatures, from 311 nM at time 0 to 251 nM and 184 nM ($p < 0.05$) after 7 days storage at +4°C and RT, respectively. Along with a decrease in thrombin generation, lag phase was prolonged from 13.8 min to 14.4 min and 19.6 min after 7 days storage at +4°C

and RT, respectively. In 1 FFP unit, after 7 days storage at RT, no clotting was observed within 60 minutes.

Activation markers of the coagulation and fibrinolytic system

FVIIa levels of single-donor FFP units varied considerably already at baseline (30-121 mIU/ml). There was a substantial activation of FVII to FVIIa in a single FFP unit (FVIIa levels increased from 69 mIU/ml to 613-3159 mIU/ml at +4°C and to 463-648 mIU/ml at RT). This correlated well with the increase of apparent FVII levels in the same FFP unit (Illustration 3). TAT and D-dimer levels remained stable during storage at +4°C, but increased significantly in single FFP units during storage at RT (TAT levels in 4 units by 180-5278%, and D-dimer levels in 2 units by 155-519%). After 7 days storage, F1+2 in single FFP units increased significantly relative to baseline (in 2 units by 151-731% at +4°C or in 5 units by 145-714% at RT), whereas in other FFP units, F1+2 levels remained stable or showed a trend towards lower levels.

Lipids, lipoproteins, turbidity and pH-values

Lipid and lipoprotein levels in the single-donor FFP units were measured at baseline levels, i.e. directly after thawing; mean levels were 144, 112, 188 and 7.6 mg/dl for cholesterol, triglycerides, phospholipids and lipoprotein (a), respectively (data not shown). There was a direct correlation between triglyceride concentrations and turbidity, i.e. clear FFP units (OD660nm 0.10-0.13) had the lowest triglyceride levels (43-62 mg/dl). The presence of a white layer and/or sediment was distinctive for turbid plasma, especially after centrifugation. Interestingly, 2 FFP units remained clear during 7 days storage at +4°C, but became extremely turbid after 7 days storage at RT (i.e. OD660nm levels increased from 0.10/0.13 to 2.36/2.19; Illustration 3). The same FFP units shifted from neutral to acidic pH values during storage at RT (pH changed from 7.4-7.6 to 5.3-5.8). Turbidity in other FFP units remained stable over time at both temperatures.

Discussion

Plasma is a complex blood product, containing numerous proteins, among them coagulation and fibrinolytic factors and protease inhibitors responsible for the regulation of the overall haemostatic balance. Any disturbances of this balance can cause hyper-coagulant or hyper-fibrinolytic activities resulting in thrombotic or bleeding risks. Although levels of haemostasis factors and inhibitors may vary significantly between individual blood donations, resulting in wide “normal ranges” for FFP, recent reports suggest that thawed FFP would comprise sufficient potential and haemostatic balance even after 5 to 7 days storage [5-9].

In similar studies, the stability of clotting factors and inhibitors during storage for 2-7 days after thawing was investigated in methylene-blue treated FFP and the pharmaceutical plasma products employing S/D treatment to increase pathogen safety. Due to more pronounced changes in clotting factor activities, the use of methylene-blue treated FFP at +4°C for 7 days could not be recommended [8,9]. For thawed S/D plasma (i.e. Octaplas®), sufficient coagulation activity of labile FV and FVIII was reported after 6 days storage at +4°C [26-27] or 5 days storage at RT [28]. Caution was warranted by decreases in protein S and plasmin inhibitor levels in Octaplas® immediately after thawing [17,26,28]. Due to changes in the manufacturing process, activities of both protease inhibitors are remarkable higher in the new generation product OctaplasLG® at baseline levels [29,30] and during storage after thawing (Keller MK, personal communication).

In our studies, single FFP units with clotting factor activities both below (e.g. for FVIII) and above (e.g. for FVII and FIX) the reference ranges [25] were found. During storage at different temperatures, significant decreases of activities of several coagulation factors and inhibitors were observed, in particular of FV, FVIII and protein S. Activity losses were more substantial at higher temperatures (i.e. at RT vs. +4°C) and longer storage periods (i.e. ≥ 3-days vs. ≤ 1-days storage). Activation markers, such as FVIIa, TAT, F1+2 and D-dimer concentrations increased variably in single FFP units, indicating activation of the coagulation and/or fibrinolytic system during storage after thawing, as described previously by different working groups [13-17]. Significant increase of apparent FVII activities (as was the case in single FFP units), together with decreased protein S activities (protein S levels declined significantly in all FFP units during storage at both temperatures), may shift the overall haemostatic balance. In addition, contact activation and/or activation of the complement system in thawed and stored plasma were reported as well. An increase in kallikrein-like activity was accompanied by a decrease in C1-inhibitor activity [15,16,18]. Activation of the complement system was evidenced by a rapid increase in the concentrations of C3 and C4 fragments (i.e. C3a-desArg and C4a-desArg) [19,20]. In our studies, the decrease of C1-inhibitor activities was also confirmed (by 5 and 39% during 7 days storage at +4°C and RT), while complement factors were not

investigated.

Parallel to single factor/inhibitor quantification and the use of established global coagulation assays, we included TGA to study the overall haemostatic potential in vitro. TGA has become state-of-the-art for the assessment of the overall haemostatic potential in vitro, supporting clinical diagnosing, optimization and monitoring of appropriate therapies including blood components. It is current understanding that the in vivo consequences of a low thrombin generation potential can result in insufficient fibrin generation, clot formation and subsequent wound closure enhancing a bleeding risk. In recent studies, Matijevic et al [21-22] showed that despite individual factor levels remained above 50% of normal levels in thawed plasma, which was stored for 5 days at +4°C, the overall haemostatic potential decreased by 58%. While activation of coagulation factors, thus marker proteins/complexes, may suggest an enhanced pro-coagulant potential, we observed substantially decreased thrombin generation capacities and a trend to prolonged lag times in thawed and stored single-donor FFP units at both storage temperatures. Most probably, major contributors to this overall reduced potential are the significantly reduced activities of the cofactors FV and FVIII.

The statistical power of the data is limited by the relatively small number of FFP units investigated. However, our studies confirmed that important coagulation factors and protease inhibitors, especially FV, FVIII and protein S, substantially decrease during storage of thawed plasma. This further increases the clotting factor and inhibitor variability between single-donor FFP units. These changes in single factor levels may be accompanied by activation of the coagulation, fibrinolytic system and/or complement system. TGA parameters reflected the impact on the overall in vitro haemostatic/coagulation potential. As this variability between single plasma units is not known to the treating physician at the time of infusion, insufficient restoration of the in vivo haemostatic capacity may result, if the plasma is stored for longer periods. Administering plasma with impaired thrombin generation capacity may provide adequate haemostasis for minor surgery or in case of single factor deficiencies, however, may be insufficient to correct bleeding and coagulopathy in cases of massive transfusion. In conclusion, based on the above data and previous reports, thawed single-donor FFP units should be administered complying with current recommendations for the use and storage of thawed plasma [1-4], at least until their effectiveness despite individually decreased haemostatic capacity was proven in clinical studies.

Acknowledgement(s)

The authors thank the laboratory staff of Octapharma's Research & Development Plasma Department (in alphabetical order: J. Brainovic, R. Cortes, R. Gruber, S. Janisch, R. Kaiser, E.-M. Kern, N. Langmeyer, A. Pfeiffer, B. Prager, H. Seidl and C. Zapfl) for their excellent technical assistance.

Reference(s)

1. O'Shaughnessy DF, Atterbury C, Bolton Maggs P, Murphy M, Thomas D, Yates S, Williamson LM; Guidelines for the use of fresh frozen plasma, cryoprecipitate and cryosupernatant. British Committee for Standards in Haematology. Blood Transfusion Task Force. Br J Haematol 2004; 126(1):11-28

2. Paul-Ehrlich-Institut, Bekanntmachung der Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie) gemäss §§12 und 18 des Transfusionsgesetzes (TFG) – Änderungen und Ergänzungen 2010 (vom 4. Mai 2010), Bundesanzeiger 2010, 101:2839

3. United Kingdom Blood Transfusion Services. Guidelines for the blood transfusion services in the United Kingdom, 7th ed. 2005, TSO

4. US Food and Drug Administration. Guidance for industry. An acceptable
circular of information for the use of human blood and blood components; 12 September 2003

5. Milam JD, Buzzuro CJ, Austin SF, Stansberry SW. Stability of factors V and VIII in thawed fresh frozen plasma units. Transfusion 1980; 20(5):546-548

6. Sidhu RS, Le T, Brimhall B, Thompson H. Study of coagulation factor activities in apheresed thawed fresh-frozen plasma at 1-6°C for five days. J Clin Apher 2006; 21:224-226

7. Von Heymann C, Keller MK, Spies C, Schuster M, Meinck K,Sander M, Wernecke KD, Kiesewetter H, Pruss A. Activity of clotting factors in fresh-frozen plasma during storage at +4°C over 6 days. Transfusion 2009; 49:913-920

8. Selleng K, Kellner S, Nauck M, Wasner C, Zimmermann K, Wessel A, Greinacher A. Liquid plasma bank: towards a timely supply in bleeding emergencies. Hämostaseologie 2011; 31(1):26-27

9. Thiele T, Kellner S, Hron G, Wasner C, Nauck M, Zimmermann K, Wessel A, Warkentin TE, Greinacher A, Selleng K. Storage of thawed plasma for a liquid plasma bank: impact of temperature and methylene

blue pathogen inactivation. Transfusion 2011, doi: 10.1111/j.1537-2995
10. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, Denny C, Silliman CC. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. Am J Surg 1999; 178(6):570-572
11. Grienwald CE, McCrindle BW, Crawford-Lean L, Holtby H, Parshuram C, Massicotte P, Van Arsdell G. Reconstituted fresh whole blood improves clinical outcomes compared with stored component blood therapy for neonates undergoing cardiopulmonary bypass for cardiac surgery: a randomized controlled trial. J Thoeac Cardiovasc Surg 2008; 136(6):1442-1449
12. Guidet B. Should fresh blood be recommended for intensive care patients Crit Care 2010; 14(3):15
13. Seligsohn U, Zivelin A, Bar-Shani S. Cold-promoted activation of factor VII: is it a problem under blood bank conditions Haemostasis 1983; 13(3):186-191
14. Blömbäck M, Chmielewska J, Netré C, Akerblom O. Activation of blood coagulation, fibrinolytic and kallikrein system during storage of plasma. Vox Sang 1984; 47(5):355-342
15. Omran S, Hallouda M, Essawi F, el Attar I, Aboul Enein A, Gaafar T, Afifi N. Effect of cold storage in the haemostatic cascade system. Transfus Sci 1996; 17(3):407-414
16. Sountaka AM, Silveura A, Söderström T, Nlombäck M. Occurrence of cold activation of transfusion plasma during storage at +4 degrees C. Vox Sang 2005; 88(3):172-180
17. Heger A, Römisch J, Svae TE. Stability od solvent/detergent-treated plasma and single-donor fresh-frozen plasma during 48h after thawing. Transfusion Apher Sci 2005; 33:257-267
18. Norda R, Knutson F, Berseus O, Akerblom O, Nilsson-Ekdahl K, Stegmayr B, Nillson B. Unexpected effects of donor gender on the storage of liquid plasma. Vox Sang 2007; 93(3):223-228
19. Schleunig M, Schmid-Haslbeck M, Utz H, Jochum M, Mempel W, Wilmanns W. Complement activation during storage of blood under normal blood bank conditions. Effects of proteinase inhibitors and leukocyte depletion. Blood 1992; 79(11):3071-3075
20. Norda R, Schött U, Berséus O, Akerblom O, Nilsson B, Ejdahl KN, Stegmayr BG, Knutson EF. Complement activation products in liquid stored plasma and C3a kinetics after transfusion of autologous plasma. Vox Sang 2011, doi: 10.1111/j.1423-0410.2011.01522.x.
21. Matijevic N, Kostousov V, Wang Y-W W, Wade CE, Wang W, Letourneau P, Hartwell E, Kozar R, Ko T, Holcomb JB. Multiple levels of degradation diminish hemostatic potential of thawed plasma. J Trauma 2011; 70(1):71-80
22. Matijevic N, Wang Y-W W, Kostousov V, Wade CE, Vijayan KV, Holcomb JB. Decline in platelet microparticles contributes to reduced hemostatic potential of stored plasma. Thromb Res 2011; 128(1):35-41
23. Heger A, Römisch J, Svae TE. A biochemical comparison of a pharmaceutically licensed coagulation active plasma (Octaplas) with a universally applicable development product (Uniplas) and single-donor FFPs subjected to methylene-blue dye and white-light treatment. Transfus Apher Sci 2006; 35(3):223-233
24. Pock K, Heger A, Janisch S, Svae TE, Römisch J. Thrombin generationcapacity is impaired in methylene-blue treated plasma compared to normal levels in single-donor fresh-frozen plasma, a licensed solvent/detergent-treated plasma (Octaplas) and a development product (Uniplas). Transfus Apher Sci 2007; 37(3):223-231
25. Beeck H, Hellstern P. In vitro characterization of solvent/detergent-treated human plasma and quarantine fresh frozen plasma. Vox Sang 119; 74(1):219-223
26. Buchta C, Felfernig M, Höcker P, Macher M, Körmöczi GF, Quehnberger P, et al. Stability of coagulation factors in thawed, solvent/detergent-treated plasma during storage at 4°C for 6 days. Vox Sang 2004; 87:182-186
27. Keller MK, Pruss A, Spies C, Sander M, Wernecke KD, von Heymann C. Comparison of Clotting Factor Stability in FFP and S/D-treated Plasma and Stored for 6 days at 4°C, Poster 691 SCCM 2009
28. Nifong TP, Light J, Wenk RE. Coagulant stability and sterility of thawed S/D-treated plasma. Transfusion 2002; 42:1581-1584
29. Heger A, Svae TE, Neisser-Svae A, Jordan S, Behizad M, Römisch J. Biochemical quality of the pharmaceuticallylicensed plasma OctaplasLG after implementation of a novel prion protein (PrPSc) removal technology and reduction of the solvent/detergent (S/D) process time. Vox Sang 2009; 97:219-225
30. Lawrie AS, Green L, Canciani MT, Mackie IJ, Peyvandi F, Scully MA, Machin SJ. The effect of prion reduction in solvent/detergent-treated plasma on haemostatic variables. Vox Sang 2010; 99(3):232-238.

Illustrations

Illustration 1

Storage of thawed FFP at 4Ã‚Â°C. Statistically significant differences relative to baseline values: *p < 0.05; #Reference range according to test kits. n.d., not determined.

Parameters	Reference range [25]	Storage time at +4°C after thawing [days]				Change [%] after 7 days
		0	1	3	7	
Factor V [IU/ml]	0.54-1.45	0.66 ± 0.12	0.60 ± 0.14	0.54 ± 0.13	0.59 ± 0.09	-11
Factor VII [IU/ml]	0.62-1.65	1.15 ±0.29	1.52 ± 1.73	1.26 ± 1.28	1.08 ± 0.65	-7
Factor VIII [IU/ml]	0.45-1.68	0.67 ± 0.22	0.45 ±0.19	0.42 ± 0.19*	0.40 ± 0.18*	-62
Factor IX [IU/ml]	0.45-1.48	0.96 ± 0.34	0.96 ± 0.39	0.91 ± 0.32	0.95 ± 0.35	-1
Factor XI [IU/ml]	0.42-1.44	0.84 ± 0.23	0.84 ± 0.23	0.84 ± 0.24	0.83 ± 0.25	-2
aPTT [sec]	28-40	34.8 ± 4.1	37.3 ± 6.2	38.1 ± 5.9	37.3 ± 5.6	+7
Protein C [IU/ml]	0.58-1.64	0.98 ±0.15	0.96 ± 0.15	0.98 ± 0.15	1.00 ± 0.13	+3
Protein S [IU/ml]	0.56-1.68	0.97 ± 0.39	0.75 ± 0.40	0.63 ± 0.32	0.53 ± 0.23*	-45
Plasmin inhibitor [IU/ml]	0.72-1.32	1.03 ± 0.13	1.00 ± 0.11	0.97 ± 0.09	0.99 ± 0.08	-3
C1-inhibitor [IU/ml]	0.60-1.24	0.96 ± 0.15	n.d.	n.d.	0.93 ± 0.26	-5
Factor VIIa [mIU/ml]	25-170	70 ± 27	431 ± 1103	255 ± 639	96 ± 209	+39
TAT [µg/l]	1.0-4.1#	3.0 ± 1.3	n.d.	n.d.	3.1 ± 1.2	+10
F1+2 [nmol/l]	0.07-0.23#	0.19 ± 0.09	n.d.	n.d.	0.28 ± 0.33	+71
D-dimer [ng/ml]	< 500#	108 ± 67	n.d.	n.d.	118 ± 72	+11

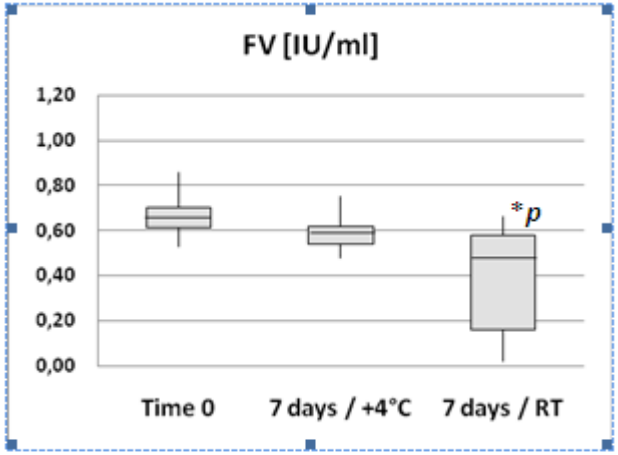
Illustration 2

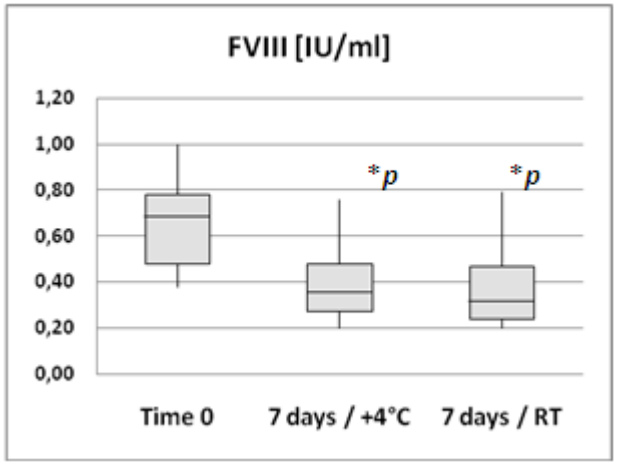
Storage of thawed FFP at RT. Statistically significant differences relative to baseline values: *p < 0.05; **p < 0.01; ***p < 0.005; #Reference range according to test kits. n.d., not determined.

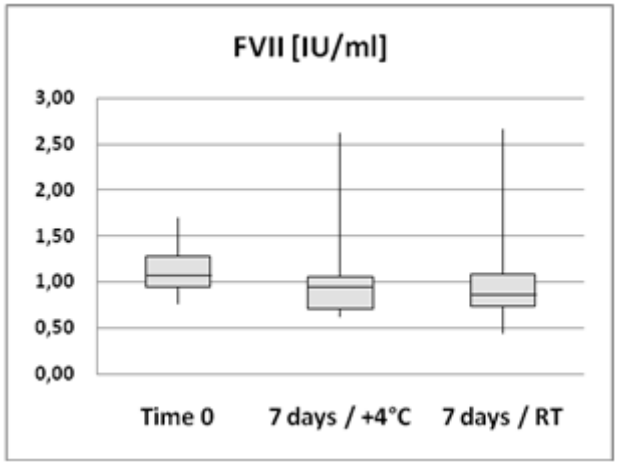
Parameters	Reference range [25]	Storage time at RT after thawing [days]				Change [%] after 7 days
		0	1	3	7	
Factor V [IU/ml]	0.54-1.45	0.66 ± 0.12	0.53 ± 0.13	0.35 ± 0.20**	0.37 ± 0.26*	-44
Factor VII [IU/ml]	0.62-1.65	1.15 ±0.29	1.06 ± 0.27	0.99 ± 0.44	1.05 ± 0.69	-10
Factor VIII [IU/ml]	0.45-1.68	0.67 ± 0.22	0.50 ± 0.20	0.40 ± 0.23	0.37 ± 0.20*	-46
Factor IX [IU/ml]	0.45-1.48	0.96 ± 0.34	0.95 ± 0.34	0.72 ± 0.42	0.81 0.45	-17
Factor XI [IU/ml]	0.42-1.44	0.84 ± 0.23	0.84 ± 0.22	0.82 ± 0.20	0.74 ± 0.26	-12
aPTT [sec]	28-40	34.8 ± 4.1	38.2 ± 4.5	41.4 ± 7.0*	34.3 ± 4.0	+2
Protein C [IU/ml]	0.58-1.64	0.98 ± 0.15	0.98 ± 0.13	0.67 ± 0.40	0.72 ± 0.35	-29
Protein S [IU/ml]	0.56-1.68	0.97 ± 0.39	0.64 ± 0.22	0.23 ± 0.24***	0.21 ± 0.17***	-76
Plasmin inhibitor [IU/ml]	0.72-1.32	1.03 ± 0.13	0.98 ± 0.08	0.80 ± 0.23*	0.74 ± 0.29*	-28
C1-inhibitor [IU/ml]	0.60-1.24	0.96 ± 0.15	n.d.	n.d.	0.62 ± 0.39*	-38
Factor VIIa [mIU/ml]	25-170	70 ± 27	56 ± 22	141 ± 158	159 ± 216	+138
TAT [µg/l]	1.0-4.1#	3.0 ± 1.3	n.d.	n.d.	30.3 ± 48.2	+1288
F1+2 [nmol/l]	0.07-0.23#	0.19 ± 0.09	n.d.	n.d.	0.52 ± 0.44	+219
D-dimer [ng/ml]	< 500#	108 ± 67	n.d.	n.d.	134 ± 87	+52

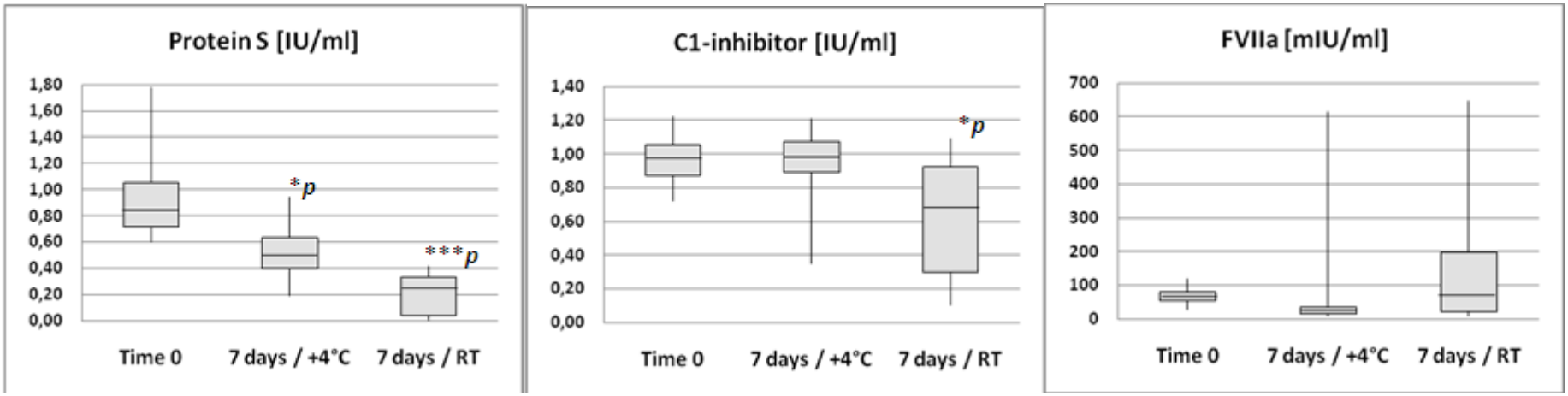
Illustration 3

Biochemical characteristics of thawed FFP during storage at 4Ã‚Â°C and RT. Median levels, 25% and 75% quartiles and minimum-maximum intervals in FFP units at time 0, as well as after 7 days storage at 4Ã‚Â°C and RT are presented. Statistically significant differences relative to baseline values: *p









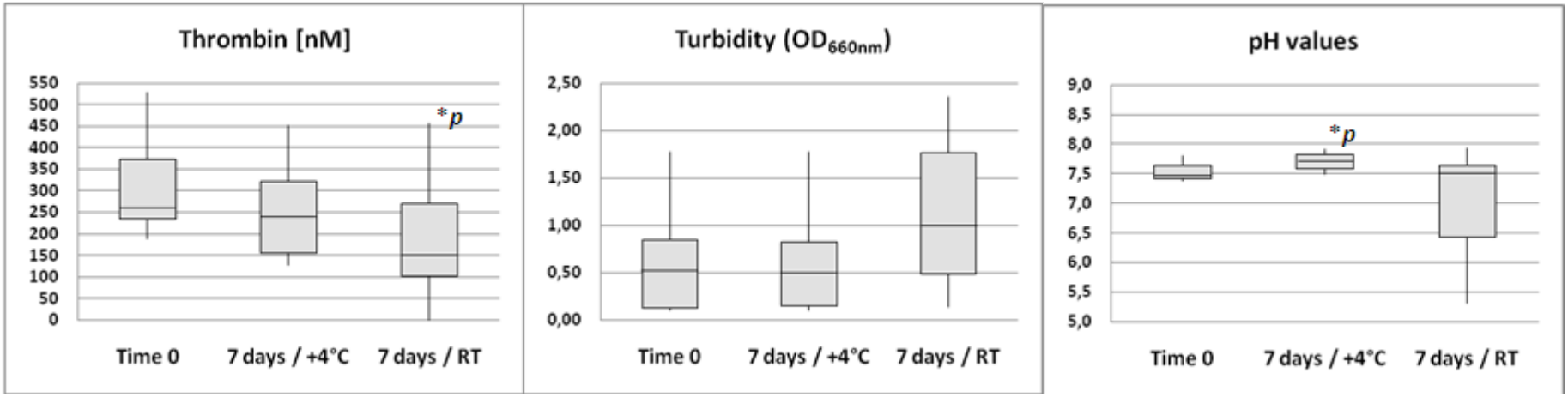
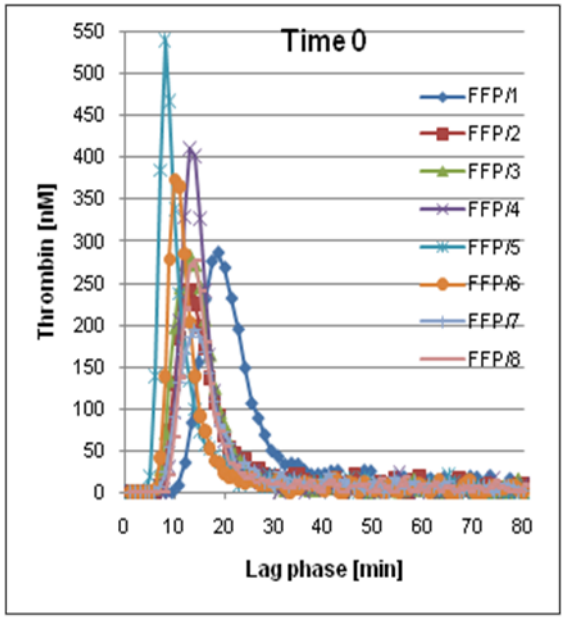
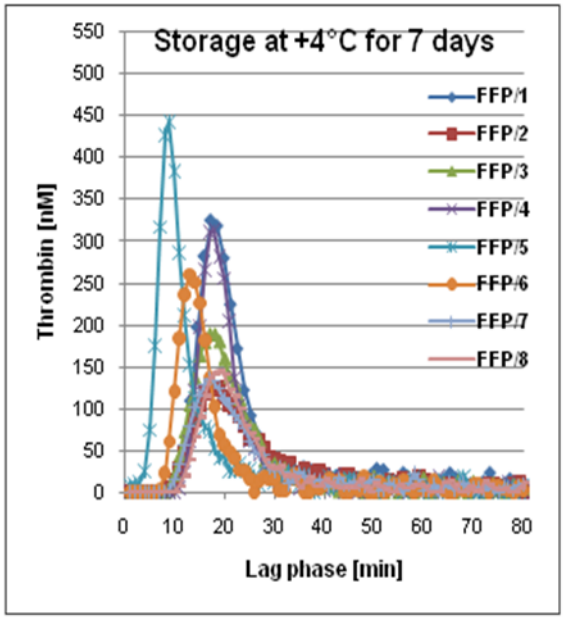
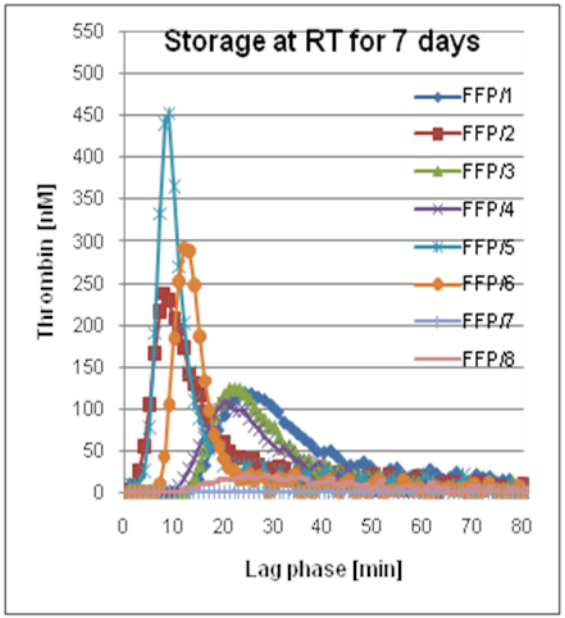


Illustration 4

Thrombin generation capacities in thawed FFP during storage at 4Ã‚Â°C and RT. Peak thrombin [nM] and lag phase [min] in single-donor FFP units at time 0, as well as after 7 days storage at 4Ã‚Â°C and RT are indicated. Statistically significant differences relative to baseline values: *p







Peak thrombin:	311 ± 111 nM	251 ± 114 nM	184 ± 152 nM *
Lag phase:	12.4 ± 1.9 min	14.4 ± 3.5 min	19.6 ± 16.4 min

Disclaimer