Prolonged (post-thaw) shelf life of −80°C frozen AB apheresis plasma

Tim W.H. Rijnhout[1,2] *Femke Noorman,*[3] *Bob De Kort,*[3] *Margreet Zoodsma,*[3] *and Rigo Hoencamp*[1,2,4,5]

BACKGROUND: Early plasma transfusion is important in the treatment of patients with major hemorrhage. Prolonged shelf life of AB type frozen −80°C and cold-stored (4°C) deep frozen plasma (DFP) will improve strategic stock management, minimize need for resupply, and make pre-hospital implementation more feasible.

METHODS AND MATERIALS: Plasma products type AB of different age and origin (−30°C Fresh Frozen [(FFP], −80°C DFP [short (±1 year) and long (±7 year)] stored) were thawed (Day 0), stored at 4°C, and sampled on Days 7 and 14. Additionally, samples of plasma containing blood products (Octaplas LG®, whole blood and platelets) were compared for coagulation factor activity, phospholipid clotting time (PPL), and kaolin TEG during 4°C or 22°C storage.

RESULTS: Coagulation profiles of FFP, short- and long-stored −80°C DFP were not significantly different after thaw. Cold storage did not affect fibrinogen, Protein C, and Antithrombin III activities whereas factor V, VII, VIII, and Protein S decreased in all blood products. After 14 days DFP still meets the guidelines for clinical use, except for Protein S (0.4 IU/mL). With exception of Octaplas LG®, phospholipid activity and TEG coagulation were similar between plasma containing blood components during storage.

CONCLUSION: AB DFP quality was unaffected by almost 7 years of frozen storage. Quality of thawed 14-day stored AB DFP met, with exception of Protein S, all minimal guidelines which implies that its quality is sufficient for use in the (pre)-hospital (military) environment for treatment of major hemorrhage.

In the treatment of major hemorrhage, the doctrine shifted from transfusing significant quantities of red blood cells (RBCs) and crystalloids before laboratory-based plasma and platelet transfusion, to immediate goal-directed resuscitation with all blood components in a ratio that equals whole blood (WB) until the patient is hemodynamically stable.[1,2] Especially during massive transfusion, rapid availability of blood components and prudent crystalloid use is advocated to prevent or ameliorate the effects of hemodilution and coagulopathy.

Early plasma transfusion is beneficial for survival in patients with major hemorrhage, it is therefore widely advocated and has been used in different forms and quantities. It contains clotting factors, clotting inhibitors, albumin, immunoglobulins, and other components essential to maintain hemostasis and vascular integrity, including the

From the [1]Department of Surgery, Alrijne Medical Center, Leiderdorp; [2]Trauma Research Unit Department of Surgery, Erasmus MC, University Medical Center Rotterdam, P.O. Box 2040, 3000 CA Rotterdam; [3]Military Blood Bank, Ministry of Defense; the [4]Department of Surgery, Defense Healthcare Organization, Ministry of Defense, Utrecht and [5]Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands.

Address reprint requests to: Tim W.H. Rijnhout, Department of Surgery, Rijnhout, Alrijne Medical Center, Simon Smitweg 1, 2353 GA, Leiderdorp, The Netherlands; e-mail: twhrijnhout@alrijne.nl.

Presentations: Dutch traumadays November 2019; European Congress of Trauma and Emergency Surgery

Abstracts-poster presentation AABB 2019, F. Noorman et al. Transfusion 59, Suppl S3,pp 202A

Sources of support

Dutch Department of Defense and the Dutch Army Health Insurance Foundation (SZVK).

Received for publication April 7, 2020; revision received May 20, 2020, and accepted June 3, 2020.

doi:10.1111/trf.15963

TRANSFUSION 2020;60;1846–1855

regulation of coagulation and fibrinolysis. However, due to the limited shelf life of 3-4 weeks at 4°C, plasma is mostly frozen as soon as possible after donation to extend its shelf life. With a thawing time of approximately 15-35 minutes (pending on volume and temperature frozen) it is not immediately available.

Availability of thawed plasma offers opportunities to reduce time to first plasma transfusion and to implement plasma transfusion in the (pre)-hospital setting.[3-5] Multiple study groups have shown that prolonged 4°C storage of thawed plasma led to a reduction in clotting factors and argued that quality is still sufficient for treatment of major hemorrhage.[6-14]

The Dutch Ministry of Defense (D-MOD) has used −80°C AB type apheresis deep frozen plasma (DFP) since 2001 and its use is shown to be safe and effective.[15,16] Currently, DFP can be stored frozen (−80°C) for up to 7 years and cold stored (+4°C) up to 7 days post-thaw.[15,17] Introduction of 7-day cold-stored DFP in Afghanistan resulted in 140 (1-5 per patient) transfused units with an average product age of 4 days. Additionally, DFP use resulted in earlier plasma and platelet use which was associated with reduced mortality.[15]

The aims of this study are to extend shelf life of both frozen and thawed DFP for the treatment of major hemorrhage. Increased shelf life of frozen and thawed DFP will result in more efficient strategic stock management and create possibilities to reduce production frequency to maintain a stockpile of DFP, minimize need to resupply hospitals, reduce the logistic burden and improve the availability of universal donor plasma for early use in (pre-) hospital care.

METHODS AND MATERIALS

Study design

Both long and short −80°C stored (1 vs. almost 7 years) AB DFP are compared to fresh thawed AB FFP. Additionally, AB DFP quality is compared with other plasma containing universal donor type blood products (AB Octaplas®, O WB, A platelets) used during resuscitation. To test the hypothesis that the maximum allowed −80°C and post-thaw 4°C shelf life can be doubled, short- and long-stored products were compared with each other and with the European Directorate for the Quality of Medicines (EDQM) guidelines for fresh frozen plasma (FFP) or pathogen-reduced FFP (PR-FFP) (see Appendix S1, available as supporting information in the online version of this paper.).[18]

Comparison of plasma storage results

Short- (±1 year) and long- (±7 years) stored units of −80°C DFP were thawed and compared to ±1 year 30°C stored FFP directly after thaw. Additional testing was performed for both types of DFP on Day 7 (D7) and Day 14 (D14) of storage at +4°C after thaw.

DFP vs. other blood (components)

As plasma of platelets and WB contribute to the treatment of bleeding patients these products are included in this study. DFP was compared to −30°C S/D treated AB type plasma (omniplas (OMN); Octaplas® LG made from Dutch FFP, Octapharma GmbH, Langenfeld Germany), cold-stored O type CPD WB without (WB0) and with leukodepletion (WBF), and A type leukodepleted pooled buffy coat thrombocyte concentrates in plasma (BTC) or in additive solution (BAS). BAS units contain 35% plasma and 65% Platelet Additive Solution-E (PAS-E). All products were analyzed and compared during 4°C/22°C storage.

Based on the Blood Supply Act, Sanquin is the only organization in the Netherlands authorized to manage the need for blood and blood products. For this reason, Octaplas LG ® and DFP are made from FFP that is procured from Sanquin. WB and platelet products used in this study were obtained from Sanquin after informed consent from the donors. Eight samples (N = 8) of each product was compared.

Production and quality control of DFP

Leukocyte-depleted apheresis plasma (AB-Rh D positive and negative) is fresh frozen and stored at −30°C for a minimum of 4 months by Sanquin (FFP). After release from quarantine and delivery to the Military Blood Bank (MBB) the product is stored at −30°C till production of DFP; FFP is thawed in a temperature controlled 37°C water bath until a temperature of 28-32°C is reached and subsequently transferred into a new 600 mL PVC bag (Macopharma, Utrecht, The Netherlands), vacuum sealed in an overwrap, packed in a cardboard box and re-frozen on the bottom of an empty −86°C chest freezer (Snijders Labs, Tilburg, The Netherlands) to freeze the products at a rate of 1-5°C/min to −80°C. Hereafter the DFP are transferred to upright −86°C freezers (Snijders) for long term −80°C storage. Temperatures of freezers are continuously monitored to guarantee a temperature below −65°C at all times.

Periodically, samples are drawn from the FFP during production of DFP for quality control (QC) purposes. Similarly, DFP produced by the MBB was periodically thawed for QC purposes. After −80°C storage, units of DFP were thawed for 25-35 minutes in a temperature-controlled water bath (37°C) until a temperature of 28-32°C is reached and after sampling the product was stored at 4°C and sampled again on D7 and D14. QC data of the period 2018-2019 are used in this study.

Blood product comparison study

For the comparison experiment (period 2018-2019), n = 8, short-stored DFP were compared to AB OMN (2 different batches, ±2 yr frozen and ± 4 yr frozen, N = 4/batch) and the supernatant plasma of three different plasma containing blood components. Fresh thawed DFP and OMN were split

in equal portions in 150 mL PVC sample bags (TerumoBCT Leuven, Belgium) and analyzed on Day 0 (D0) and after 4°C storage D7 and D14. WB products (n = 16) were shipped 1 day after donation and donor testing to the MBB; half of the units were leukodepleted by filtration (WBF) with a platelet sparing filter (Imuflex® WB-SP TerumoBCT, Leuven Belgium), the other half was not leukodepleted (WB0). Subsequently, all units were split and stored in 150 mL PVC bags at 4°C before analysis on Day 2 (D2), D14, and Day 21 (D21). BTC and BAS units (n = 8 each) were shipped to the MBB on Day 1 (D1) after donation, donor testing, and processing by Sanquin. One ±120 mL sample was stored for 14 days at 4°C and the rest of the product was stored in the original bag at 22°C under constant agitation before sampling and analysis on D2 and D7.

Sample analysis

All measurements were performed within 6 hours of sampling/removal from storage. The number of products measured each day did not exceed 12 and the same reagent lot numbers were used for QC and comparison studies. QC samples of FFP, DFP, and OMN were analyzed without preanalysis centrifugation or filtration. In the comparison study, all product samples were filtered with a 200 µm transfusion filter (Codan transfusion set, Lensahn, Germany) prior to analysis. FFP, OMN, and DFP were analyzed directly, whereas WB and BTC samples were first centrifuged (2500 g, 15 min) to obtain supernatant plasma for coagulation analysis. Samples were analyzed with the Sta Compact Max 3 (Stago BNL, Leiden, The Netherlands) for coagulation factor profiles according to the manufacturers' instructions. Furthermore, the Sysmex XS1000i Hematological analyzer (Sysmex, Etten-Leur, the Netherlands) was used to measure concentration. Stago phospholipid (PPL) activity (measures coagulation time of a sample diluted in buffer and PPL deficient plasma, activated with Calcium and activated factor X) and Kaolin TEG (TEG 5000, Haemonetics, Breda, the Netherlands) were measured of pure plasma and of microparticle rich supernatant of plasma (1 × 2500 g 15 min) or microparticle rich supernatant of cellular blood products (2 × 2500 g 15 min).[19] Not all QC samples were measured for all coagulation factors daily, as a result of temporarily insufficient consumable items on different QC days.

Outcome measures

Coagulation factor activators and inhibitors (factor V, VII, VIII, Protein C, Protein S, Antithrombin III [ATIII]) were compared with European EDQM guidelines 2017 for fresh FFP; average factor VIII activity ≥0.7 IU/mL in ≥90% of the products measured within 1 month of −30°C frozen storage and with guidelines for PR-FFP; average factor VIII activity ≥0.5 IU/mL and fibrinogen activity ≥60% of fresh donor plasma, in ≥90% of the products measured within 1 month of −30°C frozen storage. In the accompanying text it is stated that PR-FFP "*contains, on average, about 50 to 70% of the labile coagulation factors and naturally occurring inhibitors present in fresh unfrozen/thawed plasma.*"[18] The percentage of products that comply with these guidelines was determined for all product categories. In addition, coagulation times APTT, INR, phospholipid (PPL) clotting time, and TEG-profiles were assessed.

Statistical analysis

Statistical calculations were performed using SPSS (version 25, IBM or higher). Shapiro–Wilk test was used to assess the normality of the data distribution. Normal distributed data are expressed with mean and 95% confidence upper and lower limits, t-tests were used to determine significant differences. Not normally distributed data are expressed as median and 95% confidence upper and lower limits, Mann

TABLE 1. Quality control data 2018-2019; FFP and DFP coagulation factor profiles after thaw; effect of −80°C storage duration

	FFP (n = 68)	DFP short stored (n = 37)	DFP long stored (n = 42)
Years at −30°C	0.6 [0.6-0.6] †,‡	0.7 [0.7-0.8] *,‡	1.0 [0.9-1.0] *,†
Years at −80°C	ND	0.1 [0.1-0.2] *,‡	5.8 [5.7-6.1] *,‡
Volume mL	305 [304-306] †,‡	294 [291-297] *,‡	299 [296-301] *,†
Plt count 10^9/L	10 [7-13]	14 [10-21]	13 [7-20]
MPV (fL)	10.1 [9.8-10.3] †, ‡	7.6 [6.9-8.2] *	7.4 [7.3-7.6] *
APTT sec	31 [31-32] ‡	31 [31-32]	32 [32-33] *
INR	1.1 [1.1-1.2]	1.1 [1.1-1.2]	1.1 [1.1-1.2]
Fibrinogen gr/L	3.0 [3.0-3.1]	3.0 [3.0-3.2]	3.0 [2.8-3.5]
FV IU/mL	1.0 [1.0-1.1] n = 64	0.9 [0.9-1]	1.0 [1.0-1.1]
FVII IU/mL	1.0 [1.0-1.1] n = 60	1.0 [1.0-1.1]	1.0 [1.0-1.1]
FVIII IU/mL	1.2 [1.2-1.3] n = 49	1.1 [1.1-1.4]	1.0 [0.9-1.2]
Protein S IU/mL	1.0 [1.0-1.1] n = 51	1.0 [1.0-1.1]	1.0 [1.0-1.2]
Protein C IU/mL	1.2 [1.2-1.3] n = 45	1.2 [1.2-1.3]	1.2 [1.2-1.3]
ATIII IU/mL	1.0 [1.0-1.1] n = 59	1.0 [1.0-1.1]	1.0 [1.0-1.1]

Data are presented as median-[95%LCL-UCL]); All products are within EDQM guidelines for 1 month stored FFP (<10% of units. Coagulation factors<0.65 IU/mL). Bonferroni correction p value< alpha 0.05/3 = 0.0167.
*,†,‡ = p < 0.0167 Mann Whitney test vs. respectively FFP, DFP short and long stored at −80°C.
FFP = fresh frozen plasma; DFP = deep frozen plasma.

TABLE 2. Quality control data 2018-2019; FFP and DFP plasma coagulation factor profile; effect of post-thaw 4°C storage on thawed DFP

	FFP n = 68	DFP = 79 (short and long stored at −80°C)		
Days at 4°C	0	0	7	14
plt count 10^9/L	10 [7-13] *,†	14 [10-20]	20 [15-24]	21 [15-28]
MPV (fL)	10.1 [9.8-10.3] *,†	7.4 [7.3-7.6]	7.6 [7.2-8.0] *	7.6 [7.1-8.0] †
Fibrinogen gr/L	3.0 [3.0-3.1]	3.0 [2.9-3.2]	3.1 [3.0-3.3]	3.0 [3.0-3.2
APTT sec	31 [31-32] *,†	32 [32-33] §,ll	35 [35-36] ‡	35 [35-36] ‡
FVIII IU/mL	1.2 [1.2-1.3] *,†	1.0 [1.0-1.1] §,ll	0.7 [0.7-0.8] ‡	0.7 [0.7-0.9] ‡
INR	1.1 [1.1-1.2] *,†	1.1 [1.1-1.2] §,ll	1.3 [1.3-1.4] ‡	1.3 [1.3-1.4] ‡
FV IU/mL	1.0 [1.0-1.1] *,†	1.0 [1.0-1.1] §,ll	0.8 [0.8-0.9] ‡,ll	0.7 [0.7-0.8] ‡,§
FVII IU/mL	1.0 [1.0-1.1] *	1.0 [1.0-1.1] §,ll	0.8 [0.8-0.9] ‡,ll	0.9 [0.8-1.0] ‡,§
Protein S IU/mL	1.0 [1.0-1.1] *,†	1.0 [1.0-1.1] §,ll	0.6 [0.6-0.7] ‡,ll	0.4 [0.4-0.5] ‡,§
Protein C IU/mL	1.2 [1.2-1.3] †	1.2 [1.2-1.3]	1.2 [1.2-1.4]	1.1 [1.1-1.2]
ATIII IU/mL	1.0 [1.0-1.1]	1.0 [1.0-1.1]	1 [1-1.1]	1.0 [1.0-1.1]

Data are displayed as median 95% LCL-UCL; Bonferroni correction p value< alpha 0.05/6 = 0.0083.
Exact n value of DFP not shown, for exact number measured see Table 3.
FFP *,† = p < 0.0083 Mann Whitney test FFP vs. respectively DFP Day 7 or 14.
DFP ‡,§,ll Mann Whitney test p < 0.0083 DFP vs. respectively DFP day 0,7 or 14.

TABLE 3. QC data 2018-2019; Nr and percentage of FFP and DFP products not within EDQM guidelines after thaw and during 4°C post-thaw storage

	FFP	DFP (short and long stored at −80°C)		
Days at 4°C	0	0	7	14
Platelets<50 10^9/mL *, §	2/68 (3%)	2/79 (3%)	9/79 (11%)	12/67 (18%)
FVIII<0.65 IU/mL †	0/49 (0)	0/79 (0)	26/79 (33%)	30/79 (38%)
FVIII<0.45 IU/mL §	0/49 (0)	0/79 (0)	1/79 (1%)	2/79 (3%)
FV < 0.45 IU/mL §	0/64 (0)	0/79 (0)	1/79 (1%)	2/79 (3%)
FVII<0.45 IU/mL §	0/60 (0)	0/79 (0)	1/79 (1%)	0/79 (0)
FVII≥1.45 IU/mL ‡	0/60 (0)	1/79 (1%)	3/79 (4%)	12/79 (15%)
Protein S < 0.45 IU/mL §	0/51 (0)	0/79 (0)	10/79 (13%)	58/78 (74%)
Protein C < 0.45 IU/mL §	0/45 (0)	0/77 (0)	0/79 (0)	0/70 (0)
Fibrinogen<1.75 gr/L §	0/68 (0)	0/79 (0)	0/79 (0)	0/79 (0)
ATIII <0.45 IU/mL §	0/59 (0)	0/79 (0)	0/79 (0)	0/79 (0)

Number of products not within guidelines.
* Guidelines EDQM-FFP before freezing.
† Guidelines EDQM-FFP after 1 month at −30°C.
‡ Not in EDQM guidelines.
§ EDQM-pathogen reduced FFP /total number of products after 1 month at −30°C(%).

TABLE 4. QC data 2018-2019; FFP and DFP Phospholipid (PPL) coagulation time and kaolin-TEG profile after thaw and during 4°C post-thaw storage

	PPL* Sec	TEGR min	TEG angle °	TEG MA mm
FFP day 0 n = 68	41 (40-43) †	9 [8-9] †	68 [67-71] †	28 [27-30]
DFP <5y day0 n = 37	46 (43-48)	9 [9-10]	65 [64-68]	27 [25-30]
DFP >5y day0 n = 42	49 (47-51)	10 [9-10]	65 [62-68]	27 [25-31]
DFP day 0 n = 79	47 (46-49) ‡	9 [9-10] ‡	65 [64-68]	27 [26-30] ‡
DFP day 7 n = 79	57 (55-59) ‡	11 [10-11] ‡	63 [62-66]	28 [28-30]
DFP day14 n = 67	64 (62-66)§	11 [10-11] ‡	65 [64-67]	29 [29-32] ‡

Data are presented as median [95% Lower confidence interval – Upper confidence interval].
* PPL normally distributed data represented as mean - (95% Lower confidence interval – Upper confidence interval) Bonferroni correction p value< alpha 0.05/6 = 0.0083.
† p < 0.0083 Mann Whitney. test FFP vs. DFP (short or long stored, Day 0,7 or 14 (PPL t-test).
‡ p < 0.0083 Mann Whitney. DFP day 0 vs. DFP Day 7 or 14 (PPL t-test).
§ p < 0.0083 t-test DFP day 7 vs. DFP Day 14 (PPL).

Whitney tests were used to determine significant differences.

RESULTS

Presentation of results is separated in quality control (QC) data (68 units of FFP, 37 units of short stored DFP, and 42 units of long stored DFP) and data from product comparisons of DFP, OMN, and supernatant plasma from WB0, WBF, BTC, and BAS (8 units per products). Data were not distributed normally with exception of PPL clotting time and are presented accordingly.

Effect of frozen storage on DFP quality

Table 1 compares product characteristics and coagulation factor activity between FFP and short and long stored DFP.

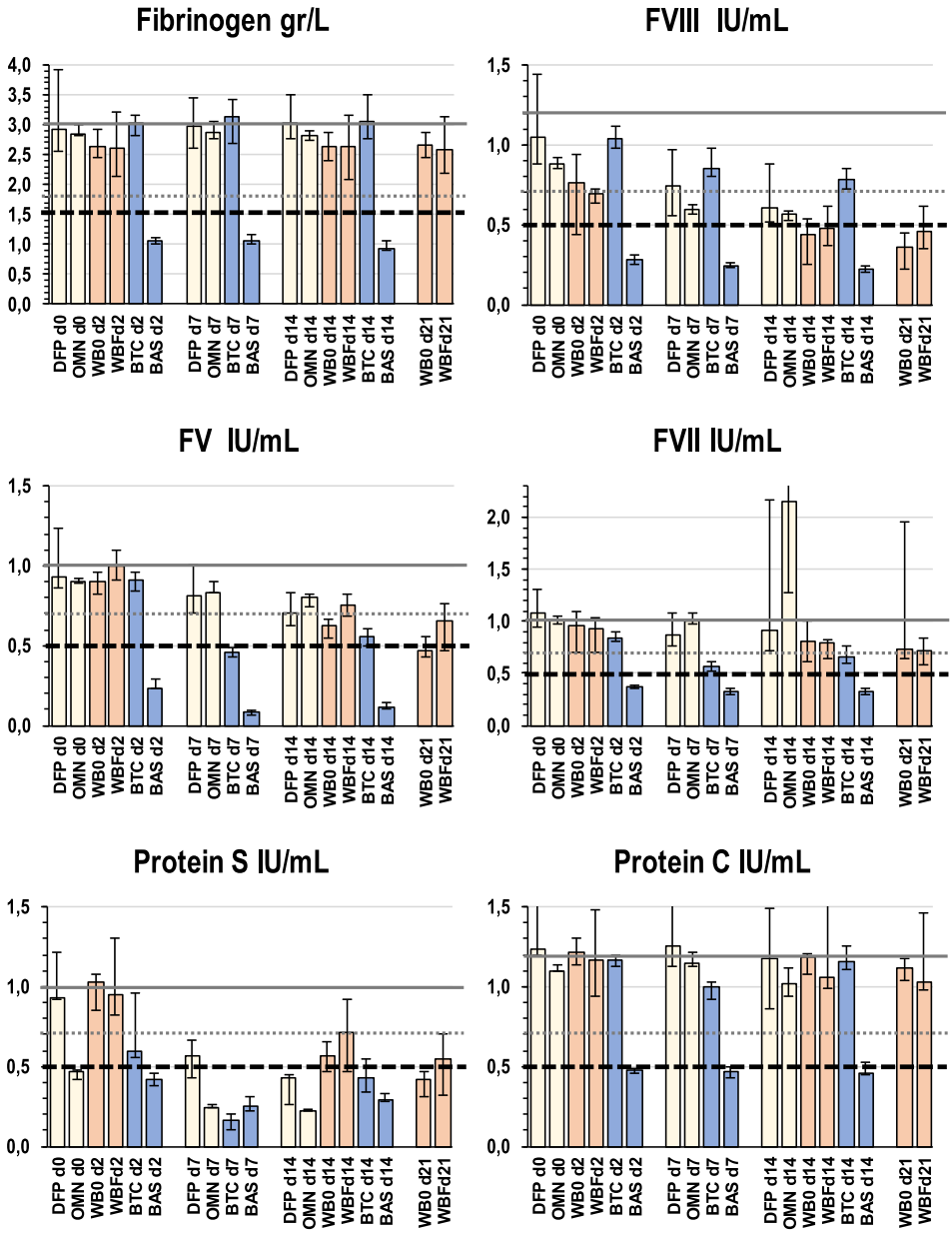

Fig 1. Coagulation factors in plasma, comparison of products (n = 8). Coagulation factor activities measured in plasma (DFP, OMN) and supernatant plasma of 1 × 2500 g 15 min centrifuged cellular blood components. DFP, −80°C apheresis plasma; OMN, omniplasma; WB0, whole blood; WBF, leukodepleted whole blood; BTC, leukodepleted buffy coat pool platelets in 100% plasma; BAS, leukodepleted buffy coat pool platelets in 65% platelet additive solution E/35%plasma. Gray line = QC FFP value after thaw (see Table 1), dotted line = 70% activity (0.7 IU/mL) intermittent fat line = Minimum EDQM guideline PR-FFP: 50% activity (0.5 IU/mL) or 60% fibrinogen (1.8 gr/L). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 5. Nr of plasma containing blood components not within EDQM guidelines after thaw and during (post-thaw) storage (n = 8/product)

	FVIII <0.65 IU/mL *	FVIII <0.45 IU/mL †	FV <0.45 IU/mL †	FVII ≥1.45 IU/mL ‡	ProtS <0.45 IU/mL †
DFP after thaw	-	-	-	-	-
DFP 7 days 4°C	3	-	-	-	2
DFP 14 days 4°C	5	1	-	2	7
OMN after thaw	-	-	-	-	**3**
OMN 7 days 4°C	8	-	-	-	8
OMN 14 days 4°C	8	-	-	6	8
WB0 2 days 22/4°C	3	2	-	-	-
WB0 14 days 4°C	8	4	-	-	1
WB0 21 days 4°C	8	7	4	1	6
WBF 2 days 22/4°C	2	-	-	-	-
WBF 14 days 4°C	7	3	-	-	1
WBF 21 days 4°C	8	4	1	-	2
BTC 2 days 22°C	-	-	-	-	1
BTC 7 days 22°C	1	-	4	-	8
BTC 14 days 4°C	-	-	-	-	5
BAS 2 days 22°C	8	8	8	-	6
BAS 7 days 22°C	8	8	8	-	8
BAS 14 days 4°C	8	8	8	-	8

DFP, −80°C apheresis plasma; OMN, omniplasma; WB0, whole blood; WBF, leukodepleted whole blood; BTC, leukodepleted buffy coat pool platelets in 100% plasma; BAS, leukodepleted buffy coat pool platelets in 65% platelet additive solution E/35%plasma.
* Guidelines EDQM-FFP after 1 month at −30°C.
† EDQM-pathogen reduced FFP total number of products after 1 month at −30°C (%).
‡ Not in EDQM guideline.

Median storage time at −30°C was significantly longer in both short- (0.7 year) and long-stored DFP (1 year) in comparison with FFP (0.6 years) ($p < 0.0167$). Median additional −80°C storage time was 0.1 and 5.8 years, which added up to a median total storage time of 0.9 and 6.8 years for short- and long-stored DFP respectively. Immediately after thaw, only APTT was significantly increased (1 second) in longer stored DFP. All FFP and DFP units contained an average coagulation factor activity of ≥0.7 IU/mL after thaw.

Effect on DFP quality during post-thaw +4°C storage

During post-thaw cold storage there were no significant differences between short and long stored DFP (data not shown). Table 2 compares clotting factors, inhibitors. and clotting times between fresh thawed FFP and thawed DFP (short and long stored) during post-thaw cold storage. No statistically significant differences were found between DFP and FFP on D0 after thaw except a decreased mean platelet volume (MPV) in DFP. There were significant reductions in coagulation activity of factor V, VII and VIII, Protein S, and significant increases in APTT and INR during post-thaw cold storage of DFP, whereas platelet count and MPV non-significantly increase and fibrinogen levels, Protein C and ATIII remained stable.

Table 3 summarizes the number and percentage of units that did not meet minimum quality thresholds set by the EDQM for fresh thawed (PR-)FFP. At D7 and D14, units of DFP did not meet these guidelines for platelet count (11-18%) and Protein S (13-74%). In some plasma units factor VII increased to above normal during cold storage. Table 4 shows the phospholipid coagulation times and kaolin-TEG profiles. PPL clotting time, TEG R time, and TEG MA significantly increased during post-thaw storage ($p < 0.0083$).

Comparison of plasma containing blood products

Figure 1 and Appendix S2, available as supporting information in the online version of this paper, lists main characteristics and trend of clotting factor profile in plasma containing blood components. The total number of products not meeting quality thresholds is shown in Table 5. ATIII and Protein C activities were not affected by cold storage in any product, and except in BAS, both inhibitor activities were between 0.9-1.3 IU/mL (data not shown). Directly after thaw, Protein S in OMN is below the minimum EDQM threshold for PR-FFP (0.5 IU/mL). At D7, OMN Protein S, BTC factor V, and Protein S no longer met these guidelines. After 14 days of cold storage, factor VIII was below this guideline in all products except in OMN and BTC. BAS did not meet (PR-)FFP guidelines at all. In some DFP, almost all OMN and one WB0 factor VII increased to above normal during cold storage.

OMN showed no clot formation in TEG within 30 minutes and no clot formation in PPL within 120 seconds (see Appendix S3, available as supporting information in the online version of this paper.). Supernatant PPL clotting time increased in thawed DFP and decreased for both types of WB and platelets during storage, however, supernatant TEG R time was not significantly shortened in the cellular blood products during storage. Supernatant TEG angle reduced for DFP and showed an increase for BAS during storage.

DISCUSSION

Patients with major hemorrhage are in need of massive volume resuscitation. Rapid initiation of a 1:1:1 massive transfusion protocol with early transfusion of (pre-thawed) blood components and reduced use of crystalloids improve chances of survival.[20] Thawing of plasma often causes delay, making it difficult to start balanced resuscitation with all blood components. Plasma transfusion remains essential since additional medication such as prothrombin complex concentrate and/or fibrinogen concentrate is not sufficient to restore or compensate all diluted or lost coagulation factors.[21,22]

We demonstrated that DFP quality after almost 7 years of frozen storage was equal in clotting factors and inhibitors in comparison with fresh thawed 1-year stored FFP and DFP. The higher platelet count and decreased MPV in thawed cold stored DFP compared to FFP is probably related to formation of small microparticles. During cold storage the platelet count and MPV slightly increase which indicates swelling and/or fusion of microparticles, thus becoming large enough to be detected as a 'platelet' in the hematological analyzer. Given the fact that plasma quality was not affected by 7 years of frozen storage, it is expected that extending the shelf life at −80°C beyond 7 years will also not affect plasma quality. This is consistent with results presented in 2005 by Valeri and colleagues who showed that 14-year −80°C storage of plasma did not cause significant changes in coagulation factor V, VIII, and fibrinogen levels.[23] We show that there is no reason to discard 7-year stored AB DFP. Therefore, the MBB has prolonged its storage time to 14 years for now and will continue to monitor the oldest and newest DFP units in stock with periodical QC measurements (±6 times/year). We will report on outcome in future studies.

European guidelines for PR-FFP guidelines indicate that it should contain about 50-70% of labile coagulation factor and inhibitor activities compared to fresh donor plasma for clinical use. Guidelines for fresh thawed FFP are set higher, since this product is also used as a source for the production of isolated coagulation factors.[24] For this reason, European guidelines state that 90% of 1 month stored −30°C FFP units should contain factor VIII activity ≥0.7 IU/mL. Similarly, UK guidelines stipulate a minimum level of 0.7 IU/mL factor VIII activity in 75% of all plasma units tested, furthermore units found to have <0.3 IU/mL should not be issued for transfusion.[25]

There are no guidelines for the minimum quality of cold-stored (PR-)FFP at the end of storage time. Currently thawed (PR-)FFP can be stored for 5 days at 4°C. However, particularly in smaller trauma centers, thawed plasma wastage will increase as demand is often too low.[6,26] During (post-thaw) storage all blood products show decreased clotting factor activities and increased clotting times. DFP coagulation factor activity still meets fresh thawed PR-FFP requirements after 14 days of cold storage, except for Protein S (0.4 IU/mL) which is in line with other studies.[6-9,11,13,27-31]

BAS products contain 35% plasma and 65% PAS-E which explains the low level of clotting factors in this product. It is important to consider clinical implications of the reduced plasma volume when switching to additive solutions for platelet suspension. BAS is still suitable for transfusion since it is primarily transfused for platelet support and has a higher safety profile, however the product will contribute less to patient's clotting factor activities compared to platelets in plasma. Similarly, it can be argued that although clotting factors are reduced, 14-day cold-stored thawed DFP will be primarily used in the early resuscitation of major hemorrhage instead of crystalloids. Its implementation can thus result in better patient outcome. In addition, it can be used by forward surgical teams and reduce the frequency of resupply, which is especially in military environments advantageous to minimize risks for material and personnel.

It has been argued that plasma with coagulation factor activity above 30% of normal is sufficient to support clinical or surgical hemostasis.[32] Most products described in the current study would comply with the latter prerequisite except BAS, 7-14-day cold stored OMN, 7 day 22°C stored BTC and 21 days cold stored WB. Regarding European guidelines, DFP Protein S activity is below 0.5 IU/mL in more than 10% of units on day 7 and 14 of cold storage. However, fresh thawed OMN also did not meet this guideline, probably because the OMN used in this study had been stored for 2-4 years at −30°C. According to the OMN insert, Protein S level is at 0.6 ± 0.1 IU/mL (n = 5) with a guaranteed level above 0.2 IU/mL. Low Protein S activity is caused by the solvent-detergent (S/D) treatment of Octaplas® and has been improved with the introduction of Octaplas LG®.[11,14] Despite the low Protein S activity, thawed S/D plasma (e.g., OMN) is successfully used for years in many countries including The Netherlands.[14,33] Although 14-day cold-stored thawed DFP is inferior to fresh thawed DFP, pre-hospital availability of hemostatic capacity is important and can be followed with in-hospital transfusion of fresh thawed plasma. The complex question whether the early transfusion of cold stored DFP offers advantages rather than disadvantages because of reduced Protein S activity remains to be investigated.

A potential concern is the increase in factor VII activity at D14 in 3 units of DFP, 7 units of OMN and 1 unit of WB0. Cold activation is associated with an increase in contact and coagulation systems, however, adverse events in patients receiving cold activated plasma have not been demonstrated.[33,34] The occurrence of cold activation was previously reported for all plasma containing blood components and is more frequent in female plasma, but no related clinical side effects have been reported.[35,36] Furthermore, beyond the scope of this study, blood components are cold stored in PVC bags containing the plasticizer di(2-

ethylhexyl) phthalate (DEHP), which is soluble in oil and slowly leaks into plasma during 4°C storage.[28,37] There are several concerns regarding the deleterious effects of DEHP, but toxicology in humans has not been demonstrated and leakage will occur in all plasma containing blood products that are cold stored in PVC.

Despite the lack of clinical data to support limitation of its storage time after thaw and lack of EDQM guidelines for plasma quality in any cold-stored blood product, the current guidelines do mention a maximum 5-day shelf life for (PR-)FFP after thaw. In the 1980s it was already shown that prolonged storage time of thawed FFP at +4°C for up to 7 or 14 days is acceptable for clinical use.[7,38] More recently, Tholpady and colleagues argued that clotting factor activity remained at sufficient levels for up to 10 days.[39] Furthermore never frozen plasma can be stored in the United Kingdom for 7 days because of concerns for contact activation, in Sweden for 7 to 14 days whereas in the US it is approved for 26 days by the Food and Drug Administration. In 2017, ±83000 units were distributed and ± 14000 units of never frozen plasma were transfused in the US.[40] The current data of 14 days cold-stored AB DFP show similar clotting factor activities as reported for 20 days never frozen plasma.[41,42]

Cold-stored thawed DFP is indeed inferior in comparison with fresh thawed DFP, but similar to that of plasma in cold-stored WB and platelets. Leukodepletion of WB with a platelet sparing filter did not affect plasma quality, which has been observed with other leukodepletion filters.[43,44] Based on the data of this study, WB has equal fibrinogen content as a unit of apheresis plasma despite its higher plasma volume, which is due to the higher citrate dilution of WB compared to apheresis plasma.[45] Furthermore universal donor type O required for early transfusion of WB, has lower factor VIII activities compared to other blood groups.[46] In clinical practice these small differences in coagulation factor activity will probably have minimal impact on the clinical outcome of patients with major hemorrhage. The reduction of plasma in PAS stored platelets may have a significant effect compared to platelets stored in plasma in this patient category, though this has yet to be shown.

Supernatant PPL clotting time is performed in buffer to which sample, activated factor X and PPL deficient plasma is added. The clotting time decreases with increased microparticle concentration in plasma samples.[47] Microparticle content of plasma is thought to be beneficial in the treatment of bleeding trauma patients. This study shows significant shorter PPL-time, TEG R time, and TEG angle at baseline in FFP in comparison with both fresh thawed short- and long-stored DFP. Likely, this results from the extra thaw–freeze cycle to produce DFP from FFP, as PPL activity was not significantly different between short- and long-stored DFP. It is however questionable if the absence of PPL activity in OMN, the reduced PPL activity in cold-stored thawed DFP and fresh cellular blood products compared to fresh thawed FFP and cold-stored cellular blood products, will have clinical effects in the treatment of these patients as multiple products of different ages are given in a fixed 1:1:1 ratio.[13,27]

Strength and limitations

Different ages and blood types of products makes interpretation of the data presented more difficult. For example, plasma frozen within 24 hours after extraction from 1-6°C WB or room temperature stored WB, differ in coagulation factor VIII and Protein C activity.[48,49] In the current study WB was stored at room temperature overnight before leukodepletion, splitting and storage at 4°C and BTC were prepared and stored at 22°C under continuous rotation and the 4°C sample was drawn and stored 1.5 days after donation. Routinely processed cold-stored platelets and WB are likely to be stored at 4°C more quickly after production. Thus, quality results of routinely processed products may be better. Furthermore, in the comparison study, all products (with exception of 22°C stored BTC and BAS) were stored in sample bags and the smaller volume may have influenced outcome, although no significant differences were observed between DFP stored in 600 mL bags or 150 mL bags during cold storage.

The strength of this study is that only blood types and products were studied that are actually used in acute resuscitation. Furthermore, the current study measured all clotting factors without first freezing samples prior to analysis thus eliminating effects of an additional freeze–thaw step.

CONCLUSION

Coagulation factor activities were not affected by frozen storage duration of almost 7 years. Future research will determine at which cut-off −80°C shelf life, the quality of DFP is reduced compared to ±1 year −30°C stored FFP. Clotting factor activity in cold-stored thawed AB DFP still meets minimum quality guidelines after 14 days and its quality is similar to plasma quality of other cold-stored blood components. Therefore 14-day cold-stored thawed AB DFP can be implemented for the early treatment of major hemorrhage.

ACKNOWLEDGMENTS

The authors wish to thank KJCM Thielen, Sgt1 SAG Petrelli, Sgt. LJHC Boudewijns, and Sgt. JW Buis for their technical assistance.

CONFLICTS OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471-82.

2. Roback JD, Caldwell S, Carson J, et al. Evidence-based practice guidelines for plasma transfusion. Transfusion 2010;50: 1227-39.
3. Sperry JL, Guyette FX, Brown JB, et al. Prehospital plasma during air medical transport in trauma patients at risk for hemorrhagic shock. N Engl J Med 2018;379:315-26.
4. Teixeira PG, Inaba K, Shulman I, et al. Impact of plasma transfusion in massively transfused trauma patients. J Trauma 2009; 66:693-7.
5. Menzies Wojtowicz M, Motum P, Dhir V. Extended life plasma: blood wastage and opportunities. Transfus Med 2020;30(2): 161-3.
6. Downes KA, Wilson E, Yomtovian R, et al. Serial measurement of clotting factors in thawed plasma stored for 5 days. Transfusion 2001;41:570.
7. Lamboo M, Poland DC, Eikenboom JC, et al. Coagulation parameters of thawed fresh-frozen plasma during storage at different temperatures. Transfus Med 2007;17:182-6.
8. von Heymann C, Keller MK, Spies C, et al. Activity of clotting factors in fresh-frozen plasma during storage at 4 degrees C over 6 days. Transfusion 2009;49:913-20.
9. Sheffield WP, Bhakta V, Yi Q-L, et al. Stability of thawed apheresis fresh-frozen plasma stored for up to 120 hours at 1°C to 6°C. J Blood Transfus 2016;2016:6260792.
10. Neisser-Svae A, Trawnicek L, Heger A, et al. Five-day stability of thawed plasma: solvent/detergent-treated plasma comparable with fresh-frozen plasma and plasma frozen within 24 hours. Transfusion 2016;56:404-9.
11. Keller MK, Krebs M, Spies C, et al. Clotting factor activity in thawed Octaplas(R) LG during storage at 2-6 degrees C for 6 days from a quality assurance point of view. Transfus Apher Sci 2012;46:129-36.
12. Noordin SS, Karim FA, Mohammad W, et al. Coagulation factor activities changes over 5 days in thawed fresh frozen plasma stored at different initial storage temperatures. Ind J Hematol Blood Transfus 2018;34:510-6.
13. Matijevic N, Kostousov V, Y-WW W, et al. Multiple levels of degradation diminish hemostatic potential of thawed plasma. J Trauma 2011;70:71-80.
14. Buchta C, Felfernig M, Höcker P, et al. Stability of coagulation factors in thawed, solvent/detergent-treated plasma during storage at 4 °C for 6 days. Vox Sang 2004;87:182-6.
15. Noorman F, van Dongen TT, Plat MJ, et al. Transfusion: −80 degrees C frozen blood products are safe and effective in military casualty care. PLoS One 2016;11:e0168401.
16. Lelkens CC, Koning JG, de Kort B, et al. Experiences with frozen blood products in The Netherlands military. Transfusion Apher Sci 2006;34:289-98.
17. Holley A, Marks DC, Johnson L, et al. Frozen blood products: clinically effective and potentially ideal for remote Australia. Anaesth Intensive Care 2013;41:10-9.
18. European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS). Guide to the preparation, use and quality assurance of blood components. 19th ed.; 2017). Strasbourg: Council of Europe.
19. Lacroix R, Judicone C, Mooberry M, et al. Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. J Thromb Haemost 2013;11:1190-3.
20. Radwan ZA, Bai Y, Matijevic N, et al. An emergency department thawed plasma protocol for severely injured patients. JAMA Surg 2013;148:170-5.
21. Jehan F, Aziz H, O'Keeffe T, et al. The role of four-factor prothrombin complex concentrate in coagulopathy of trauma: a propensity matched analysis. J Trauma Acute Care Surg 2018; 85:18-24.
22. Rizoli SB, Scarpelini S, Callum J, et al. Clotting factor deficiency in early trauma-associated coagulopathy. J Trauma 2011; 71Suppl 1:S427-34.
23. Valeri CR, Ragno G. The effect of storage of fresh-frozen plasma at −80 degrees C for as long as 14 years on plasma clotting proteins. Transfusion 2005;45:1829-30.
24. Acker JP, Marks DC, Sheffield WP. Quality assessment of established and emerging blood components for transfusion. J Blood Transfus 2016;2016:4860284.
25. NationalBloodService. Guidelines for the blood transfusion services in the United Kingdom. 8th ed. Norwich: TSO (The Stationery Office); 2013.
26. Sheffield WP, Bhakta V, Yi QL, et al. Stability of Thawed Apheresis Fresh-Frozen Plasma Stored for up to 120 Hours at 1 degrees C to 6 degrees C. J Blood Transfus 2016;2016: 6260792.
27. Matijevic N, Wang YW, Kostousov V, et al. Decline in platelet microparticles contributes to reduced hemostatic potential of stored plasma. Thromb Res 2011;128:35-41.
28. Sheffield WP, Bhakta V, Mastronardi C, et al. Changes in coagulation factor activity and content of di(2-ethylhexyl)phthalate in frozen plasma units during refrigerated storage for up to five days after thawing. Transfusion 2012;52:493-502.
29. Yazer MH, Cortese-Hassett A, Triulzi DJ. Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6 degrees C. Transfusion 2008;48: 2525-30.
30. Nifong TP, Light J, Wenk RE. Coagulant stability and sterility of thawed S/D-treated plasma. Transfusion 2002;42:1581-4.
31. Thiele T, Iuga C, Janetzky S, et al. Early storage lesions in apheresis platelets are induced by the activation of the integrin alphaIIbbeta(3) and focal adhesion signaling pathways. J Proteomics 2012;76 Spec No.:297-315.
32. Murray DJ, Olson J, Strauss R, et al. Coagulation changes during packed red cell replacement of major blood loss. Anesthesiology 1988;69:839-45.
33. Cardigan R, Green L. Thawed and liquid plasma–what do we know? Vox Sang 2015;109:1-10.
34. Suontaka AM, Silveira A, Soderstrom T, et al. Occurrence of cold activation of transfusion plasma during storage at +4 degrees C. Vox Sang 2005;88:172-80.
35. Huish S, Green L, Curnow E, et al. Effect of storage of plasma in the presence of red blood cells and platelets: re-

evaluating the shelf life of whole blood. Transfusion 2019;59: 3468-77.
36. Backholer L, Green L, Huish S, et al. A paired comparison of thawed and liquid plasma. Transfusion 2017;57:881-9.
37. Sampson J, de Korte D. DEHP-plasticised PVC: relevance to blood services. Transfus Med 2011;21:73-83.
38. Nilsson L, Hedner U, Nilsson IM, et al. Shelf-life of bank blood and stored plasma with special reference to coagulation factors. Transfusion 1983;23:377-81.
39. Tholpady A, Monson J, Radovancevic R, et al. Analysis of prolonged storage on coagulation Factor (F)V, FVII, and FVIII in thawed plasma: is it time to extend the expiration date beyond 5 days? Transfusion 2013;53:645-50.
40. Novak DJ, Bai Y, Cooke RK, et al. Making thawed universal donor plasma available rapidly for massively bleeding trauma patients: experience from the pragmatic, randomized optimal platelets and plasma ratios (PROPPR) trial. Transfusion 2015; 55:1331-9.
41. Gosselin RC, Marshall C, Dwyre DM, et al. Coagulation profile of liquid-state plasma. Transfusion 2013;53:579-90.
42. Matijevic N, Wang YW, Cotton BA, et al. Better hemostatic profiles of never-frozen liquid plasma compared with thawed fresh frozen plasma. J Trauma Acute Care Surg 2013;74(1):84-90; discussion 90-81.
43. Li DY, Zhang HW, Feng QZ, et al. Impacts of leukocyte filtration and irradiation on coagulation factors in fresh frozen plasma. Exp Ther Med 2015;9:598-602.
44. Afifi OAH, Abdelsalam EMN, Makhlouf A, et al. Evaluation of coagulation factors activity in different types of plasma preparations. Indian J Hematol Blood Transfus 2019;35: 551-6.
45. Rijnhout TWH, Noorman F, Bek A, et al. Massive transfusion in The Netherlands. Emerg Med J 2020;37:65-72.
46. Liu X, Chen X, Yang J, et al. Association of ABO blood groups with von Willebrand factor, factor VIII and ADAMTS-13 in patients with lung cancer. Oncol Lett 2017;14:3787-94.
47. Bohling SD, Pagano MB, Stitzel MR, et al. Comparison of clot-based vs chromogenic factor Xa procoagulant phospholipid activity assays. Am J Clin Pathol 2012;137:185-92.
48. Scott E, Puca K, Heraly J, et al. Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6 degrees C storage. Transfusion 2009;49:1584-91.
49. Smith JF, Ness PM, Moroff G, et al. Retention of coagulation factors in plasma frozen after extended holding at 1-6 degrees C. Vox Sang 2000;78:28-30.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Appendix S1: Supporting information
Appendix S2: Supporting information.