ORIGINAL ARTICLE

Evaluation of coagulation factor activity and sterility of thawed fresh frozen plasma during storage up to 5 days at 4°C

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Abstract

Introduction: Fresh frozen plasma (FFP) is a blood component containing functional quantities of all coagulation factors stored at -18°C or below. FFP has to be thawed and transfused as soon as possible to prevent the loss of certain coagulation factor activities and to minimise microbial contamination. *Materials and Methods:* Thirty units of FFP kept at -20°C were thawed using a 37°C water bath and immediately sampled for baseline Factor II (FII), Factor VIII (FVIII) and fibrinogen activity levels and sterility testing. Each unit was then divided into two smaller bags (i.e. Bag I and Bag II) and kept at 4°C. At 6 hours and Day 3, representative samples were taken from Bag I for coagulation factor activity assays, while at Day 5 representative samples were taken from Bag II for coagulation factor activity assays and sterility testing. *Results:* FII activities at the four time points were 73.43%, 73.73%, 71% and 69.8%, respectively, while FVIII activities were 177.63%, 144.37%, 80.8% and 70.97%, respectively. Fibrinogen levels at the four time points were 3.24 g/L, 3.24 g/L, 3.21 g/L and 3.20 g/L, respectively. All samples were free from microbial contamination even at Day 5. *Conclusion:* The mean reduction in FII and fibrinogen activities on Day 5 was 5% and 1%, respectively. However, FVIII activity declined significantly by approximately 60% at Day 5. Despite these reductions, thawed plasma stored for up to 5 days at 4°C is still suitable for use as the coagulation factor activity levels still exceed the minimum release criteria recommended in quality assurance regulations.

Keywords: coagulation factors, thawed fresh frozen plasma, FII, FVIII, fibrinogen, sterility

INTRODUCTION

Fresh Frozen Plasma (FFP) is a blood component prepared either from whole blood or from plasma collected by apheresis, and frozen within a period of time to a temperature that adequately maintains the labile coagulation factors in a functional state.[1] FFP contains normal levels of stable clotting factors, protease inhibitors, immunoglobulins and albumin.[2] According to national guidelines, plasma should be transfused as soon as the thawed unit is received from the hospital blood bank.[3] The current standard of practice in Universiti Kebangsaan Malaysia Medical Centre (UKMMC) is to discard post-thawed plasma which is not transfused within 4-6 hours to avoid unnecessary adverse effects of transfusion to patients.

Unpublished data from the Blood Bank of UKMMC showed that the average usage of FFP was 5,000 units per year. Unfortunately, about 150-200 units were unused and discarded annually, which accounted for about 3-4% of the total units used. These thawed FFPs were unused primarily because of unnecessary requests for non-indicated cases or due to technical issues preventing their transfusion within 6 hours. In UKMMC, thawed but unused FFP is discarded after 6 hours mainly due to the doubtful storage conditions of FFP in the ward as well as uncertainty about the quality of thawed FFP. Thus, unused FFP has led to wastage of precious plasma products. If these products could be used beyond this time frame of 4-6 hours, the wastage of resources could be minimised. Moreover, if thawed plasma stored at 4°C is usable, it can be made available for urgent transfusion

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quicker by avoiding the time-consuming thawing procedure.[4] Therefore, the objective of this study was to evaluate the coagulation factor activities and to check for microbial contamination of FFP stored at 4°C for 5 days in order to assess the suitability of using such FFP products.

MATERIAL AND METHODS

This cross-sectional study was done in 2016 in the Blood Bank Unit of UKMMC on 30 units of FFP samples previously prepared following national blood banking guidelines and kept at -20°C for not more than 3 months. Plasma was thawed and kept at 4°C for 5 days and checked for FII, FVIII and fibrinogen activity levels as well as sterility. This research project was approved by the Research & Ethics Committee of UKM Medical Centre.

The FFP units were thawed using a calibrated water bath maintained at 37°C for approximately 20-25 minutes. Samples were tested at four time points: 0 hours, 6 hours, Day 3 and Day 5. After thorough mixing of the thawed plasma, representative samples were taken for baseline (Day 0) coagulation factor activity levels and tests for bacterial contamination. Each FFP unit was then divided into two smaller bags: Bag I and Bag II, and kept at 4°C in a calibrated blood fridge until further testing was needed. Representative samples of each FFP unit were collected from Bag I at 6 hours and at Day 3 and tested for coagulation factor activities. Representative samples were only taken from Bag II at Day 5 and tested for coagulation factor activities as well as sterility testing. To minimise the time factor effects on the coagulation factor activities, the representative FFP samples were run in batches of 5-6 units in each batch.

Coagulation factors were measured using a commercially available automated coagulation analyser, STA Compact Max, (Paris, France). FII (reference range: 70-120%) and FVIII (reference range: 50-193%) activities were analysed using a clotting-based test with specific factor-deficient commercial plasma. Fibrinogen (reference range: 1.36-4.65 g/L) was measured by the Clauss method. The formation of the fibrin clot was detected by electromechanical clot detection through changes in viscosity (movement of a metal ball). The reference range for FII was derived from the manufacturer's test assay while the reference ranges for FVIII and fibrinogen were based on a local population study by UKMMC's haemostasis laboratory.

Sterility checks were performed by incubating 7.5 ml of thawed samples in BACTEC Plus Aerobic/F culture vials (Becton, Dickinson and Company, USA) at 35°C in the BD BACTEC™ FX blood culture system with continuous monitoring of the vials for up to 5 days. Gram staining was only performed on samples which were flagged as positive by the incubator. Cultured vials which were not flagged as positive by the system at the end of the 5-day incubation period were regarded as negative for bacterial growth and therefore sterile.

Data were collected and analysed using Statistical Package for Social Sciences (SPSS) for Windows, version 23. Coagulation factor levels were expressed as mean ± SD. In order to show the differences in coagulation parameters at four time points, the results were shown as percentages of baseline values. Nonparametric multivariate analyses of small sizes were done to compare the coagulation factor levels at four different time points with time as longitudinal measurement. Any P-value <0.05 was considered as statistically significant.

RESULTS

The FII, FVIII, and fibrinogen activity levels and sterility assessment results of thawed plasma stored at 4°C for 5 days are summarised in Table 1. All parameters were within the normal range at all the four different time points although the activity means were lower at Day 5. There was no significant statistical difference in the case of FII and fibrinogen, but FVIII showed a statistically significant reduction over 5 days. Although the changes were statistically significant for FVIII, the level of activity was still above the minimum requirement of ≥70% as suggested by the national blood banking guidelines.

With regards to sterility testing, blood cultures did not show any bacterial growth in all samples throughout the study period. Table 2 shows the activities of different coagulation factors at 6 hours, Day 3 and Day 5, relative to their baseline levels at Day 0. At Day 3 and Day 5, FII and fibrinogen levels remained above 95% compared to their baseline levels. For FVIII, 81% of its activity remained at 6 hours of storage, but this was not a significant reduction. However, on Day 3 and Day 5, 46% and 40% of the baseline activity remained, respectively, indicating a drop of 54-60%.

TABLE 1: Coagulation factor activity and sterility assessment results

Parameter	Day 0	6 hours	Day 3	Day 5	P-value	Range
Factor II activity, mean ± SD, %	73.43 ± 9.95	73.73 ± 10.54	71.00 ± 10.51	69.80 ± 10.77	0.396	70-120%
Factor VIII activity, mean ± SD, %	177.30 ± 40.98	144.37 ± 42.19	80.80 ± 17.09	70.97 ± 14.95	0.001	50-193%
Fibrinogen activity, mean ± SD, g/L	3.24 ± 0.42	3.24 ± 0.45	3.21 ± 0.44	3.20 ± 0.49	0.976	1.36-4.65 g/L
Microbial growth	Negative	-	-	Negative	-	-

DISCUSSION

Quality assurance regulations require the activity of coagulation factors to be at least 70%, especially for FVIII.[1,3] The shelf life of FFP is up to 3 months when stored at -18°C to -25°C; and up to 36 months when stored at or below -25°C.[3] This plasma product is indicated in cases with demonstrable multi-factor deficiencies associated with severe bleeding and/ or disseminated intravascular coagulation, or when there is a deficiency of a single coagulation factor but no suitable pathogen-reduced coagulation factor concentrate is available. FFP is also used for plasma exchange in thrombotic thrombocytopenic purpura cases, or to reverse warfarin anticoagulation in over warfarinised patients with severe bleeding or who require emergency surgery or an invasive procedure. FFP is advocated by some for the prevention of bleeding in patients with liver disease and prolonged prothrombin time.[5,6]

For transfusion, FFP must be thawed between 30-37°C in a water bath under continuous agitation or using another system that can ensure a controlled temperature.[7] Once thawed, the FFP should be transfused as soon as possible to prevent the loss of coagulation factors and to minimize microbial contamination. Labile coagulation factors, e.g. Factors V and FVIII in FFP can be lost during thawing or over storage.[8] Plasma is rarely associated with bacterial contamination because it is a cell-free product and is stored frozen.[9] Its storage below freezing point and short interval between thawing and transfusion are unfavourable conditions for survival and proliferation of bacteria.[10,11] However, plasma can be contaminated during thawing in a water bath.[9,12]

We reviewed the recommended timing of transfusion of thawed plasma. The British Society of Haematology Guidelines state that thawed, standard FFP may be stored at 4 ± 2°C for up to 24 hours. Pre-thawed plasma may also be stored at 4 ± 2°C for up to 120 hours for use only in patients with unexpected major bleeding following trauma or other reasons.[13] In the United States, thawed FFP has a shelf life of 24 hours at 1-6°C.[14] Thawed plasma held longer than 24 hours must be relabelled as "thawed plasma", and can be stored for an additional 4 days at 1-6°C.[14] In the German setting, it is recommended that FFP be administered as quickly as possible after thawing.[15] According to Dutch guidelines, thawed FFP should be used within 6 hours if stored at room temperature, or within 24 hours if stored between 1-6°C.[16] Italian guidelines recommend that thawed plasma must be transfused as soon as possible, but in any case, within 24 hours if stored at 4 ± 2°C.[7]

This study has shown that the baseline levels

TABLE 2: Activities of coagulation factors compared to their baselines at Day 0

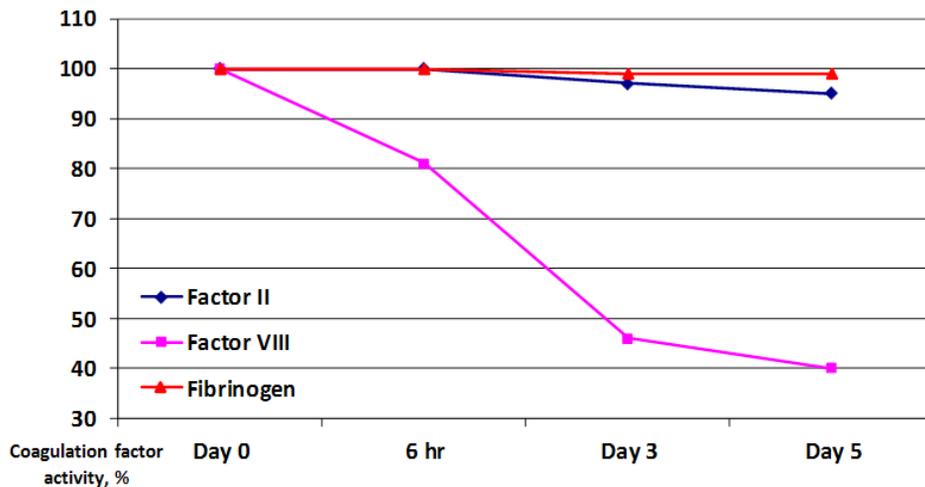| Factor	6 hours	Day 3	Day 5	% reduction at Day 3	% reduction at Day 5
Factor II, %	100	97	95	3	5
Factor VIII, %	81	46	40	54	60
Fibrinogen, %	100	99	99	1	1

FIG. 1: Factor II, Factor VIII and fibrinogen activities compared to their baseline levels at Day 0.

of FII, FVIII and fibrinogen in thawed plasma stored at 4°C were within the normal reference ranges. At Day 3 and at Day 5, FII and fibrinogen levels remained > 95% of the baseline level. The mean percentage reduction of FII and fibrinogen were 5% and 1%, respectively, at Day 5 compared to immediately post thawed levels. The reduction in the activities of FII and fibrinogen at four different time points were not statistically significant. Both FII and fibrinogen were found to be stable at all time points. Other studies have also found that FII and fibrinogen are relatively stable clotting factors.[15,17] In this study, FVIII activity was 81% at 6 hours of storage, which showed no significant reduction from the baseline. However, the activity dropped to 46% and 40% at Day 3 and Day 5, respectively, resulting in residual FVIII levels of 80.80 ± 17.09% and 70.97 ± 14.95% at Day 3 and Day 5, respectively.

Despite the significant decrease in FVIII activity on Day 3 and Day 5 relative to when the FFP was first thawed, the residual FVIII level was still above the minimum product release requirement of ≥70%. Thus, an important finding of this study is the mean FVIII activity was still at least 70% 5 days after thawing. This minimum level of FVIII activity is required to meet quality assurance regulations in Malaysia as well as in other European countries.[1,3] Similar results were reported by Von Heymann *et al*. (2009) where the level of FVIII was 78% up to 6 days after thawing.[15] The reduction in the activity of FVIII in our study has also been reported by other studies.[16-18] Lamboo *et al*. (2007) reported a reduction of 16% at 6 hours of storage at room temperature and 45% at 2 weeks of storage at

4°C.[16] Downes *et al*. (2001) reported a drop of 28% within the first 24 hours of storage at 1-6°C while the mean change from Day 1 to Day 5 was 35% for blood group B, and 41% for blood groups A and O.[17] Similar results were obtained by Sheffield *et al*. (2016), where the FVIII activity declined by 54.6% by Day 5.[18]

With regards to sterility, this present study did not show any sign of bacterial contamination in all thawed plasma samples. Blood cultures taken immediately after thawing and again on Day 5 were negative for bacterial growth. In this study, we only looked for contamination by aerobic bacteria. Anaerobic culture vials were not used in this study due to the aerobic storage conditions of FFP and thawed plasma which do not favour the growth of obligate anaerobes. Our results were supported by previous studies by von Heymann *et al*. (2009) who evaluated sterility of thawed plasma in 4°C which showed no bacterial contamination on Day 6.[15] Standard operating procedures for thawing FFP needs to be periodically reviewed to ensure the sterility of thawed plasma.

CONCLUSION

This study has demonstrated that the storage of thawed plasma for up to 5 days at 4°C maintained the recommended factor levels and did not show any evidence of microbial contamination. Although the labile FVIII level was significantly reduced over a period of 5 days, its activity was still above the minimum release criteria recommended in quality assurance regulations. Therefore, it can be concluded that thawed plasma stored at 4°C is suitable for use for up to
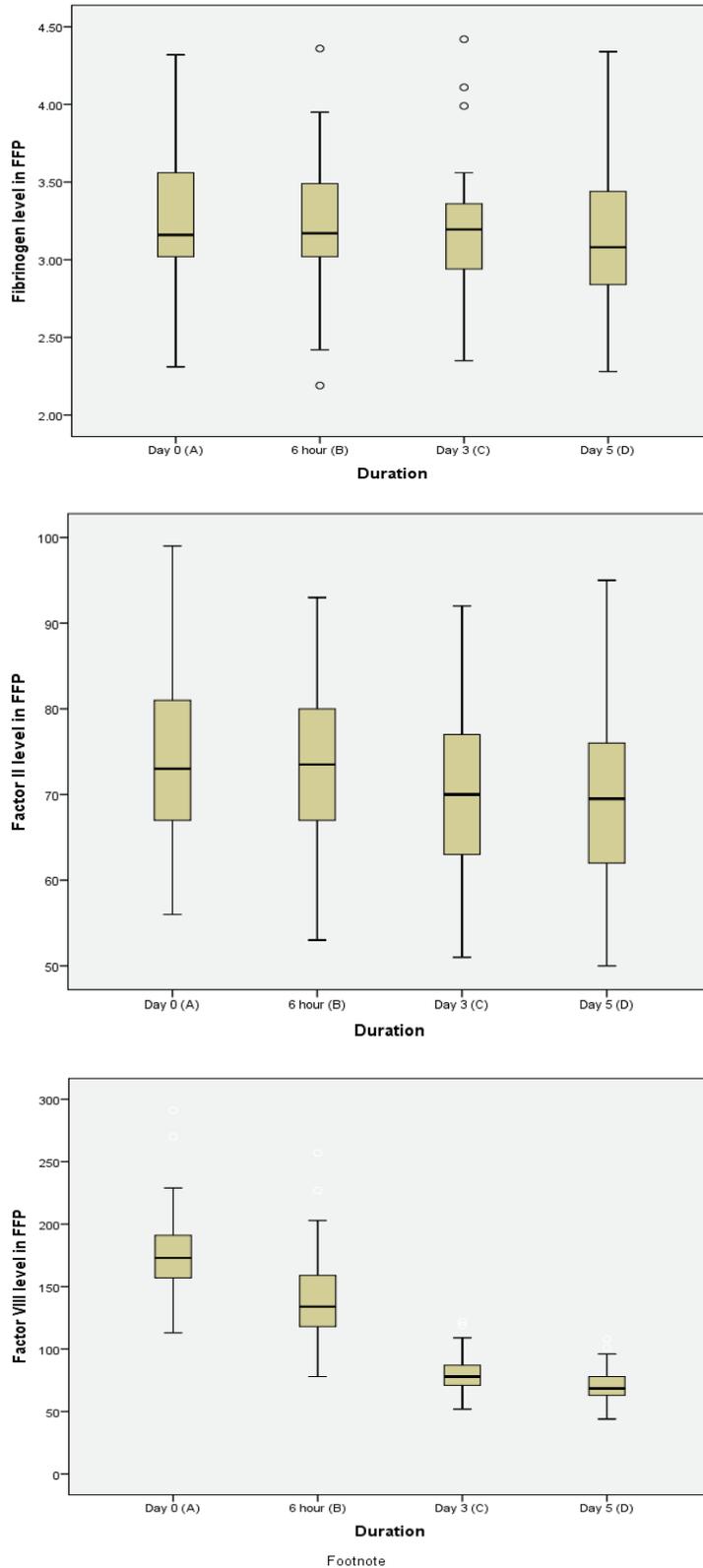
FIG. 2: Box and Whisker Plots for fibrinogen, Factor II and Factor VIII levels in thawed FFP obtained immediately after thawing (A), at 6 hours (B), at Day 3 (C), and at Day 5 (D) of storage at 4°C. Coagulation factor levels are displayed as median levels, quartiles of 25% and 75%, and minimum to maximum intervals.

5 days. However, despite the favourable outcome of the study, the decision to change the storage duration of thawed FFP for clinical use needs to be made after consideration of all the factors involved. The calibration and maintenance of the plasma thawer are important to ensure the quality of thawed plasma products.

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