AJLS

Asean Journal of Life Sciences, Vol 3(1), 2023, pp. 7-17

Accepted date: 20 May 2023
Publish date: 30 June 2023

Comparison Of Coagulation FVIII Content Between Blast-Frozen Fresh Frozen Plasma And Non-Blast Frozen Fresh Frozen Plasma

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Abstract

The fresh frozen plasma quality can be affected by many variables and factors. We investigated the effect of different freezing techniques and ABO blood groups on Fresh Frozen Plasma (FFP) quality concerning coagulation FVIII(FVIII) level. Sixty pints of whole blood each collected from the blood donation campaign were processed for FFP preparation. The sample from the FFP preparation is collected and counted for the baseline value of the FVIII level. The FFP from blood groups A, B and O were split equally to freeze using a blast freezer and conventional freezer (non-blast frozen). Then the FVIII level in FFP was analysed using a single-stage clotting assay (Post-FVIII level). The mean post-FVIII levels in blast, frozen FFP and non-blast frozen FFP were 0.86 ± 0.29 IU/ml and 0.66 ± 0.19 IU/ml. The mean percentage recovery of FVIII levels in blast-frozen FFP and non-blast-frozen FFP was 85.35% ± 9.65% and 68.43% ± 13.31%, respectively. The FFP prepared using the blast freezer had a higher per cent recovery of FVIII level than that of Fresh Frozen Plasma prepared using a conventional freezer ($P < 0.000$). The mean baseline FVIII level in blood group A and B (1.08 IU/ml) was higher than that of FVIII level in the blood group O Fresh Frozen Plasma (0.79 IU/ml). The FVIIIrecovery in Fresh Frozen Plasma improves significantly with rapid freezing using a blast freezer.

Keywords: Fresh Frozen Plasma, Blast Freezer, Non-blast Freezer, FVIII(FVIII), Freezing

Introduction

Fresh frozen plasma (FFP) is defined as the fluid portion of 1 unit of human blood that has been centrifuged, separated, and frozen solid. FVIII(FVIII) is a labile coagulation factor, and all steps of FFP production should be optimised to prevent a reduction in FVIII activity. Factors such as donor variation in FVIII levels, time and temperature between donation and the start

of the freezing process, the rate of freezing, and the blood group of the donor may affect the FVIII levels in Fresh Frozen Plasma (Dhantole et al., 2019).

Impaired haemostasis is one of the primary indications for transfusion of fresh-frozen plasma. However, there is limited knowledge of the clinical importance of the different plasma constituents and how they are influenced by various techniques for the preparation and freezing of plasma. Other studies demonstrate the importance of the freezing rate and the preservation temperature to maintain coagulation FVIII (Subramaniyan et al., 2017). Fresh-frozen plasma contains the labile and the stable components of the coagulation, fibrinolytic, and complement systems, the proteins that maintain oncotic pressure and modulate immunity, and other proteins that have diverse activities.

FFP is always used for hereditary and acquired coagulation disorders, thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulation (DIC), warfarin reversal, and massive transfusion. The quality of FFP directly affects the efficiency of clinical transfusion, and usually, the blast-frozen FFP preserved a better coagulation factor in FFP (Dhantole et al., 2019).

The study was conducted to assess the quality of FFP by measuring the factor VIII: C levels concerning freezing techniques using Conventional freezer versus blast freezer and on different ABO blood groups. Since coagulation FVIII (FVIII: C) is a labile haemostatic protein, care needs to be taken to avoid a reduction in FVIII activity during manufacturing (Farrugia et al., 2009).

This study compares the FVIII content in blast-frozen fresh frozen plasma and non-blast-frozen fresh frozen plasma. The FVIII contents quality in FFP was analysed using the blast freezer and conventional freezer. Further analysis to measure the FVIII recovery yield in blast-frozen and non-blast frozen Fresh Frozen Plasma and to compare the ABO blood group association with FVIII recovery yield levels in blast-frozen and non-blast frozen Fresh Frozen Plasma

Methodology

Ethical Consideration

The study was submitted to the Ethics Committee of Universiti Selangor, and has obtained the appropriate approval with Reference Number J160247E.

Sample Size

The sample size is calculated based on the National Blood Center Transfusion Guidelines 2016 in quality parameters. The frequencies of monitoring for blood and frequency of control suggested are ten units in the first month of storage. There were 60 samples taken between October 2019 and December 2019 (Dogra et al., 2015). The inclusion criteria for the one-stage clotting assay ACL TOP 350 CTS system data were Fresh Frozen Plasma samples from A blood group, B blood group and O blood group. The inappropriate specimen collection and

handling were discriminated against to avoid any contamination. Samples that came with insufficient quality or quantity of samples, wrong type of tube used, and lipemic were excluded.

Specimen Types and Collection

The types of samples used in this study were from the fresh frozen plasma. The plasma was collected in a blood bag and transferred to a plain tube for a quick FVIII level one-stage clotting assay test for baseline, and the sample for post-freezing after the FFP was thawed. The donor was selected randomly and collected using phlebotomy after the donor passed the blood donation criteria. The whole blood unit was collected using triple blood bags (Terumo penpol Ltd) of 450 ml collection. The entire blood unit collected from the blood donation mobile campaign was then transported while maintaining the storage temperature using the ice gel packs and digital thermometer logger. Subsequently, the blood grouping was done from the donor samples using pilot tubes. 20 donors were selected for each blood group A, blood group B and blood group O making a total of 60. The component preparation was done by following several steps. First, the whole blood units were weighed and balanced before centrifugation and tapped correctly to avoid red cell contamination in the plasma bag. Then, using a refrigerated centrifuge (HETTICH ROTO SILENTA 630RS, Germany), these whole blood (WB) units were separated into packed red blood cells (RBC) and platelet-rich plasma (PRP) using the light spin (2500 rpm for 10 min) at a temperature of 22°C with acceleration and deceleration values of 9 and 4, respectively. The plasma then was expressed into the individual's satellite bag with the help of a manual plasma expression. Before quarantining the stock, the plasma weight is recorded and ready for blast freezing or non-blast freezing.

Sampling of plasma before freezing

The transfer segments tube of the bag containing plasma was adequately stripped to ensure that there was a continuous column of plasma in the tubing segments. This segment containing the plasma was detached from the bag tubing, and the plasma bag was then frozen. After cutting the end of this tube segment with a scissor, the plasma (2 ml) was transferred into a plain tube container and used to determine FVIII levels using the ACL TOP 350 CTS system. Then, the plasma was tested on the same day of collection and separation. These FVIII levels were taken as the baseline values and counted immediately as FVIII is a labile coagulation factor and easily degraded.

Freezing of plasma units

Within each of the blast-frozen and non-blast frozen, half of the plasma units (n = 30) were frozen using a blast freezer (Dometic, MBF21, Luxembourg, Europe) for 30 minutes and the remaining half (n = 30) were frozen using conventional deep freezer at −40°C (Panasonic freezer) in 6 to 8 hours. Then, 10 frozen plasma units for each of the blood groups A, B and O were immediately transferred to a deep freezer and stored at −40°C. All these units were labelled as fresh frozen plasma (FFP).

Thawing of fresh frozen plasma units and sampling of plasma after freezing

Thawing was done using a 37°C agitating water bath (HETTICH plasma thawed) by packing FFP units in individual polythene wrappers and placing them vertically inside it for about 12 minutes. The endpoint of thawing was considered when there was a clear plasma formation in these units. The thawed plasma was transferred from the blood bag into the plain tube and 2-3 mL of the samples were taken for sampling.

Measurement of factor VIII: C levels in fresh frozen plasma

FVIII: C was measured using ACL TOP 350 CTS system, a one-stage clotting assay. Factor VIII: C was analyzed using the one-stage clotting assay-activated partial thromboplastin time (aPTT) method. The percentage recovery of factor VIII: C levels in FFP were also calculated.

Data Collection and Analysis

For data collection and analysis, this research study conducted the measurement of FVIII level from one stage clotting assay ACL TOP 350 CTS system, and Statistical Package for Social Science (SPSS) version 24 was used to analyse all data in the present study to measure descriptive analysis, independent t-test, and ANOVA association. Parameters like ABO blood group and different freezing techniques of FVIII level outcome of results were also collected to observe and compare blast-frozen FFP and non-blast frozen FFP.

Result and Discussion

Baseline Value for FVIII Levels in Plasma

The result of the study showed mean (±SD) FVIII: C levels in plasma in blast-frozen and non-blast-frozen were 0.98997 ± 0.281603 IU/bag, respectively, with A blood group 1.08895 ± 0.238746 IU/bag, B blood group 1.08935 ± 0.30837 IU/bag and O blood group 0.7960 ± 0.179358 IU/bag (Table 1).

Table 1. Baseline levels of FVIII in plasma by ABO Blood Group

Blood group	N	Mean	Std Deviation
A	20	1.08895	0.238746
B	20	1.08935	0.308137
O	20	0.79160	0.179358

Total	60	0.98997	0.281603

SD = standard deviation

The mean (±SD) for baseline FVIII levels in blast freezer and conventional freezer were 0.9995 ± 0.2983 and 0.9804 ± 0.2686 (Table 2). It demonstrates that in terms of baseline values for different freezing methods, there is little difference between a blast freezer and a conventional freezer.

Table 2. Baseline levels of FVIII: C in plasma before freezing using blast freezer and conventional freezer

Freezing method	N	Mean	SD
Blast freezer	30	0.9995	0.2983
Non-blast freezer	30	0.9804	0.2686

SD=Standard deviation

Many studies have assessed the effect of different freezing techniques and ABO blood groups on FVIII levels in plasma. FFP has approximately 50–80% of the FVIII and 30–50% of the fibrinogen of the starting plasma. Because of its low volume of isoagglutinin and absence of red blood cells, FFP can be transfused without regard for the ABO group or Rh type of the original unit, though ABO compatibility is preferred in neonates. FFP is an invaluable source of FVIII in patients with haemophilia (Subramaniam et al., 2015). The results from the study showed that the A and B blood groups have the highest baseline levels of FVIII in plasma compared to the O blood group. There are no significant differences in A and B blood groups toward baseline FVIII level but there are significant differences between A and O blood groups with p-values of 1.000 and 0.001 respectively using the ANOVA and post hoc analysis.

The results indicate that the A and B blood groups are the best blood groups that can be used for FFP preparation. This is because A and B have higher baseline levels compared to the other blood groups. The higher blood group baseline levels of FVIII can contribute to higher recovery of FVIII levels post freezing whether it uses blast freezing or non-blast freezing method. (Song et al., 2015) stated that the A and B blood groups have higher FVIII levels and other proteins such as VWF in all genders by race group and the lowest in the blood group O. They also suggest that variance ABO blood groups influence the FVIII levels activity. As such, the higher baseline levels of FVIII levels in blood groups A and B can contribute to higher post-freezing FVIII levels and percentage recovery of FVIII levels.

Effect of Freezing Technique on FVIII Levels in FFP

The mean for FVIII levels post-freezing in FFP were 0.6591 ± 0.1943 and 0.8583 ± 0.2969 using conventional and blast freeze techniques, respectively which was statistically significantly different at P-value = 0.003 (Table 3).

Table 3. The mean of factor viii post freezing for non blast and blast freezing

Freezing technique	Mean	SD	P-value
Non-blast	0.6591	0.1943	0.003
Blast	0.8583	0.2969	

Note: significant difference at $p < 0.05$
SD = Standard deviation

This study reveals the difference of effect in different freezing techniques using blast freezer and non-blast freezer on FVIII levels in FFP. The blast freezer has a higher mean of FVIII levels post-freezing compared to non-blast freezing (conventional freezing). The results showed a significant difference in the mean FVIII levels at 0.8583 ± 0.2969 and 0.6591 ± 0.1943 between the blast freezer and non-blast freezer. These results suggested that the blast freezing method is better compared to conventional freezers in terms of FVIII preservation.

(Philip et al., 2013) found that the FVIII levels in FFP improved significantly with higher baseline FVIII levels from blood group A donors and rapid freezing using a blast freezer. Rapid freezing also increases the fibrinogen yield. The freezing method is proven to be one of the common factors that can influence FVIII activity and must be employed in the blast freezer to avoid FVIII and other coagulation factors and activity loss related to plasma protein degradation. The storage temperature and time also can affect the stability of thawed FFP in terms of FVIII levels and other coagulation factors (Sheffield et al., 2016). Based on this study, it is suggested that the FVIII minimum levels should be at least 0.7 IU/bag and stored at 1 to 6 degrees Celsius for 120 hours. However, the coagulation factors will be degraded even though it is still in the minimal range (Philip et al., 2014).

Effect of Freezing Technique on FVIII Recovery Levels in FFP

The mean percentage recovery of FVIII levels in FFP were 68.4 ± 13.3 and 85.4 ± 9.7 (Figure 3) using conventional and blast freeze techniques, respectively which was statistically significant at p-value = 0.0001 (Table 4).

Table 4. The mean percentage recovery of FVIII

Freezing technique	Mean	SD	P-value
Non-blast	68.4327	13.3144	0.0001
Blast	85.3505	9.65146	

Note : significant different at $p < 0.05$
SD = Standard deviation

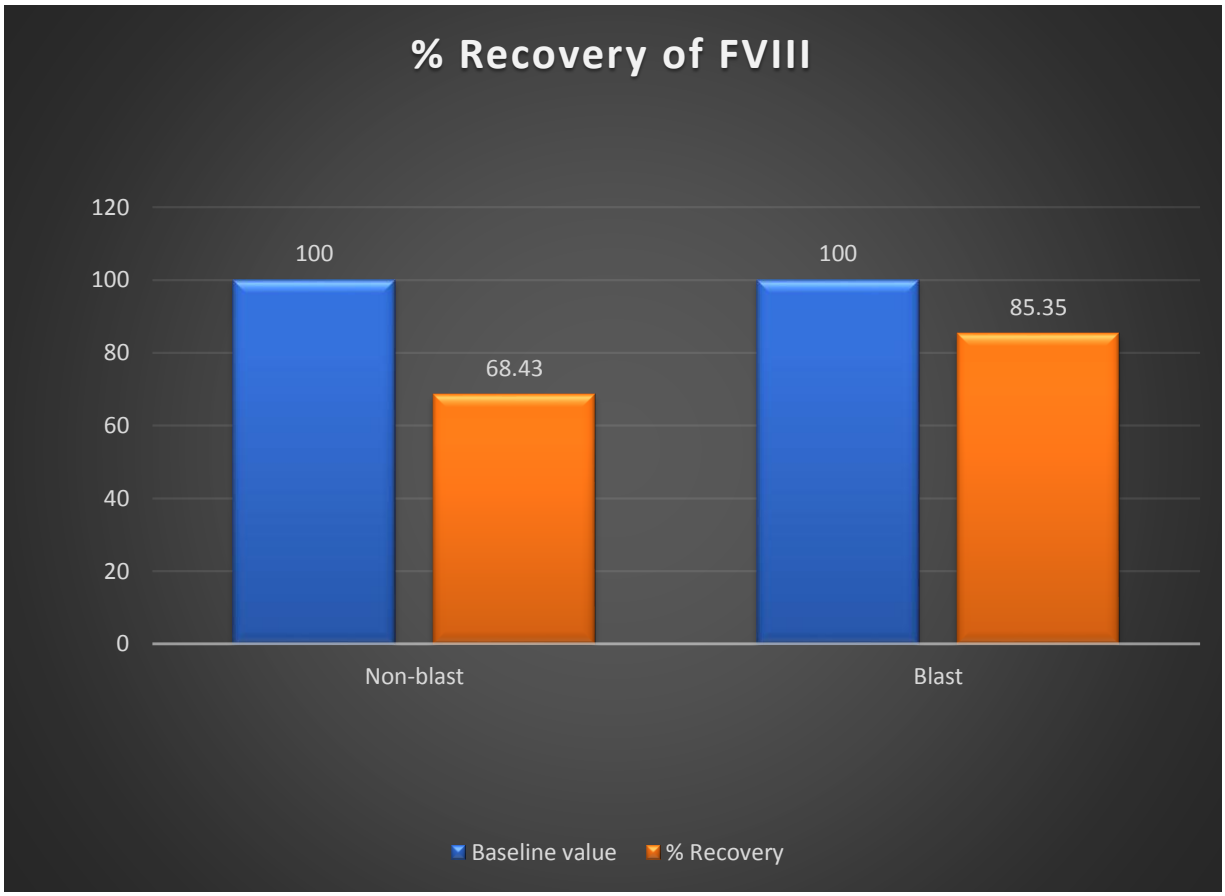

Figure 3. The mean percentage recovery of FVIII

The peak levels of FVIII activity recovery can be found in the blast freezing method in terms of factor viii recovery level compared to the non-blast freezing method. The mean percentage recovery of FVIII levels in FFP were 68.4 ± 13.3 and 85.4 ± 9.7 using conventional and blast freeze techniques, respectively which was statistically significantly different with $p = 0.0001$. This study evaluates the percentage of FVIII recovery levels from different freezing techniques using the blast freezer and conventional freezer. In comparison to the conventional way of freezing, the blast freezing method demonstrates a significant difference in the percentage of FVIII recovery in FFP. The blast freezer has a higher percentage of FVIII levels by 85.4 % mean compared to the conventional freezer which is 68.4 %. A previous study evaluated that since coagulation FVIII (FVIII: C) is the most labile haemostatic protein, where the freezing process of the plasma needs to be optimized for the best yield of FVIII recovery. The FFP FVIII levels also can be easily degraded due to storage temperature and freezing method (Acker et al., 2016). The stability of thawed FFP is limited to a few hours and thus, to avoid the loss of FVIII activity, the test to measure the FVIII levels must be run in a short time (Caudill et al., 2009).

Association of Freezing Method and ABO Blood Groups on The Quality of FFP

The result for the post-freezing method using the blast freezer showed that blood group B had the highest mean value of FVIII at 1.0245 IU/bag followed by blood group A at 0.9143 IU/bag. Blood group O FFP contains the lowest FVIII level post-freezing at 0.6360 IU/bag as shown in [Figure 4].

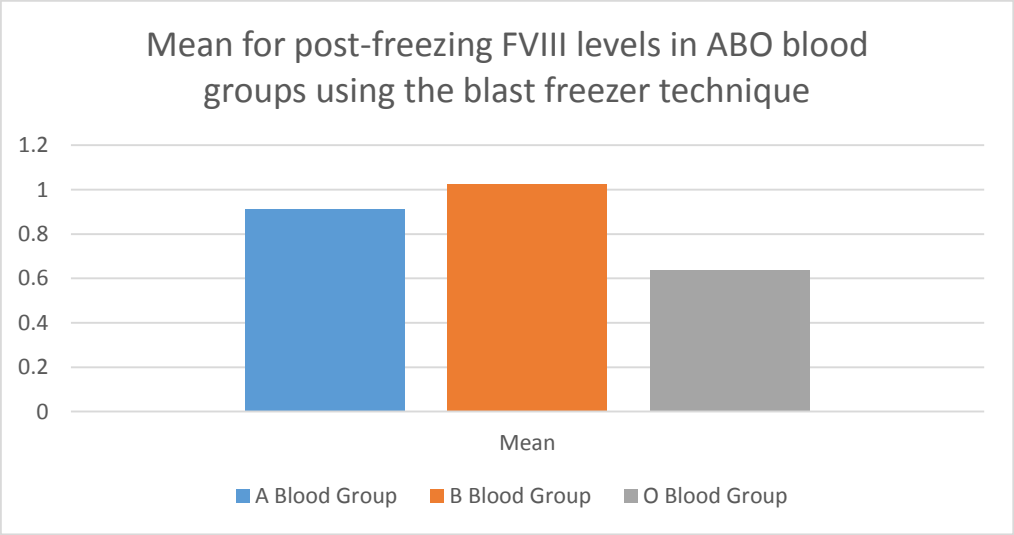

Figure 4. Mean for post-freezing FVIII levels in ABO blood groups using the blast freezer technique

The result for the post-freezing method using the non-blast freezer showed the B blood group with the highest mean value of FVIII at 0.7165 IU/bag followed by the A blood group at 0.6661 IU/bag. The O blood group FFP had the lowest FVIII level post-freezing at 0.5946 IU/bag as shown in [Figure 5].

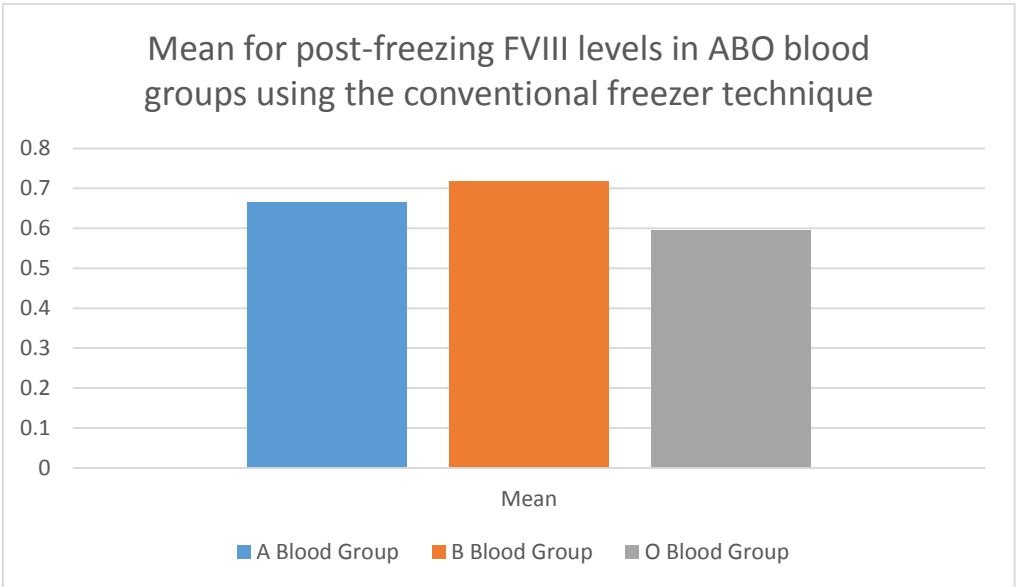

Figure 5. Mean for post-freezing FVIII levels in ABO blood groups using the conventional freezer technique

This study found that there is an association between the freezing method and the influence of ABO blood groups on the quality of FFP but (Wang et al., 2017 also reported that high altitude and low altitude can also affect the quality of fresh frozen plasma. Plasma that is frozen rapidly through a contact shock freezer had significantly lower aPTT and higher levels of FV and FVIII compared to mechanical freezing. Rapid freezing is recommended for optimum preservation of coagulation factors, especially FVIII levels (Dhantole et al., 2019). ABO blood groups are known to influence the VWF plasma level, but the link between ABO blood group and the most essential coagulation factor in FFP, FVIII levels, is less well understood (Song et al., 2015). The A and B blood groups have higher FVIII levels in plasma compared to the O blood group. FVIII levels are somewhat higher in the B blood group than A blood group, although the difference is not significant. The higher baseline from the blood groups A and B can affect the post-FVIII levels and recovery percentage of FVIII levels.

Another study found that when comparing blast freezing and non-blast freezing, the freezing method has a significant impact on FVIII recovery, which affects FVIII levels in FFP. In terms of preservation condition, a study done by Subramaniam et al., 2015 claimed that the blast freezer showed a substantial difference in FVIII recovery in cryopreservation related to FVIII recovery in FFP when compared to a conventional freezer. Furthermore, it is also shown that plasma frozen rapidly through a blast freezer or contact shock freezer had significantly lower aPTT and better levels of FV and FVIII compared to non-blast freezing (Yazer et al., 2010). There were no significant differences between PT, aPTT, fibrinogen, FV, FVII and FVIII levels of FFP thawed at 37°C and 45°C. The mean thawing time was 28 minutes at 37°C and 17 minutes at 45°C (Wilsher et al., 2008). The FFP from A and B blood groups which were prepared using the blast freezer has the best quality of FFP in terms of FVIII levels. The best quality of FFP in terms of FVIII levels is, the FFP from blood groups A and B that were prepared using that blast freezer. FVIII has a higher baseline level in A and B blood groups which can impact the FVIII recovery. Meantime the blast freezer is the best method of freezing because it can preserve better FVIII levels that can affect the FVIII recovery levels after the plasma has been thawed.

Limitation of the Study

Since the budget for this study was limited, only a small size of samples was used to observe the FVIII levels for baseline value and post-freezing effects. Selection and collection of the FFP samples were restricted only from November 2019 until December 2019 due to the collection site exchange for a brand-new machine for the coagulation test. The measurement test for FVIII is not a routine test but a special test, thus, it needs a special QC reagent. The FVIII level needs to run for testing as soon as possible because the FVIII is a labile coagulation factor and can be degraded in 4 hours. The blast freezer also needs a special machine that is used for FFP freezing before the FFP can be kept for storage.

Conclusion

From this study, freezing the FFP of the A and B blood groups in a blast freezer is the best method for recovering high levels of FVIII in plasma. The O blood group FFP and the conventional freezing method appear to be ineffective in preserving the quality of FVIII level in plasma and its recovery yield. Thus, the component preparation for FFP using the blast

freezer is the best method because it can effectively recover the FVIII level, and the A and B blood groups can also affect the FVIII as the baseline level is higher than blood group O FFP.

Further research using a bigger sample size and the addition AB blood group should be conducted to improve the accuracy and precision of the evaluation. Future studies should include other coagulation factors in FFP such as fibrinogen and von Willebrand factor to observe the total composition in FFP. The study can also be conducted at various collection sites as the altitude can also affect the baseline FVIII level and the quality of FFP. The Head of Department (HOD), with the help of the employees, shall make an ongoing effort to improve the quality of FFP preparation for the benefit of the patients. Rather than using a conventional freezer, a blast freezer is required to boost the FVIII recovery yield levels in FFP.

Acknowledgment

We are thankful to the Pathology Department staff in Hospital Sultanah Nur Zahirah who has helped in conducting the research.

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