

In Vitro Hemostatic Properties of French Lyophilized Plasma

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ABSTRACT

Background: French lyophilized plasma (FLyP) is used routinely by the French Armed Forces in war settings. The authors compared concentrations of coagulation proteins and global *in vitro* hemostatic properties in FLyP and in the same plasma before lyophilization to assess the impact of lyophilization on coagulation properties.

Methods: Twenty-four batches of plasma before and after lyophilization were tested for coagulation proteins. Thrombin generation time, thrombin antithrombin concentration, prothrombin fragment 1 + 2, and thromboelastography were assessed. Finally, the efficiencies of FLyP and plasma before lyophilization were compared on a hemorrhagic shock hemodilution model and tested on TEG[®] (Haemoscope Corporation, Glenview, IL).

Results: Prothrombin time ratio (1.1 ± 0.1 vs. 1.2 ± 0.1) and activated partial thromboplastin time (35 ± 1.3 vs. 39 ± 2.4 s) were significantly increased in FLyP ($8 \pm 3\%$, $P < 0.05$ and $11 \pm 5\%$, $P < 0.001$, respectively). Activity of factors V (85 ± 18 vs. 51 ± 16 UI/ml) and VIII (0.77 ± 0.11 vs. 0.62 ± 0.10 UI/ml) was also diminished ($25 \pm 12\%$ and $20 \pm 7\%$, respectively); however, activity of other factors was preserved. The authors observed no alteration in the thromboelastographic parameters. Thrombin generation was preserved when induced with 5 pM tissue factor *in vitro* but significantly reduced when using 1 pM tissue factor. The thrombin-antithrombin complex and prothrombin fragment 1 + 2 attested for the absence of coagulation activation. This hemodilution model showed no significant difference before and after lyophilization.

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What We Already Know about This Topic

- Fresh frozen plasma (FFP) is widely used to correct coagulopathy in trauma patients with hemorrhagic shock. However, FFP has some logistic limitations particularly in war settings. The French Military Blood Institute has manufactured a lyophilized plasma product.

What This Article Tells Us That Is New

- Although prothrombin time and activated partial thromboplastin times were slightly increased, *in vitro* hemostatic properties of plasma were not different after lyophilization using thromboelastography and an hemodilution model. Clinical trials are now required to assess the clinical efficacy of lyophilized plasma.

Conclusions: The study results account for a significant decrease of factors V and VIII in FLyP. However, the global capacity to induce clot formation *in vitro* seems to be preserved. The clinical relevance of these decreased factors is not known.

THE correction of traumatic coagulopathy is a key issue in the treatment of hemorrhagic shock.¹ Plasma infusion in this setting helps to maintain clotting factors at a normal level. Various experimental and clinical data have provided evidence that the volume resuscitation with crystalloids or colloids can induce a coagulopathy characterized by a compromised clot development, whereas the initiation of coagulation is unaffected.^{1,2} Thus, a large amount of data advocate for liberal and early use of plasma in a concept of “damage control resuscitation”^{3–5} to manage massive hemorrhage. To foster these new guidelines and to deliver plasma earlier in the emergency setting, many centers are using thawed plasma for the resuscitation of major trauma.^{5,6} The use of fresh frozen plasma (FFP) has some logistical limitations, especially in war settings or other austere environments: storage at -20°C (maximum 1 yr), refrigerated transportation, 30 min to thaw, and optimal use within 6 h of thawing.⁷ In addition, because acute coagulopathy appears early in the course of acute severe trauma, an innovative product for the early treatment of coagulopathy should be useful.^{8,9} Building on the U.S. military experience in World War II, the French Military Blood Institute (FMBI) has manufactured a freeze-dried plasma product since the late 1940s. This product was improved continuously for more than half a century as technology and legislation dictated.

When French legislation on blood products required its approval in the beginning of the 1990s, the FMBI provided biologic and pharmaceutical data to the French Agency for Sanitary Safety of Health Products, who approved the product in 1994. Finally, in 2010 the FMBI decided to go further on pathogen safety and turned to a pathogen inactivation process, rather than the quarantine strategy in use until then. This modification in the manufacturing process required a new agreement, including clinical and biologic studies. The current work presents here the biologic part of this work. French lyophilized plasma (FLYP) is shelf stable, can be stored at room temperature, has a 2-yr shelf life, is available in less than 6 min, has ABO universality, and is secured by a photochemical process that inactivates any RNA or DNA pathogens.¹⁰ The French Military Health Service and the FMBI have produced specific guidelines for the treatment of war casualties based on damage control surgery and resuscitation using FLYP, erythrocytes, and fresh whole blood. We hereby report the manufacturing of this FLYP, followed by *in vitro* evaluation. The aim of the study was to test the hypothesis that the *in vitro* properties of FLYP and FFP are not different. Thus, we performed *in vitro* measures to further characterize the concentration of coagulation proteins and the clot formation process before lyophilization (considered FFP) and after the process of freeze-drying (FLYP). We used standard tests as well as an *in vitro* model of hemodilution adapted from Bolliger *et al.*^{11,12} A crystalloid, lactated Ringer's solution (LR), was investigated in the same manner as control.

Materials and Methods

Preparation of Freeze-dried Plasma

French lyophilized plasma is obtained from apheresis plasma; donors are all male or female volunteers, and a rigorous medical selection is applied according to European and French law regarding donor selection.¹³ Only plasma that provides more than 0.9 UI/ml coagulation factor VIII (FVIII) will undergo the lyophilization process. Females with previous pregnancy are tested for antihuman leukocyte antigen antibodies and excluded if the antibodies are found. Apheresis plasma is leukoreduced by centrifugation and filtration. Final leukocyte count is certified to be less than 1,000/l. Plasma benefits from a viroinactivation process based on photochemical treatment: plasma is stored in a additive solution with amotosalen (Intercept®, Cerus Corporation, Concord, CA) and subsequently illuminated by long-wavelength ultraviolet light. Amotosalen is a compound that intercalates and cross-links double helix or single strand hairpin loop of nucleic acids upon illumination with long-wavelength ultraviolet light. This prevents replication or transcription of any pathogen. Residual amotosalen is removed by specific adsorption, and final amotosalen concentration is certified to be less than 2 μ M. The process is performed entirely in a closed system or under sterile conditions, meeting the guidelines of good manufacturing practices. Frozen plasma (stored

at -25°C) is thawed and incubated at 30°C for 15 min. For lyophilization preparation, 3,000 ml from a maximum of 10 different donors' plasma (mixed of A, B, and AB blood group) are placed in a sterile 3 l-plastic bag. Then, plasma is distributed aseptically into individual flasks for cryodesiccation, a desiccation process by sublimation of ice under vacuum. The flasks are placed into the freeze dryer, where they are quickly frozen before being freeze-dried for 4 days. Because a large amount of the carbon dioxide in FLYP is evaporated during the process, the pH after rehydration is slightly alkaline (pH = 8). After dilution, as much as 50% in a citrated whole blood (pH = 6.73), alkalization increased less than 3% (pH = 6.97). Thus, we considered its buffer effect null with no need for additional compound. FLYP is stored at ambient temperature ($2-25^{\circ}\text{C}$) until the day of its use and then reconstituted with 200 ml medical sterile water (210 ml is available after rehydration). FLYP can be reconstituted in less than 6 min (169 ± 57 s).

Coagulation Factors and Inhibitors

Plasma samples ($n = 24$ before lyophilization paired after lyophilization) were analyzed for a set of coagulation factors and physiologic inhibitors on the Star-Compact coagulation analyzer (Diagnostica Stago, Asnières, France). Measurements included prothrombin time, activated partial thromboplastin time, fibrinogen concentration, activity of FV, FVIII, FXI, FXIII, protein C, free Protein S activity, anti-thrombin, and α_2 antiplasmin.

Thrombin Generation Assay

We analyzed the hemostatic properties of every FFP and FLYP batch during a 12-month period ($n = 24$) by thrombin generation assay¹⁴ using the calibrated automated thrombogram (Thrombinoscope; Synapse BV, Maastricht, Netherlands), with platelet-poor-plasma and platelet-poor plasma low reagents (Diagnostica Stago, Parsippany, NJ), providing reaction concentrations of 5 and 1 pM tissue factor, and 4 μ M phospholipids. The calibrated automated thrombogram assay generates a curve that gives information on several parameters, including the lag time (representing the time until initial thrombin had formed [min], the thrombin peak height [nM thrombin], the endogenous thrombin potential [reflects the area under the curve], and the rate of thrombin generation [mean slope = peak height/(time to peak – lag time)]). Thrombin generation was also assessed by quantifying the thrombin-antithrombin (TAT) complex and prothrombin fragment 1 + 2 (PF1 + 2). In the process of conversion of prothrombin to thrombin, PF1 + 2 is released and thus reflects the degree of thrombin generation. Some of the thrombin generated is inactivated by circulating antithrombin, leading to formation of an inactive protease—the inhibitor TAT complex. Both assays reflect the degree of coagulation activation.¹⁵ These results show the amount of prothrombin activation during the manufacturing of FFP or FLYP. TAT and PF1 + 2 concentration measurement

was performed using commercially available enzyme-linked immunosorbent assay kits (Enzygnost TAT, Dade Behring, Deerfield, IL).

Thromboelastography

Plasma samples before ($n = 24$) and after lyophilization ($n = 24$) were equally diluted in Control-level I[®] (Haemoscope Corporation, Glenview, IL) for TEG[®] (Haemoscope Corporation), a dried whole blood that includes stabilizers and buffers. This reagent allows us to produce a clot with the to-be-tested plasma by providing platelets from a standardized blood with a better reproducibility than that from volunteers. The test was performed at 37°C on a computerized Thrombelastograph TEG 5000 (Haemoscope) coagulation analyzer according to manufacturer instructions, using kaolin as the coagulation activator. Analyzed parameters included R time (reaction time to clot initiation), α angle (kinetic of clot formation), and maximal amplitude (maximum clot firmness, maximum amplitude). The estimation of the number of subjects needed to detect a significant difference was calculated (n more than 15). In addition, blood samples were obtained from 17 consenting healthy volunteers, 10 men and 7 women, with a mean age of 36 yr (range, 25–55 yr). The volunteers had normal results of coagulation tests: hemoglobin 14.1 ± 1.2 g/dl, hematocrit $42.6 \pm 3.4\%$, platelet count 237 ± 49 g/l, prothrombin time 12.8 ± 0.3 s, activated partial thromboplastin time 33.1 ± 0.6 s, fibrinogen 2.7 ± 0.3 g/l, and FVIII 1.2 ± 0.2 U/l. They had no history of coagulopathy, and none had received anticoagulant or antiplatelet medication during the 2 weeks before blood sampling. This study was performed under consent given by volunteers and approved by the local ethics committee (HIA Percy, Clamart, France). Blood was collected in silicone-coated tubes containing 4.5 ml 0.129 M-buffered sodium citrate. After a sample was harvested, the blood was diluted by 60% using an equal amount of LR (containing Na^+ 130 mM, Cl^- 115 mM, K^+ 4 mM, Ca^{2+} 3 mM, and lactate 28 mM) and either FFP, FLYP or LR. Basically, to obtain 1,000 μl diluted blood, 400 μl citrated blood was diluted with 300 μl LR, and the remaining 300 μl were represented by FFP, FLYP or LR as control (summarized in fig. 1). Then, 340 μl diluted and celite-activated sample was added to 20 μl 0.1 mM calcium chloride in a prewarmed disposable plastic cup. The samples were run at patient temperatures to accurately reflect their *in vivo* coagulation status. Thromboelastography was performed after dilution, within 1 and 2 h after collection. We decided to induce dilution-dependent impairment of the clotting process with 60% dilution to observe the clinically relevant reduction of clot firmness and prolonged initiation of coagulation that are to be expected during profound dilution only.¹⁶ In addition, especially when hemostatic competence is severely compromised, such as in trauma patients, effective substitution is mandatory to reduce additional blood loss. Our model was adapted from Bolliger *et al.*^{11,12} Our dilutions correspond to

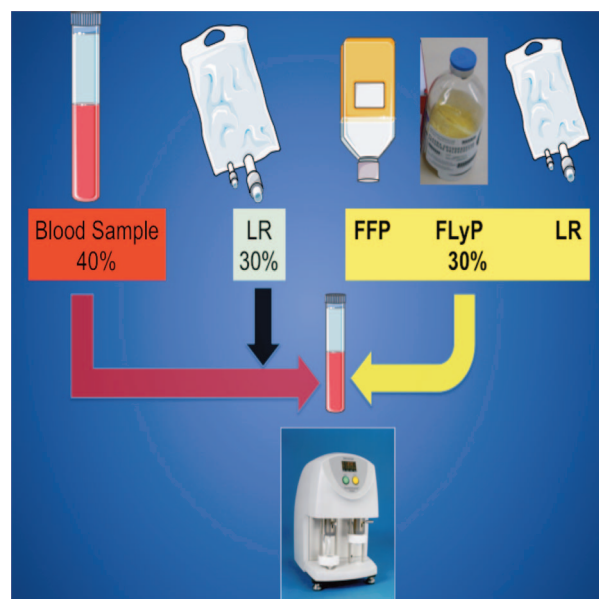


Fig. 1. Representation of TEG analyses performed on whole blood of healthy donors. FFP = fresh frozen plasma, representing plasma before lyophilization; FLYP = French lyophilized plasma, representing plasma after lyophilization; LR = lactated Ringer's; TEG = thromboelastometric.

approximately 25 ml/kg plasma (FLyP or FFP) in a patient of 70 kg body weight.

Data Analysis

The results are expressed as mean with SD (mean \pm SD) after testing by Kolmogorov-Smirnov statistics for normal distribution. When FLYP was compared with FFP, statistical comparisons of mean were accomplished using a two-tailed paired Wilcoxon *t* test assuming either equal or unequal variance, as appropriate. In hemodilution experiments with FLYP in comparison with hemodilution using LR or FFP, the between-group differences were compared using two-tailed Friedman test; *post hoc* correction of *t* tests were performed by Bonferroni-Dunn test. Statistical analyses were performed with Prism[®] v5.0 software (GraphPad, San Diego, CA). A *P* value < 0.05 was considered statistically significant.

Results

Coagulation Factors and Inhibitors

The results for mean concentration of coagulation proteins and physiologic inhibitors in FFP and FLYP, expressed in international units per liter, and the percentages of change in factors between FFP and FLYP are presented in table 1. During lyophilization, some factors decreased. We observed significant changes in two of nine proteins in FLYP compared with FFP. The decreases were noted for FV ($25 \pm 12\%$) and FVIII ($20 \pm 7\%$). There were no significant changes in fibrinogen, FXI, FXIII, antithrombin, protein C, protein S, and α_2 antiplasmin. As expected, a longer activated partial

Table 1. Coagulation Factors and Inhibitors in FFP versus FLYP

Analyte	FFP (n = 24)	FLyP (n = 24)	Reference Range	% Variation	P Value
Prothrombin time (ratio test/control)	1.1 ± 0.1	1.2 ± 0.1	<1.5	8 ± 3	<0.05
Activated partial thromboplastin time (s)	35.0 ± 1.3	39.0 ± 2.4	30–40	11 ± 5	<0.001
Fibrinogen (g/l)	2.4 ± 0.2	2.4 ± 0.3	2–4	0	0.39
Factor VIIIc (U/ml)	0.77 ± 0.11	0.62 ± 0.10	0.5–1.5	–20 ± 7	<0.001
Factor V (U/ml)	85 ± 18	51 ± 16	70–120	–25 ± 12	<0.05
Factor XI (U/ml)	76 ± 12	79 ± 11	50–140	6 ± 5	0.74
Factor XIII (U/ml)	101 ± 17	103 ± 12	20–120	3 ± 7	0.94
Protein C (U/ml)	96 ± 8	96 ± 9	70–120	0	0.72
Protein S (U/ml)	84 ± 13	77 ± 16	70–140	–7 ± 12	0.12
Antithrombin (U/ml)	103 ± 4	101 ± 5	80–120	–3 ± 2	0.18
Alpha ₂ antiplasmin (U/ml)	94 ± 3	95 ± 3	80–120	1 ± 3	0.67

Results are expressed as mean ± standard deviation.

FFP = fresh frozen plasma, representing plasma before lyophilization; FLYP = French lyophilized plasma, representing plasma after lyophilization.

thromboplastin time (35 ± 1.3 vs. 39 ± 2.4 s, $P < 0.001$) and prothrombin time (ratio 1.1 ± 0.1 vs. 1.2 ± 0.1, $P = 0.001$) were observed related with FV and FVIII decrease.

Thrombin Generation Assay

Analysis of thrombograms showed no significant differences in all parameters between FFP and FLYP when triggered by 5 pM tissue factor, whereas some significant changes were observed with the lower dose of 1 pM (table 2). At the lower dose, lag time was increased (18 ± 15%), endogenous thrombin potential decreased (30 ± 18%), and the peak thrombin declined (50 ± 23%). At the tissue factor dose of 5 pM, thrombograms demonstrated no statistically significant decline in all parameters. Analysis of PF1 + 2 revealed non-significant changes (143.3 ± 25.5 vs. 129.7 ± 16.7 pM, $P = 0.09$). After lyophilization, the TAT complex significantly decreased (4.1 ± 1.3 vs. 3.2 ± 1.5 µg/l, $P < 0.05$). Both tests revealed values in the respective reference ranges provided by the manufacturer (69–229 pM and 2–4.2 µg/l). However, clinical and biologic meaning of this statistically significant difference is irrelevant because the coefficient of variation of the test is approximately 10%.

Thromboelastometric Results

Dilution in Control-level I[®] revealed no significant modification in the thromboelastographic parameters (R, α angle, and maximum amplitude) between FFP and FLYP (table 3). At baseline, all thromboelastographic and standard laboratory variables were comparable among healthy volunteers and within the normal range. Figure 2 illustrates characteristic dynamic whole blood coagulation profiles in whole blood at baseline as well as whole blood diluted with FFP, FLYP, and LR. Compared with hemodilution by LR, dilution by both FFP solutions and FLYP did not affect the initiation phase of coagulation, as attested by a nonsignificantly different R time. R time was significantly increased by LR dilution compared with whole blood at baseline (8.8 ± 1.5 vs. 7.6 ± 1.5 s, $P < 0.001$) and compared with FLYP (7.6 ± 1.2 s) or FFP (7.4 ± 0.9 s). We found no difference between FLYP and FFP regarding R time ($P = 0.07$). Comparison of α angle between FFP (63.9 ± 3.4 degrees), FLYP (63.6 ± 4.5 degrees), and LR (48.4 ± 6.6 degrees) revealed smaller α angle (ANOVA, $P < 0.001$), indicating its better ability to form a clot. In addition, there was no significant differences between FFP and

Table 2. Thrombin Generation Results in FFP versus FLYP

	FFP (n = 24)	FLyP (n = 24)	P Value
Triggering with 5 pM TF			
Lag time (min)	2.2 ± 0.4	2.1 ± 0.2	0.52
Endogenous thrombin potential (nM/min)	1,525 ± 131	1,440 ± 203	0.25
Peak thrombin (nM)	263.5 ± 27.5	264.3 ± 22.0	0.93
Triggering with 1 pM TF			
Lag time (min)	5.1 ± 0.8	6.2 ± 1.0	<0.01
Endogenous thrombin potential (nM/min)	1,382 ± 124	926 ± 224	<0.01
Peak thrombin (nM)	195.5 ± 80.9	79.2 ± 23.1	<0.05
Fragment 1 + 2 (pM)	143.3 ± 25.5	129.7 ± 16.7	0.09
TAT complex (µg/l)	4.1 ± 1.3	3.6 ± 1.6	<0.05

Data are presented as mean ± standard deviation.

FFP = fresh frozen plasma, representing plasma before lyophilization; FLYP = French lyophilized plasma, representing plasma after lyophilization; TAT = thrombin-antithrombin complex; TF = tissue factor.

Table 3. Thromboelastometric Results of FFP versus FLYP

	FFP	FLyP	P Value
TEG analyses			
R time (s)	1.9 ± 0.4	2.0 ± 0.4	0.07
α angle (degrees)	78.8 ± 2.4	79.4 ± 2.4	0.09
Maximum amplitude (mm)	41.1 ± 2.6	40.7 ± 1.6	0.45

Measurements of R time in seconds representing time to clot initiation, α angle in degrees representing kinetic clot formation, and maximum amplitude in millimeters representing clot firmness. Measurements were performed in Control-level I, after 50% dilution with plasma before lyophilization (FFP) or after lyophilization (FLyP). n = 24 in each group. Results are expressed as mean ± standard deviation.

FFP = fresh frozen plasma; FLyP = French lyophilized plasma; ns = not significant; TEG = thromboelastometric.

FLyP. Furthermore, hemodilution with LR significantly depressed clot strength, as indicated by a significant decrease in maximum amplitude (38.9 ± 5.9 mm). This decrease was significant between LR, FLyP, and FFP (ANOVA, $P < 0.001$) but not between FFP and FLyP (52.1 ± 3.9 vs. 53.4 ± 4.1 mm, $P = 0.06$).

Discussion

Our main finding is that the *in vitro* hemostatic properties of plasma after lyophilization are associated with a longer prothrombin time or activated partial thromboplastin time, a significant decrease of FV and FVIII, and less thrombin generation when triggered with a low dose of tissue factor compared with the same plasma before this process. However, every coagulation parameter remained in the reference ranges, and global assessment of clot formation by thromboelastography accounts for reasonable alteration of *in vitro* hemostatic properties of plasma. This finding is consistent with much older data available after the first uses of lyophilized plasma during World War II and reported from the 1950s in the literature.¹⁷ In addition, a high correlation has been shown between the activity of lyophilized and fresh plasma from healthy volunteers.¹⁸ Our data are also consistent with more recent clinical data. Lyophilized plasma has been transfused to manage successfully bleeding episodes in patients with hemophilia.^{19,20} In a porcine trauma model, treatment with FLyP resulted in excellent early survival.²¹ Multiple injuries and hemorrhagic shock cause an increase as great as 70% in prothrombin time, and both lyophilized plasma and FFP have been shown to be equally effective in correcting the coagulopathy.¹⁷ Because some concerns arose about the ability of the process to perform viral inactivation in the pooled plasma, its use decreased during the 1960s and 1970s. Fortunately, viral inactivation methods have evolved, and the amotosalen process, based on psoralen compound and long-wavelength ultraviolet light, is

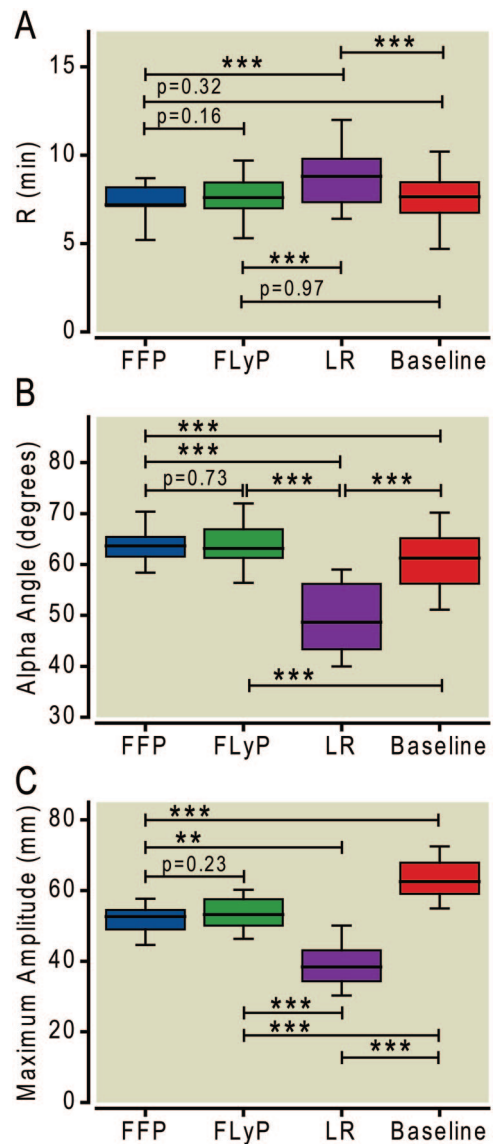


Fig. 2. TEG analysis: measurements of R time in seconds (A), α angle in degrees (B), and maximum amplitude in millimeters (C). Measurements were performed in blood at baseline and after 60% dilution with lactated Ringer's solution and after substitution with plasma before lyophilization or after lyophilization. *** $P < 0.001$. ** $P < 0.05$. FFP = fresh frozen plasma, representing plasma before lyophilization; FLyP = French lyophilized plasma, representing plasma after lyophilization; LR = lactated Ringer's; TEG = thromboelastometric.

able to inactivate any pathogen of RNA and DNA.^{22,23} The same process is now used for viral inactivation during the production of the FLyP.

The aim of the current study was to test the hypothesis that the lyophilization process did not impair hemostasis compared with the same plasma before lyophilization, using standard coagulation assays, thrombinography and thromboelastographic analysis, and *ex vivo* dilution performed on blood samples from healthy volunteers. We demonstrate that lyophilization of human plasma maintains clotting capac-

ity when this FLYP is measured by *in vitro* assays after reconstitution.

First, we tested coagulation proteins before and after lyophilization. The influence of the freeze-drying process on selected coagulation proteins in drawn plasma samples was described recently.²⁴ Assay of clotting factors selected to represent the extrinsic, intrinsic, and common arm of the coagulation cascade revealed only a mild decrease in the FLYP samples. In concordance with previous observation, we found an increased prothrombin time and activated partial thromboplastin time in the same proportions.²⁵ This was in relation to a significant decrease in the activity of FVIII and FV. However, all parameters met the quality requirements of French regulations for plasma²⁶ and were within the physiologic ranges for coagulation factors (FIX, FXI, FXIII, fibrinogen, antithrombin, protein C and S, α_2 antiplasmin). FVIII is a sensitive factor that is the routine quality marker of plasma in France and Europe.²⁷ The observed decrease in FVIII activity in our study is in agreement with data obtained from FFP or solvent-detergent plasma.^{28–31} Several studies examined coagulation factors in thawed plasma at baseline or during storage and demonstrated that FVIII showed the greatest decrease of activity, followed by FV.^{29–32} In our study, FVIII activity decreased to 80% of the baseline value. These FVIII values were in the same range as those reported by Steil *et al.*³³ and Theusinger *et al.*³⁴ Fibrinogen and FXIII are key determinants for clot stability.³⁵ Fibrinogen or fibrin polymerization has been singled out as the primary problem triggering dilutional coagulopathy, reversible by increasing its concentration by transfusion,³⁶ making its concentration in FFP or FLYP a key point. We demonstrated that fibrinogen was a stable protein in our study. Lower fibrinogen concentrations in freeze-dried plasma compared with FFP samples have been reported to be caused by the freeze-drying process stressing the fibrinogen molecule.²⁴ The FLYP fibrinogen concentration was higher than that observed recently by Theusinger *et al.* in a solvent-detergent plasma.³⁴ In contrast with that study,³⁴ we did not observe any greater variability of factor concentrations in FFP than in FLYP; this might be clinically relevant when only a few plasma products are transfused, so the response likely will be more predictable because of few variations. Finally, coagulation inhibitors remained stable, as reported by others in studies with lyophilized plasma.^{32,33}

Second, we performed thrombinographic assays and demonstrated that lyophilization does not alter the thrombin generation when triggered by 5 pM tissue factor, and shows a significant decrease of thrombin generation when triggered by 1 pM. In this more sensitive situation, factor degradation is more detrimental: especially FVIII and FIX involved in the “Josso loop.” However, in trauma patients, concentrations of tissue factor are much greater, more than 100 pM.³⁷ Because activation of coagulation during lyophilization can be a major concern, we address this issue through TAT and PF1 + 2. Results of the TAT assay and PF1 + 2 account

for a nonactivation of coagulation because values remained in the reference ranges.

Finally, in addition to the usual parameters, we used thromboelastography to measure the coagulation profile. This method assesses the viscoelastic properties of whole blood and provides functional information about the interactions among erythrocytes, platelets, and clotting factors. Thromboelastography renders a tracing accounting for the different elements of coagulation; many studies explain them in detail. Dilution in a standardized blood showed no significant difference between FLYP and FFP. We decided to challenge FFP and FLYP in an *in vitro* model of hemodilution. Other investigations have demonstrated that hemodilution by approximately 60% with crystalloids induces impairment of coagulation,³⁸ and our results were in accordance with these observations. We demonstrated that FLYP *in vitro* hemostatic properties were not different from the same plasma before lyophilization. Indeed, a significant improvement of clot initiation and speed of clot formation were assessed with FFP or FLYP equally. This correction of coagulation impairment was sufficient to trigger the clot formation in the same manner as in undiluted blood. This might be in relation with the selection of plasma donors providing enough coagulation factors to induce the clot formation even in a hemodiluted state.

Our study has the limitations of *in vitro* models: it lacked the contribution of endothelial and vascular factors in the clotting process and compensatory mechanisms, such as buffering, pH control, electrolyte environment, and metabolic degradation. Consequently, our findings could not be translated directly to the clinical setting.

Because lyophilized plasma does not require cost-intensive logistics for storage and transport and reconstitution requires less time than thawing of frozen plasma, lyophilized plasma with pathogen inactivation is an attractive option for providing the most important basic treatment for severe coagulopathies in emergency situations or in areas without supply chain. Indeed, hemorrhage is a leading cause of preventable death and is inadequately treated with crystalloid and colloid resuscitation; there is some evidence that aggressive administration of crystalloids decreases survival.^{2,39} Plasma is the treatment of choice to prevent or mitigate coagulopathy in trauma patients; even in the absence of transfused erythrocytes its use was shown in an animal model to be superior to a colloid at reversing the coagulopathy of trauma and to improve survival.⁴⁰ The availability of FLYP in austere settings, as well as in prehospital units, could benefit severe trauma patients, and its use could be extended to civilian centers. However, strong evidence is lacking regarding the wide use of FLYP in emergency settings, and clinical trials should be promoted to address the issue of a benefit of the use of FLYP instead of thawed FFP. These trials could test the clinical significance of the partially lower coagulation activity of FLYP. Since early 2010, the FMFI has provided this product for overseas operations under active surveillance, and

clinical trials are in progress with some very encouraging preliminary data.⁴¹

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