Original Research Article

Estimation of Fibrinogen and Factor VIII levels as a Quality Assurance activity in Fresh Frozen Plasma (FFP): Study from a Blood Bank attached with a tertiary care hospital of Konkan region, Maharashtra, India

Bhushan M Warpe[1], Shweta Joshi-Warpe[2*], Vinayak V. Haldive[3]

[1,2]Associate Professor, [3]Technician, Department of Pathology, B.K.L. Walawalkar Rural Medical College (BKLWRMC), Dervan, Ratnagiri, Maharashtra, INDIA.
Email: shwetawarpejoshi@gmail.com

Abstract

Background: As per standard guidelines, for quality assurance of FFP, 1% of all the units prepared or four units per month are tested for stable coagulation factors: Factor VIII and Fibrinogen levels. **Objectives:** To analyze the activity of factor VIII and fibrinogen level in fresh frozen plasma (FFP) with respect to age, gender, ABO blood grouping, donor demography and timing of factor testing in days with respect to separation date of FFP. **Methods:** The observational study data was collected from blood bank attached to tertiary care center of Konkan region of Maharashtra. The duration of study was four years and seven months from the period of 1st June 2016 to 31st December 2020. The overall FFP bags for Quality assurance were 3.63%. FFP was tested for fibrinogen content and factor VIII levels for quality control with the help of automated coagulometer. **Results:** The mean fibrinogen value was 262.83±55.39 mg/dl and mean factor VIII values was 110.56±31.56 %. The highest value of fibrinogen is 375 mg/dl and lowest is 139 mg/dl respectively. The minimum factor VIII value is 27.9 % and maximum value is 190% respectively. The mean fibrinogen and factor VIII values were high when there is less duration between FFP separation date and date of factors testing for quality assurance. No such study has been done in Western India. **Conclusion:** Quality of FFP prepared at our blood bank meets the international standards. Regular quality evaluation and maintenance of records helps provide quality FFP blood product to meet the patients' needs.

Keywords: Fresh-frozen plasma (FFP); Fibrinogen; Factor-VIII.

[*]**Address for Correspondence:**
Dr Shweta Joshi-Warpe, Associate Professor, Department of Pathology, B.K.L. Walawalkar Rural Medical College (BKLWRMC), Dervan, Ratnagiri, Maharashtra, INDIA.
Email: shwetawarpejoshi@gmail.com
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INTRODUCTION

Plasma is the aqueous component of blood in which many different cellular elements and macromolecules are suspended, but it is the proteins that have been the focus of interest for transfusion medicine, including specifically albumin, coagulation factors, and immunoglobulins. Transfusion of fresh-frozen plasma (FFP) is indicated to replace deficiencies of multiple coagulation factors in massive blood loss, liver disease, and disseminated intravascular coagulation (DIC).[1]

The management of inherited or acquired bleeding diseases is a real challenge in resource-limited

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countries. The lack of efficient mechanism for the management of hemorrhagic diseases could explain the high lethality associated to this epidemic. In obstetric context, post-partum hemorrhage is the leading cause of maternal mortality in resource-limited countries.[2-4] Plasma separated from whole blood, frozen within 6 to 8 hours of collection and stored at -40˚C or below is defined as fresh frozen plasma (FFP).[1,2] Usually 175-250 mL of FFP is separated from standard donation of whole blood (450 ml), containing factor VIII, factor IX, von-Willebrand factor and other plasma clotting factors.[2,3]

As the demand for FFP is increasing in day to day practice, its quality management is of utmost importance. Quality analysis of blood components including FFP depends on the concepts of quality control, quality assurance and quality management which aim at providing right blood to the right person at right place and time.[2,3] Quality control of blood and its components ensures the availability of high quality product with maximum efficacy and minimal risk to recipients.

As per standard guidelines, for quality assurance of FFP, 1% of all the units prepared or 4 units per month are tested for stable coagulation factors (200 Units of each factor); Factor VIII - 0.7 Units/ml and Fibrinogen levels 200-400 mg/dl. Factor VIII and fibrinogen levels can be measured in a standard blood bank with the help of coagulometer by clotting assay.[2] Internal quality control (IQC) is the backbone of quality assurance program. In blood banking, the quality control of blood products ensures the timely availability of a blood component of high quality with maximum efficacy and minimal risk to potential recipients.[4,5]

After literature search, though there are many studies on usage of FFP, we found just three similar studies from Indian subcontinent based on quality assurance of prepared FFP bags. The aim of present Konkan based study signifies the regular monthly monitoring of FFP bags for quality assurance based on factor assays. This study will help to provide quality FFP blood product to meet the patients' needs.

METHODOLOGY

The estimation of fibrinogen and factor VIII in 220 FFP bags was done at Shree Swami Samarth Blood Bank in Konkan region of Maharashtra. The study period was of four years and seven months duration. Outdoor camp collection as well as indoor blood collection was evaluated.

The study design is Observational Descriptive study. The duration of the study spans from 1st June 2016 to 31st December 2020 (4 years and 7 months). The sample size was 220 FFP bags (4 bags each month). The Inclusion Criteria was as follows:- 1) All donors with male and female were studied of all age groups, with or without complications. 2) The donor with no medical history, no drug history, are selected for study. 3) The donors who are eligible for blood donation and fulfill donation criteria.

The Exclusion Criteria comprised:- 1) The donors with medical and drug history are excluded. 2) The donors suffering from HIV, HCV, Syphilis, Malaria, HBV.

The materials required for FFP preparation and factor testing: Syringe of volume 2cc and needle, Sterile lancet, Cotton, Spirit or 70% alcohol, 450 – 350 ml Triple bag/ double bag, Thawing bath, Weighing machine, Refrigerated centrifuge, Lamina air flow, Refrigerator, Tube Sealer, Plasma Expresser, ACL elite Automated coagulometer by Instrumentation laboratory company.

Method :- I) Blood collection: This is the basic important part of FFP preparation or blood bag preparation. Selection of donor is done with the help of blood donation criteria or eligibility form. The syringe is inserted in median cubital vein and blood collection procedure started. After collection of sufficient blood in the blood bag, the syringe is removed and donor held under observation and blood bag stored in temperature controlled box. The blood bags are dispatched for further-procedure.

II) Fresh frozen plasma preparation:

Preparation of Packed Red Cells and F.F.P. using 'double' bags: Keep the units vertical on the Laminar air flow table for 30 minutes (Fig. 1). Process all units within 6 hours of blood collection. Keep the bags in the buckets and balance them accurately. Keep the equally balanced buckets with bags diagonally opposite in the refrigerated centrifuge ensuring that the position of the bags in buckets is parallel to the direction of the spin.

Figure 1: The photograph *(left)* shows laminar air flow used to maintain sterile environment and *(right)* show plasma expresser used for separation/expression of plasma.

Centrifuge the bags at 2500 rpm, for 11 minutes at 4° C. After centrifugation, gently remove the bags from the bucket and place them on the plasma expresser stand under the laminar air flow. Break the integral seal of the tube connecting it to the satellite. Then express the supernatant plasma into the satellite bag by using plasma expresser (Fig. 1). In case of double bag, Leave 50-60 ml of plasma back along with the red cells in the primary bag and this component is called Packed Red Cells (PRCs). Label the plasma in the satellite bag, as Fresh Frozen Plasma (FFP) if separated within 6 hours of collection and stored immediately below -40° C. Cut the segment of FFP bags.

Preparation of Packed Red Cells, Platelet Concentrate and FFP using 'triple' bags with additives solutions: Process the blood collected within 6 hours. Keep the bags erect on the laminar air flow for 30 minutes. Note the weight of the primary bag and record in the component separation register. Balance the bags in the buckets using dry rubber or unused bags. Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge. Position the bags in buckets parallel to the direction of the spin.

Figure 2: The photograph shows centrifuge programed for low spin centrifugation.

Centrifuge the bags at 1500 rpm for 15 minutes at 23° C (Fig. 2). Keep the bag on the separator on the laminar air flow. Break the seal of the tubing connecting to the satellite bag and express the plasma into the satellite bag. If the bag with additive solution is used, remove all plasma in satellite bag before clamping. Remove the clamp of the bag containing additive solution and let the additive solution slowly pass in to the primary bag containing red cells. Mix the contents thoroughly and seal the tubing and detach the bags. Place the primary bag containing packed red cells with additive solution in the untested blood Bank refrigerator kept in the Quarantine room (Fig. 3). Label the bag and take it on the inventory after the testing is over.

Figure 3: Storage chamber of deep freezer for FFP bags.

Spin the satellite bag containing platelet rich plasma (PRP) and connecting bag from which additive solution was emptied. At 22° C in refrigerated centrifuge at 3200 rpm for 11 minutes after balancing the buckets. Place the bag containing PRP on the expresser stand. Express the plasma into the empty bag leaving 50-60 ml plasma along with platelets. Seal the tubing and cut the tubing of the plasma bag short to avoid breakage during frozen storage. A small segment of tube containing platelets is prepared after mixing of the bag and keep bag in the untested platelet incubator with agitator at 20°-24° C. Keep the plasma bag in the untested compartment of the deep freezer kept in the storage room.

Products of Packed Red Cells, Platelet Concentrate and FFP using 'triple' bags:

Mother blood bag: Packed Red Cells (230- 380 ml),

Second Bag: Fresh Frozen Plasma (120 — 250 ml) (Fig. 4),

Third bag : Platelet Concentration (50-60 ml).

FFP storage: -40° C for 1 year and -80° C for 3 years.

Figure 4: A FFP bag after component separation

III) Estimation of fibrinogen and factor VIII using ACL elite (Automated Coagulometer)

After relevant testing for machine controls, standard operating procedures were used to evaluate the

coagulation factors. FFP bag was tested for coagulation factors of Fibrinogen and Factor VIII soon after thawing the bag.

Fresh frozen plasma is thawed over 20-30 minutes at a temperature between 30 and 37^0C. The activity of the labile coagulation factors, specifically factor V and VII, decreased gradually after thawing, and thus it is recommended that fresh frozen plasma is best used soon after thawing. Four FFP bags were tested for these clotting factors every month (Fig. 4).

RESULTS

In the present study, total 6053 FFP bags which were prepared by our blood bank department from 1st June 2016 to 31st December 2020. The total FFP bags evaluated for quality assurance were 220/6053=3.63% FFP bags. After literature search, though there are many studies on usage of FFP, we did not find enough cumulative studies based on quality assurance of prepared FFP bags. In present study, we have compared the 220 quality assurance bags which are prepared in our tertiary care hospital blood bank during the four year and seven months study period.

Table 1: Year wise distribution of Fresh frozen plasma bags preparation

Sr.no.	Year	Total bags collected (per year)	Total prepared – FFP bags (per year)	FFP samples for quality assurance
1	2016 (01-06-2016 to 31-12 2016)	679	547	28
2	2017	1584	1343	48
3	2018	1487	1303	48
4	2019	1671	1392	48
5	2020	1733	1468	48
Total	4 Years and 7 months	7154	6053	220

Total FFP bags prepared: 6053÷7157×100= 84.61%

Table 1 shows, in the year 2020, highest number of FFP bags has been prepared which is 1468 and lowest in 2018 which is 1303 in count.

Table 2: Distribution of blood group on FFP samples for quality assurance

Blood group	No of bags tested of blood group	Rhesus	No of FFP bags tested (%)
A	30.4%	Positive	30%
		Negative	0.40%
O	29.50%	positive	29%
		Negative	0.40%
B	29.09%	Positive	29.09%
		Negative	0%
AB	10.90%	Positive	10.90%
		Negative	0%

Table 2 shows the blood group wise distribution of quality assurance FFP samples. The blood group 'A' had least no of count which is 30.4 % and blood group 'B' and 'O' were having 29.09 % and 29.5 % bags tested respectively. This table shows 'AB' blood group bags with 10.90%.

Table 3: Distribution of Mean Fibrinogen and Mean Factor VIII levels w.r.t. blood group in 220 FFP samples for Quality Assurance.

Blood group	No of bags out of 220 (%)	Mean Fibrinogen (mg/dl)	Mean Factor VIII (%)
A' Rh Positive	66 (30.4 %)	260.65	110.08
A' Rh Negative	1 (0.45%)	195	103
B' Rh Positive	64 (29.09 %)	265.35	112
B' Rh Negative	0	0	0
AB' Rh Positive	24 (10.90%)	258.7	109.4
AB' Rh Negative	0	0	0
O' Rh Positive	64 (29.09%)	268.4	111.4
O' Rh Negative	1 (0.45%)	52.8	48

Table 3 showed the distribution of mean fibrinogen and mean factor VIII levels with respect to blood group in 220 FFP for Quality assurance. In the above table 'A,' Rh positive, 'B,' Rh positive and 'O,' Rh positive which having sample count 66, 64 and 64 respectively and mean fibrinogen value of 260.65, 265.35, 268.4 mg/dl values respectively. In 'AB,' Rh positive blood group; 24 bags has been recorded which showed mean value of 258.7 mg/dl. Rh-negative blood group bags like 'B' and 'AB' Rh negative were having no records of quality assurance sample in FFP as they were least used

for quality assurance in our set-up. This means Rh negative FFP samples should be used for quality assurance in our blood bank and other set-ups as well.

Table 4: Distribution of fibrinogen and factor VIII levels w.r.t. age group

Age group (Years)	15-30	30-45	45 and above
Sample	128	66	26
Mean fibrinogen in mg/dl	264.84	256.1	258.42
Mean factor VIII in %	112.64	108.9	108.41

Table 4 shows the distribution of age of donor in our study. The least no of fibrinogen level donors where in the age group of 15-30 years which is 264.84 mg/dl and factor VIII having value 112.64%. Out of 66 samples of age group, 30-45 years, the mean fibrinogen value was 256.07 mg/dl and mean factor VIII value was 108.93% respectively. There is no significance difference in mean fibrinogen and factor VIII values with respect to age groups. However people aged less than 30 years of age recorded slightly higher values compared to those aged above 30 years of age.

Table 5: Gender-wise Distribution w.r.t. fibrinogen and factor VIII levels

Gender	Males	Females
Total	214 (97.27%)	6(2.72%)
Mean Fibrinogen (mg/dl)	264.24	261
Mean Factor VIII (%)	111.35	95.76

In the Table 5, we showed distribution of fibrinogen and factor VIII with respect to gender. There are 214 males and 6 females in count and there is no significance difference in fibrinogen and factor VIII levels. The factor VIII level seen is slightly higher in males as compared to females but the difference is negligible. There was less participation of female FFP sample count of quality assurance samples. So further study is required for level of factor VIII and fibrinogen estimation in females as in our study there is no significance difference in gender wise analysis as the difference in values are negligible.

Demography: The highest no of quality assurance FFP bags are from Ratnagiri district. The other district based samples were few as this blood bank caters to this Ranagiri native district. Devgad taluka of Sindudurg district showed the highest fibrinogen level which was 353 mg/dl. Kudal taluka of Sindudurg district showed the highest factor VIII level which is 180 %. The minimum fibrinogen value was recorded in Shiroli taluka of Sangli district.

Highest fibrinogen value was recorded from FFP bag of Thane district which is 343 mg/dl and lowest in Sangli which is 170 mg/dl. The highest factor VIII value was recorded in Sindhudurg and Jalgaon district which is 150 % and lowest is recorded in Thane district which is 52.8 %. This needs confirmation from blood bank data from other districts with similar study. In the study, some districts and talukas contain less no of bags collected, so further study is required for analysis.

Table 6: Distribution of duration between separation date and factor testing date for FFP samples

No of days between separation date and date of factor testing for quality assurance	Fibrinogen: mean (mg/dl)	Factor VIII: mean (%)
0-10 (count-29)	279.2	110.94
011-20 (count 92)	268.5	111
21-30 (count 97)	254.67	111.24
31-60 (count-2)	161	51.55

Table 6: In this table, the distribution of numbers of days between separation date and factor testing date was done. As in the table shows the mean fibrinogen and factor VIII values were high when there is less duration of separation date and date of factor testing. Highest fibrinogen levels are recorded in 0-10 days of duration which is 279.20 mg/dl. The increase in the duration between separation date and date of factor testing leads to reduced mean fibrinogen levels. Hence 161 mg/dl is the mean fibrinogen value, when the duration is 31-60 days.

Table 7: Levels of fibrinogen Distribution in FFP sample for quality assurance.

Fibrinogen (mg/dl)	<179	180-250	251-300	301-350	>351
No of bags	8	90	64	47	11
Percentage	3.63%	40.90%	29.09	21.36%	5%
Mean	151.6	215	280	325	362

Table 7 shows the level of fibrinogen distribution in FFP samples for quality assurance. In the table, highest no of bags with fibrinogen range of 180-250 mg/dl was 90 bags in count. There were 11 bags with fibrinogen value more than 351 mg/dl. There were 8 bags with fibrinogen value less than 179 mg/dl.

Table 8: Level of factor VIII distribution in FFP samples for quality assurance

Factor VIII (%)	25-69	70-150	>151
No of bags	10	190	20
Percentage (%)	4.54	86.36	9.09
Mean	51.96	107.66	166

Table 8 shows the level of factor VIII distribution in FFP sample for quality assurance. There are 87.27% bags with 70-150 % factor VIII value and 9.09% bags with >151% factor VIII value. 4.54% FFP bags had low factor VIII values.

Table 9: Range of values of factor VIII and Fibrinogen in our study

Range of values for fibrinogen and factor VIII			
	Maximum	Minimum	Mean
Fibrinogen (mg/dl)	375	139	262.83±55.39
Factor VIII (%)	190	27.9	110.56±31.56

Table 9 shows the range of values for fibrinogen and factor VIII in our study. The mean fibrinogen value was 262.83±55.39 mg/dl and mean factor VIII values was 110.56±31.56 %. The highest value of fibrinogen is 375 mg/dl and lowest is 139 mg/dl respectively. The minimum value of factor VIII value is 27.9 % and maximum value is 190% respectively.

DISCUSSION

Utilization of fresh frozen plasma in clinical practice has been increased in recent years: Plasma for transfusion is most often used where there is abnormal coagulation screening tests, either therapeutically in the face of bleeding, or prophylactically in non-bleeding patients prior to invasive procedures or surgery.[6]

For safe and effective preparation of blood and its components, in house quality control plays a very important role. Quality concepts comprises of a triad of quality control, quality assurance, quality management and their maintenance.[7]. Quality control is the backbone of all laboratory services including blood bank. Quality testing and monitoring of blood components have led to development of safer and more potent components for transfusion practices. Factor VIII and fibrinogen levels are internal quality control parameters required for quality analysis of fresh frozen plasma as per standard guidelines.[8]

Table 10: Comparative study of Fibrinogen and factor VIII levels with other similar studies

	Dogra M, *et al.* (2015)[1]	Sultan S, *et al.* (2018)[5]	Bala G, *et al.* (2019)[2]	Our study (2021)
Fibrinogen: mean (mg/dl)	270.66±69.64	247.17±49.69	304.31±53.68	262.83±55.39
Factor VIII: mean (%)	117.205±29.01	84.24± 15.01	80±8.6	110.56±31.56

Table 10 shows our study with similar mean Fibrinogen values and Factor VIII values when compared to Dogra M, *et al.*[1]. Only three such similar Indian subcontinent-based studies have been done, till date. All these studies are from North India and South Pakistan. No such study has been done in Western India.

Our study assessed the levels of factor VIII and Fibrinogen in stored units of FFP after they were thawed for utilization. 3.63% of all units of FFP prepared in four years and seven months were evaluated and levels of both parameters were in concordance with standard guidelines. In our study, mean factor VIII levels were 110.56±31.56% and mean fibrinogen levels was 262.83±55.39 respectively.

As per Bathinda based (Punjab) study by Bala G, *et al.*[2], the mean volume of 41 FFP units tested was 217 mL with range of 192-235ml. The mean factor VIII levels were 80±8.6% and mean fibrinogen levels were 304.31±53.68 mg/dl. 97.5% of units tested had factor VIII levels above 0.7 U/mL and 100% units had fibrinogen levels more than 200mg/dl.

Similar study was done by Sultan S, *et al.*[5] at South Pakistan in which 100 units were tested for internal quality control. The mean factor VIII and fibrinogen levels were found to be 84.24±15.01% and 247.17±49.69 mg/dl for FFP respectively. Almost all donors had fibrinogen ≥150 mg/dl, while only five (5%) donors had factor VIII below the desired levels.

Dogra M, *et al.* (2015)[1] also did a study on comparative analysis of activity of Factors V and VIII and level of fibrinogen in Fresh Frozen Plasma and Frozen Plasma in GMC Jammu. They studied 100 units of FFP in which levels of fibrinogen were 270.66 ± 69.64 mg/dl and factor VIII was 117.205±29.01%.

Thus, all the above mentioned studies have evaluated quality control parameters as done in our study and results are in concordance as per standard reference parameters. FFP is generally not used in developed countries due to the availability of recombinant or factor concentrates; however, in developing countries like us utilization of FFP is more for various inherited

coagulation disorders and diseases leading to liver dysfunctions.

Internal quality control thus enhances the quality of blood products and helps in monitoring of quality standards of blood bank. Regular quality evaluation and maintenance of records helps to keep up the working standards and any deficiency can be checked and curtailed. The results derived in our study are in concordance with the national standards and other studies reviewed above, thus establishing the quality standards of our blood bank.

CONCLUSIONS

Quality of FFP being prepared at our blood bank meets the international standards. Regular update of quality assessment with respect to standard guidelines is important for effective production of blood components at all blood banks. A study of quality parameters in FFP is essential for establishment of good transfusion practices in blood banks.

LIMITATIONS

Though sample size was highest in our study amongst the compared studies, gender-wise and demographic distribution was not effectively done for quality assurance of FFP bags. Sample size was mainly comprised of male population and catered the local Ratnagiri district of Maharashtra. Rh 'negative' bags were fewer in number. Also volume of FFP bags was not done in our study.

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