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Whole Blood Holding at 22 °C: Coagulation Factor V and VIII Activity and Plasma Factor Content per Unit

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Abstract

Introduction

Platelet preparation by the platelet-rich plasma (PRP) method requires whole blood held at 20-24 °C, whereas units processed only for red cells and plasma are often refrigerated. We compared factor V and VIII activity and factor content per unit in fresh frozen plasma (FFP) from these two processing pathways.

Methods

In this single-center observational study, we analyzed 160 FFP units: 91 from whole blood held at 22 °C and used for PRP-platelet preparation and 69 from whole blood held at 4 °C without platelet preparation; all units were processed within 8 h of collection. Content per unit was calculated from activity and volume. Individual units were assessed against the Directorate General of Health Services (DGHS), India, specification, and each pathway against the Council of Europe and United Kingdom criteria.

Results

Factor V (107.4 ± 20.1 vs 110.6 ± 21.5 IU/dL; t(141.1) = −0.98, p = 0.33) and factor VIII activity (118.8 ± 32.8 vs 131.0 ± 46.1 IU/dL; t(117.1) = −1.87, p = 0.06; ABO-adjusted t(157) = −1.67, p = 0.10) did not differ significantly. Plasma volume was lower in the 22 °C arm (186 ± 8 vs 257 ± 32 mL), so factor VIII content was 35% lower (220 ± 61 vs 340 ± 141 IU per unit; t(87.3) = −6.62, p < 0.001); after adjustment for volume, the difference was not significant (t(157) = 0.85, p = 0.40). All units met the DGHS (⩾70 IU per unit), Council of Europe and United Kingdom factor VIII specifications.

Conclusion

Holding whole blood at 22 °C for platelet preparation preserved factor V and VIII activity, but the smaller plasma volume reduced factor content per unit. Plasma should be dosed and issued by volume rather than by unit count.

Categories: Hematology, Pathology, Quality Improvement
Keywords: factor v, factor viii, fresh frozen plasma (ffp), platelet-rich plasma method, whole blood holding temperature

Introduction

Fresh frozen plasma (FFP) is transfused to replace coagulation factors, and factor VIII, the most labile of them, is the quality marker used by the major specifications. The Council of Europe sets a mean factor VIII activity of at least 70 IU/100 mL with at least 90% of units above 50 IU/100 mL [1]; the United Kingdom (UK) requires at least 0.7 IU/mL in 75% of units tested [2]; and the Indian Directorate General of Health Services (DGHS) specifies at least 70 IU of factor VIII per unit [3]. The first two are concentration criteria, whereas the DGHS criterion is expressed per unit and therefore depends on plasma volume as well as activity.

Component preparation imposes conflicting temperature requirements. Platelets prepared by the platelet-rich plasma (PRP) method must come from whole blood held at 20-24 °C, while refrigeration of whole blood was historically favored to maximize factor VIII yield [4]. Subsequent studies showed that holding whole blood at ambient temperature for up to 24 h preserves most coagulation factors, with factor VIII falling by about 13-28% [5-8], and that platelets from such blood are of acceptable quality [9,10]. Most of these data concern overnight holds; fewer address the shorter holds typical of same-day processing in Indian blood centers, where units destined for PRP-platelets are kept at 22 °C and the remainder are refrigerated.

A second, less examined consequence of PRP-platelet preparation is plasma volume. A portion of the plasma is retained to resuspend the platelet concentrate, leaving a smaller volume available for FFP.

Concentration-based specifications do not capture this, yet in practice plasma is frequently ordered by unit count, and massive transfusion protocols issue plasma in fixed unit ratios. In our center, whole blood destined for PRP-platelet preparation is held at 22 °C, whereas blood processed only for red cells and plasma is held at 4 °C, so holding temperature and preparation method are linked. We therefore compared factor V and VIII activity, plasma volume and factor content per unit between these two processing pathways, without attempting to isolate the effect of temperature alone. We also assessed the compliance of individual units with the DGHS per-unit specification and of each pathway with the group-level Council of Europe and UK criteria.

Materials And Methods

Study design and setting

This observational study was conducted in the Department of Transfusion Medicine, All India Institute of Medical Sciences (AIIMS), Raebareli, India, between December 2023 and November 2024, and was approved by the Institutional Ethics Committee. FFP units prepared from whole blood donated by voluntary donors who met national donor selection criteria [3] were included consecutively as part of routine monthly quality control.

Whole blood holding and component preparation

Whole blood (450 mL) was collected into the same type of multiple blood bag system in both pathways (Polymed, Poly Medicure Ltd., India; quadruple bags with CPDA-1 anticoagulant and SAGM additive solution). Units designated for platelet preparation were held at 22 ± 2 °C and units not designated for platelets were held at 4 ± 2 °C until processing; all units were processed within 8 h of collection. In the 22 °C/PRP pathway, PRP was separated after a light spin at 1054 g for 6 min at 22 °C and centrifuged again at 2,928 ×g for 10 min at 22 °C to obtain the platelet concentrate; the platelet-poor plasma remaining after retention of (50-70) mL to resuspend the platelets was frozen as FFP. In the 4 °C pathway, plasma was separated after a heavy spin at 5076 ×g for 5 min at 4°C. All plasma was frozen within 8 h of collection and stored at ⩽−30 °C; storage duration before testing ranged from 2 to 11 months. Plasma volume was derived from net weight (specific gravity 1.03).

Coagulation assays and content per unit

Units were thawed in a water bath at 37 °C and tested within 30 min of thawing. Factor V and VIII activities were measured by one-stage clotting assays on a Sysmex CS-2500 analyzer (Sysmex Corporation, Kobe, Japan). Factor V was assayed with factor V-deficient plasma and Thromborel S thromboplastin, and factor VIII with factor VIII-deficient plasma, Actin FS activated partial thromboplastin time reagent and 0.025 mol/L calcium chloride (all reagents from Siemens Healthcare Diagnostics, Marburg, Germany). Assays were calibrated with Standard Human Plasma, Siemens Healthcare Diagnostics at each new reagent lot and when indicated by internal quality control. A normal-level control plasma (Control Plasma N, Siemens Healthcare Diagnostics) was run daily as internal quality control. Factor content per unit was calculated as activity (IU/dL) × plasma volume (mL)/100.

Quality specifications

Individual units were assessed against the DGHS specification of ⩾70 IU of factor VIII per unit [3]. The Council of Europe criteria (mean ⩾70 IU/100 mL and ⩾90% of units ⩾50 IU/100 mL) [1] and the UK criterion (⩾0.7 IU/mL in ⩾75% of units tested) [2] apply to groups of units and were therefore assessed for each pathway. The DGHS specification of 200 units per bag applies to stable coagulation factors; as factor V is labile and has no per-unit specification, it was reported descriptively.

Statistical analysis

Normality was assessed with the Shapiro-Wilk test, and data are presented as mean ± standard deviation (SD) and median (interquartile range (IQR)). Groups were compared with Welch's t-test and the Mann-Whitney U test. Because ABO blood group is a major determinant of factor VIII [11-13], differences were also estimated by linear regression adjusted for blood group (O vs non-O), with log-transformed models as a sensitivity analysis. To separate the effects of volume and temperature on content per unit, log content was modeled with log volume as a covariate. Compliance proportions are given with Wilson 95% confidence intervals (CIs) and compared with Fisher's exact test. Two further sensitivity analyses were performed: (i) 69 units were randomly sampled without replacement from the 22 °C arm and compared with the 69 units of the 4 °C arm, repeated 1,000 times, with median estimates reported; and (ii) the analysis was restricted to units collected during the same period, because all 40 units with lower numbers were in the 4 °C arm. The practical effect of volume was illustrated for a 60-kg adult receiving 15 mL/kg and for a fixed four-unit issue. Analyses were performed in Python 3.13 (SciPy 1.18, statsmodels 0.15); two-sided $p < 0.05$ was considered significant.

Results

Unit characteristics

Of 160 FFP units, 91 were prepared from whole blood held at 22 °C and 69 from whole blood held at 4 °C (Table 1). The proportion of group O units did not differ significantly between arms (34.1% vs 24.6%; $\chi^2(1) = 1.66$, p = 0.20), nor did the proportion of RhD-negative units (5.5% vs 7.2%; Fisher's exact test, p = 0.75). Plasma volume was 28% lower in the 22 °C arm (185.6 ± 7.6 vs 256.8 ± 31.8 mL; t(73.9) = −18.18, p < 0.001).

Characteristic	22 °C hold (n = 91)	4 °C hold (n = 69)	Test statistic	p-value
Platelet concentrate prepared	Yes (PRP method)	No		
Blood group A, n (%)	20 (22.0)	15 (21.7)		
Blood group B, n (%)	40 (44.0)	36 (52.2)		
Blood group O, n (%)	31 (34.1)	17 (24.6)	$\chi^2(1) = 1.66$	0.20
Blood group AB, n (%)	0 (0)	1 (1.4)		
RhD negative, n (%)	5 (5.5)	5 (7.2)	Fisher's exact	0.75
Plasma volume, mL, mean ± SD	185.6 ± 7.6	256.8 ± 31.8	t(73.9) = −18.18	<0.001
Plasma volume, mL, median (IQR)	184 (182–188)	265 (230–277)	U = 102	<0.001
Plasma volume, mL, minimum	164	181		
Plasma volume, mL, maximum	218	316		

TABLE 1: Characteristics of FFP units by whole blood holding temperature

FFP: fresh frozen plasma; PRP: platelet-rich plasma; SD: standard deviation; IQR: interquartile range. The chi-square test compares group O with non-O units. t: Welch's t-test with Welch–Satterthwaite degrees of freedom; U: Mann–Whitney U test.

Factor V and VIII activities

Factor V activity was 107.4 ± 20.1 IU/dL in the 22 °C arm and 110.6 ± 21.5 IU/dL in the 4 °C arm (difference −3.2 IU/dL, 95% CI −9.8 to 3.3; t(141.1) = −0.98, p = 0.33; Mann-Whitney U = 2,818, z = −1.11, p = 0.27), and factor VIII activity was 118.8 ± 32.8 and 131.0 ± 46.1 IU/dL (difference −12.2 IU/dL, 95% CI −25.1 to 0.7; t(117.1) = −1.87, p = 0.06; Mann-Whitney U = 2,740, z = −1.37, p = 0.17) (Table 2). Non-O units had 21.4 IU/dL higher factor VIII than group O units (t(157) = 3.26, p = 0.001), whereas factor V did not differ by blood group (t(157) = −0.80, p = 0.42). After adjustment for blood group, the differences were −3.5 IU/dL for factor V (95% CI −10.1 to 3.0; t(157) = −1.06, p = 0.29) and −10.2 IU/dL for factor VIII (95% CI −22.2 to 1.8; t(157) = −1.67, p = 0.10); the 22 °C:4 °C ratio on the log scale was 0.97 (t(157) = −0.94, p = 0.35) and 0.94 (t(157) = −1.28, p = 0.20), respectively.

Parameter	22 °C hold (n = 91)	4 °C hold (n = 69)	Difference, 22 − 4 °C (95% CI)	Test statistic	p-value
Factor V, IU/dL, mean ± SD	107.4 ± 20.1	110.6 ± 21.5	−3.2 (−9.8 to 3.3)	t(141.1) = −0.98	0.33
Factor V, IU/dL, median (IQR)	104.8 (91.8–122.5)	110.6 (96.5–122.8)		U = 2,818	0.27
Factor VIII, IU/dL, mean ± SD	118.8 ± 32.8	131.0 ± 46.1	−12.2 (−25.1 to 0.7)	t(117.1) = −1.87	0.06
Factor VIII, IU/dL, median (IQR)	113.3 (93.7–139.2)	122.8 (95.0–157.7)		U = 2,740	0.17
Factor V content, IU per unit, mean ± SD	199.0 ± 36.1	285.2 ± 70.6	−86.2 (−104.7 to −67.7)	t(94.7) = −9.26	<0.001
Factor V content, IU per unit, median (IQR)	194.6 (171.9–226.0)	275.8 (236.1–321.4)		U = 859	<0.001
Factor VIII content, IU per unit, mean ± SD	220.4 ± 60.7	340.2 ± 141.0	−119.8 (−155.8 to −83.9)	t(87.3) = −6.62	<0.001
Factor VIII content, IU per unit, median (IQR)	206.9 (176.1–262.0)	312.7 (220.2–415.8)		U = 1,331	<0.001

TABLE 2: Factor V and VIII activities and content per unit

SD: standard deviation; CI: confidence interval; IQR: interquartile range. Differences are unadjusted mean differences with Welch 95% CI. t: Welch's t-test with Welch–Satterthwaite degrees of freedom; U: Mann–Whitney U test. Blood group-adjusted estimates are given in the text.

In 1,000 equal-sized comparisons (69 vs 69), the median Welch t was −0.90 (median p = 0.37) for factor V and the median Mann-Whitney U was 2,076.5 (median p = 0.20) for factor VIII; the factor VIII difference was significant in 1.2% of draws by the Mann-Whitney test. When restricted to units collected during the same period (91 vs 29), the blood group-adjusted differences were −1.3 IU/dL for factor V (95% CI −10.2 to 7.6; t(117) = −0.30, p = 0.77) and −5.6 IU/dL for factor VIII (95% CI −21.1 to 9.8; t(117) = −0.72, p = 0.47).

Factor content per unit

Factor VIII content was 220.4 ± 60.7 IU per unit in the 22 °C arm and 340.2 ± 141.0 IU in the 4 °C arm, and factor V content was 199.0 ± 36.1 and 285.2 ± 70.6 IU per unit (t(87.3) = −6.62 and t(94.7) = −9.26, respectively; both p < 0.001; Table *2*, Figures *1A*-*1D*). The blood group-adjusted differences were −115.6 IU (95% CI −147.7 to −83.5; t(157) = −7.11, p < 0.001) for factor VIII and −87.2 IU (95% CI −104.3 to −70.2; t(157) = −10.13, p < 0.001) for factor V. Volume accounted for about three-quarters of the difference in factor VIII content on the log scale. After adjustment for volume, the 22 °C:4 °C ratio of factor VIII content was 1.09 (95% CI 0.90-1.32; t(157) = 0.85, p = 0.40), and 1.15 (95% CI 0.96-1.39; t(156) = 1.49, p = 0.14) with additional adjustment for blood group; the corresponding ratio for factor V was 0.99 (95% CI 0.88-1.13; t(156) = −0.10, p = 0.92). Within the 22 °C arm, plasma volume was not correlated with factor VIII activity (Spearman ρ = −0.09, n = 91, p = 0.38).

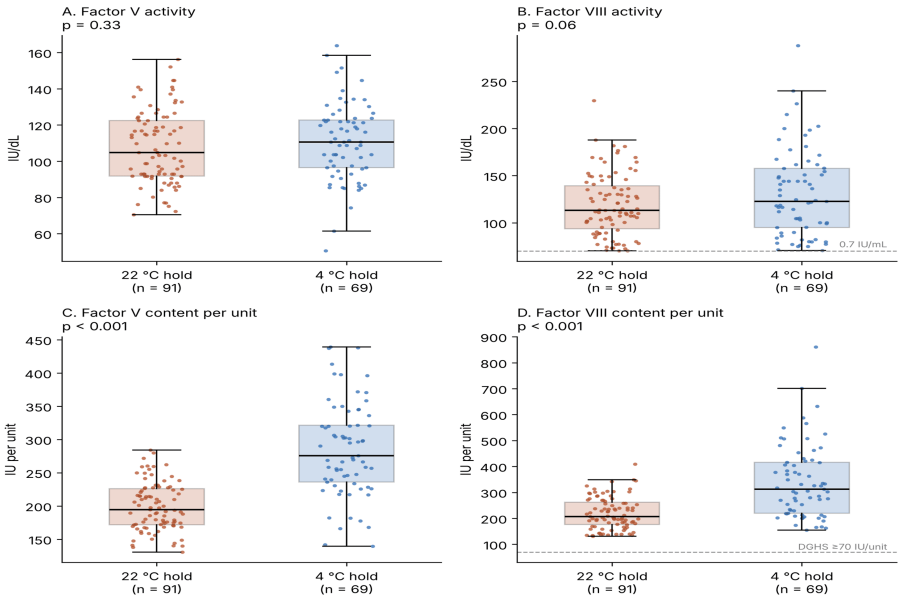

FIGURE 1: Factor V and VIII activity (A, B) and content per unit (C, D) in FFP

Factor V and VIII activity (A, B) and content per unit (C, D) in FFP prepared from whole blood held at 22 °C (with PRP-platelet preparation; n = 91) or 4 °C (n = 69). Boxes show median and interquartile range, whiskers 1.5 × IQR, and points individual units. Dashed lines mark 0.7 IU/mL (B) and the DGHS specification of 70 IU per unit (D). p-values are from Welch's t-test; the corresponding t-statistics are given in Table 2.

FFP: fresh frozen plasma

Compliance with specifications

All units in both arms met the DGHS specification of ⩾70 IU per unit (lowest value 131 IU in the 22 °C arm and 155 IU in the 4 °C arm; 100%, 95% CI 95.9-100 and 94.7-100, respectively), the UK criterion and both Council of Europe criteria (mean factor VIII 118.8 IU/dL, 95% CI 112.0-125.6, and 131.0 IU/dL, 95% CI 119.9-142.1). As compliance was complete in both arms, no between-arm test was applicable. These results were unchanged in every equal-sized resampled comparison.

Practical effect of plasma volume

For a 60-kg adult prescribed 15 mL/kg (900 mL), five units of 22 °C plasma or four units of 4 °C plasma were required (Table 3). Conversely, a fixed issue of four units delivered 743 mL and 882 IU of factor VIII from the 22 °C arm against 1,027 mL and 1,361 IU from the 4 °C arm, a shortfall of 28% in volume and 35% in factor VIII; about 5.5 units of 22 °C plasma were needed to match four units of 4 °C plasma.

Scenario	22 °C hold	4 °C hold	Difference, %
Mean volume per unit, mL	185.6	256.8	−28
Weight-based: units for 15 mL/kg in a 60-kg adult (900 mL)	5	4	+25
Fixed issue of four units: plasma volume, mL	743	1,027	−28
Fixed issue of four units: factor VIII, IU	882	1,361	−35
Fixed issue of four units: factor V, IU	796	1,141	−30
Units needed to match the volume of 4 units of 4 °C plasma	5.5	4	+38

TABLE 3: Illustrative effect of plasma volume on dosing (mean values)

Calculated from arm means; factor content is the sum of mean content per unit. Figures are illustrative and do not model in vivo recovery. The exact requirement was 4.85 units (22 °C) and 3.50 units (4 °C), rounded up to whole units.

Discussion

In this study, FFP prepared from whole blood held at 22 °C and processed within 8 h for PRP-platelet preparation showed no statistically significant difference in factor V or VIII activity compared with FFP from whole blood held at 4 °C, and all units met the DGHS, Council of Europe, and UK factor VIII specifications. Plasma from the 22 °C/PRP pathway was, however, smaller in volume and contained about one-third less factor VIII per unit.

The 9% lower mean factor VIII activity in the 22 °C/PRP pathway did not reach statistical significance and is smaller than the 13-28% reductions reported after overnight ambient holds [5-8], consistent with the shorter hold. It was further attenuated by adjustment for blood group and by restriction to contemporaneous units, suggesting that donor ABO distribution and collection period contributed to the crude difference. Factor V showed no significant difference, in agreement with studies reporting that it is largely stable during ambient storage of whole blood [5,8]. These findings should not be read as evidence of equivalence: the study was not designed to test equivalence, and the CI for factor VIII does not exclude a clinically relevant reduction. Moreover, because holding temperature and preparation method differed together, any effect of temperature alone cannot be isolated.

The lower factor content per unit in the 22 °C/PRP pathway was accompanied by a smaller plasma volume, as a portion of the plasma is retained to resuspend the platelet concentrate. Since content was calculated from activity and volume, and activity did not differ significantly, the lower content is consistent with the smaller volume; however, the independent contributions of holding temperature and preparation method could not be determined. Because the DGHS specification is expressed per unit, it is in principle sensitive to volume, but the margin observed here was wide; even the smallest PRP-pathway unit contained 131 IU of factor VIII. The concentration-based Council of Europe and UK criteria are independent of volume and were met by both pathways.

The difference in volume may be relevant to how plasma is prescribed and issued. Guidelines recommend weight-based dosing [14], under which smaller units require more units to deliver the same volume, at the cost of additional donor exposures. When plasma is ordered by unit count, as is common in routine practice and in fixed-ratio massive transfusion protocols, units from the PRP pathway would supply less plasma; in our illustrative calculation, four such units supplied 284 mL less plasma and about 480 IU less factor VIII than four FFP-only units. Early plasma delivery has been associated with improved hemostasis and reduced death from exsanguination in major trauma hemorrhage [15,16], but our study did not evaluate hemostatic efficacy, correction of coagulopathy, or patient outcomes. Labeling each unit with its volume and prescribing plasma by volume are therefore practical implications that warrant clinical evaluation rather than validated recommendations.

Limitations

The strengths of this study include assessment of both activity and content per unit, comparison with three quality specifications, and adjustment for ABO blood group. Its limitations should be noted. First, the processing pathway was determined by the need for platelets rather than by randomization, so holding temperature and preparation method were linked and their separate effects cannot be determined. Second, no baseline sample was taken before processing, and the study was not designed to test equivalence, so the absence of a statistically significant difference does not establish preserved or equivalent activity. Third, the earliest units were all from the 4 °C pathway; we addressed this with a sensitivity analysis restricted to the overlapping collection period. Fourth, only factors V and VIII were measured, at a single time point after one

storage interval, so effects on fibrinogen, other factors, and longer holds cannot be inferred. Fifth, individual holding times within the 8-h window were not recorded, and volume was derived from weight. Finally, the study was conducted at a single center and did not assess clinical outcomes.

Conclusions

FFP prepared from whole blood held at 22 °C for PRP-platelet preparation and FFP from whole blood held at 4 °C showed no statistically significant difference in factor V or VIII activity, and all units met the DGHS, Council of Europe, and UK factor VIII specifications. Because holding temperature and preparation method differed together, these findings describe two processing pathways rather than the effect of temperature alone, and they do not establish equivalence.

Plasma from the PRP pathway had a smaller volume and lower factor content per unit. This may matter when plasma is issued by unit count, including in massive transfusion protocols, and labeling units with their volume and prescribing plasma by volume are practical implications that warrant clinical evaluation.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Critical review of the manuscript for important intellectual content: Trupti Lokhande, Dnyaneshwar Patale, Vinita Paswan, Arunpreet Kaur

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Bioethics Cell, AIIMS, Raebareli issued approval 2023 -7 -IMP- 3. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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