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Effect of storage of plasma in the presence of red cells and platelets: re-evaluating the shelf life of whole blood.

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Background:

There is renewed interest in administering whole blood (WB) for the resuscitation of bleeding trauma patients. The shelf-life of WB was established decades ago, based on the viability of red cells. However, plasma quality during WB storage is not established.

Study Design and Methods:

Leucocyte- and Platelet- depleted WB (WB-PLT) was prepared using standard processes and compared to WB processed using a platelet-sparing leucocyte depletion filter (WB+PLT). WB (+/- PLT) was held at 2–6 ºC for 35 days alongside control units of red blood cells in SAGM (RBC(SAGM)) and liquid plasma (LP). A series of assays explored the coagulation potential and red cell quality.

Results:

Whilst fibrinogen and a_2-antiplasmin remained unaffected by storage, other factors varied between components, or over time at 2-6 °C. At 14 days FV, FVII, a2-AP and free protein S antigen remained on average >0.50 IU/ml or 50 %, as appropriate, in WB+/-PLT. FVIII was on average 0.49 IU/mL in WB+PLT, and 0.56 IU/mL for WB-PLT. Free protein S activity decreased significantly in all arms but remained on average >40% at day 14. Contact activation was not demonstrated before day 14. Thrombin generation in plasma remained relatively stable to day 35 in all arms.

Conclusions:

FV, FVII, FVIII and proteins S activity reduce over storage in all arms but are mostly ≥ 0.50 IU/mL, or 50%, at day 14. Further data on platelet function in WB+PLT is needed to inform its shelf-life.

KEYWORDS: Blood Component Preparations, Haemostasis, Whole Blood, Major Haemorrhage, Trauma

Introduction

Recent randomised controlled trials have shown that early administration red blood cells and fresh frozen plasma (FFP) reduce mortality in trauma patients who are bleeding, and as such, there is renewed interest in the availability of a whole blood component which could facilitate the 1:1:1 ratio of RBC, FFP and platelet and provide logistical advantages particularly in the pre-hospital setting. [1,2]

Whole blood (WB) was used routinely by the military between 1940 and 1960 to treat soldiers during World War II, Korean War and Vietnam War.[3] By 1965 it's use reduced significantly due to the introduction of blood components (i.e. RBC, FFP, platelet concentrates and cryoprecipitate). WB is not used routinely in many countries, and the introduction of universal leucocyte depletion by many blood operators has meant that most whole blood collections also remove platelets. Hence, the filtered WB component contains only red cells and plasma. An alternative platelet-sparing leucocyte depletion filter is available in the IMUFLEX-WB-SP collection set from Terumo. The duration of WB storage currently permitted was based mainly on the viability of red cells.[4,5] However, considering that WB transfusion in major bleeding setting requires that there is also an optimum plasma content, it is important to assess the quality of plasma within the WB over time when stored at 2 – 4°C.

This work compared coagulation potential of WB produced using the standard collection set (WB-PLT), and the platelet-sparing Imuflex WB-SP kit (WB+PLT), to understand the effect of storing plasma with platelets and/or red cells. We therefore examined concurrently in this study the effect of storing plasma on its own, or with red cells ± platelets. We also assessed basic parameters of red cell quality to understand how the presence of plasma and platelets affects these. This work was undertaken to inform an appropriate shelf life of WB-PLT in the UK, which may be used in future in the pre-hospital setting. Thromboelastography of whole blood components were also assessed to provide initial data on the impact of platelet number/function. However, further analysis of platelet function during whole blood storage will be the subject of future laboratory studies.

Materials and methods

Liquid plasma (LP) was used as a comparator in this study to understand the effect of storing plasma at 4 °C in the presence of red cells (+/-) platelets. Although thawed plasma (TP) stored up to 5 days is also standard of care in some jurisdictions for the treatment of major haemorrhage, we have previously shown that there is little difference between TP and LP over the first 7 days of storage.[6,7] Therefore, the data in this study on LP are comparable to that of TP.

Blood processing and sampling

Forty-eight units of whole blood (WB) from male donors, 475±47.5 mL, were collected into 66.5 mL CPD in top-and-top collection packs (MacoPharma, Twickenham, UK) following standard operating procedures, and stored at 20-24 °C for approx. 20 hours. Of these units, 32 were leucocyte depleted (LD) using the WB LXT filter integral to the collection pack to produce WB-PLT component. The final 16 units were LD and then stored in the transfer pack contained in an Imuflex WB-SP platelet-sparing filter kit (Terumo BCT, Larne, UK) to produce WB+PLT units. Sixteen of the units processed with the LXT LD filter, were further processed into red cells in SAGM (RBC(SAGM)) and liquid plasma (LP) via centrifugation (4950 g, 15 min, Room Temperature) in a RC3BP+ SORVAL (Thermo Scientific, Hemel Hempstead, UK). Separation of the plasma was made using an automated press (Optipress-II Fresenius-Kabi, Runcorn, UK). In total, 11 out of 16 WB-PLT and WB+PLT units and 12 out of 16 RCC(SAGM)/LP units were group O, with the remainder being group A.

Samples were taken from all units immediately following processing via sterile docking of a sample pouch. Once processed and sampled, all units were placed at 2-6°C and further sampled on days 5, 9, 14, 21, 28 and 35 of storage. A sample of the plasma from WB+/-PLT arms was separated via centrifugation (2862 g) and stored at ≤-30°C before testing as described below. Further samples of WB+/-PLT were deproteinised and stored at ≤-30°C for testing of ATP and 2,3 DPG.[8] The supernatant from RBC(SAGM) were similarly obtained, stored and tested. LP units were stored at 4 °C and measured alongside WB+/-PLT for all coagulation parameters.

Coagulation Assays

FV, FVII, FVIII and protein S were measured as they are known to be labile factors in stored plasma.[6] Global haemostatic potential was assessed with thromboelastography and thrombin generation in plasma. To investigate the impact on the fibrinolytic pathway, another important aspect of trauma-induced coagulopathy, α_2-antiplasmin, the key fibrinolysis inhibitor, was also measured. The chromogenic substrate, S-2302, assessed kallikrein-like activity as a measure of contact activation, known to be consequence of storing plasma at 4 °C.

Coagulation assays were carried out on an ACLTOP550 Coagulometer (Instrumentation Laboratory UK Ltd, Cheshire, UK). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed using HemosIL RecombiPlasTin 2G and SynthASil reagents respectively (Instrumentation laboratory). FV, FVII and FVIII were performed using one-stage clotting assays with the corresponding HemosIL factor deficient plasma (Instrumentation Laboratory). Free protein S (antigen and activity), α_2-antiplasmin, as well as fibrinogen (Clauss) were performed using HemosIL reagents (Instrumentation Laboratory) according to manufacturer's instructions. Assays were standardised using HemosIL calibration plasma (Instrumentation laboratory).

The chromogenic substrate S-2302 (Quadratech Diagnostics Ltd., Surrey, UK) was used to detect the presence of kallikrein-like activity, as previously described in Gallimore et al, 1982.[9]. Kallikrein-like activity was defined as Max V increase of more than 1.0 mOD/min from baseline value (day 1).

Thromboelastrography was performed on a TEG5000 (Haemonetics, Hartlepool, UK) according to manufacturer's recommendations using a commercially available kaolin reagent (Haemonetics).

Thrombin generation was performed using the Calibrated Automated Thrombogram system (Thrombinoscope BV, Maastricht, The Netherlands) as described by Hemker et al, 2003 in conjunction with the manufacturer's PPP reagents, which gave reaction concentrations of 5 pmol/L tissue factor and 4 µmol/L phospholipid (Thrombinoscope BV). [10]

Red Cell Function

A full blood count (FBC) was performed on WB+/-PLT units before and after leucocyte depletion (LD), and RCC(SAGM) units following processing, using a XN-1000 haematology analyser (Sysmex, Milton

Keynes, UK), and at all sampling points during storage. For platelet counts, an impedance count was performed on a sample (500 µL) that had been incubated (RT, 30 min, roller mixer) in an EDTA tube (1.3 mL, Sarstedt, Leicester, UK) before analysis. For 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) assays, samples were deproteinised with perchloric acid as described previously and stored below –70 °C before testing.[8] Levels of 2,3-DPG were measured with a commercially available kit (Roche Diagnostics), and ATP was measured with a coupled enzyme method adapted from Lagerberg et al, 2007, using a microplate reader (Synergy HT, BioTek).[11] The extent of haemolysis throughout storage was determined by measuring supernatant haemoglobin (Hb) levels as previously described.[8]

Statistical Analysis

Coagulation factors were analysed using univariate statistical methods. Analysis of variance (ANOVA) was performed, comparing concentration of each factor across arms (WB-PLT, WB+PLT, LP) and over time. A random effect was included in each model to allow for the correlation between results from the same donor. Post-tests were performed to compare, within each arm of the study, concentration on each subsequent day to the concentration on day 1, and to compare WB+/-PLT to LP on days 9 and 14. P-values for post-hoc comparisons were adjusted to take into account the multiple tests performed using Dunnett's method.

Results

Red cells

Haemolysis increased over time for all arms, and up to day 21 was similar between the three arms (WB+PLT, WB-PLT and RBC(SAGM)). Beyond day 21 the increase in haemolysis was higher for WB+PLT, followed by WB-PLT and RBC(SAGM) (Table 1). Potassium leakage into the supernatant was similar in all arms. Red cell ATP and 2,3 DPG decreased over time in all arms. However, compared with both WB arms, RCC(SAGM) had an overall higher ATP and lower 2,3 DPG values. At day 14, 100% of WB+/-PLT had a level of ATP greater than 2.3µg/g Hb which dropped to 75% by day 21.

Coagulation profile of WB+/-PLT

There was evidence that concentration of all coagulation factors decreased over time ($p<0.0001$). However, the extent of change with time was different between arms and dependent upon which factor was measured except for a2-antiplasmin. For factors, except fibrinogen and a_2-antiplasmin, within each arm, concentrations on subsequent days were different from day 1.

APTT ratio increased during storage in all units (Fig. 1a), corresponding with a decrease in FVIII activity (Fig. 1b). This was also true when the group O units, known to have lower FVIII levels and the ABO group that would be used in whole blood transfusion, were analysed individually (Fig. 1c) (include ref). FVIII was higher in WB-PLT than LP at day 14, but not in WB+PLT (p-value <0.05).

PT increased during storage for all arms, with the change being significantly greater in WB+PLT than LP and WB-PLT (Fig. 1d). This corresponded with a decrease in FV levels (Fig. 1e) which was markedly greater in WB+PLT arm. A general loss of FVII activity was also seen in all arms (Fig. 1f). However, in two WB-PLT units an increase in FVII activity was demonstrated from day 14 compared to day 1, suggesting the generation of activated FVII, a known consequence of contact activation (Figure 1g).[12] In four LP units, FVII activity increased from day 21. All increases in FVII levels corresponded with a decrease in PT ratio at equivalent time points.

Free protein S antigen remained high throughout storage for LP (Fig. 2). In WB+/-PLT, there was a significant decrease over time. In all arms, free protein S activity decreased dramatically over the storage period, and by day 35 was below the level of detection (<10%) in WB+/-PLT, and marginally higher in LP.

Overall, at 14 days FV, FVII, a2-AP and free protein S antigen remained on average ≥0.50 IU/ml (or 50 %) in WB+/-PLT. At day 14, FVIII was on average 0.49 IU/mL in WB+PLT, and 0.56 IU/mL for WB-PLT. Protein S activity remained on average >40% at day 14 in WB+/-PLT. By day 21, FVII and a2-AP were still on average ≥0.50 IU/ml (or 50 %) in WB+/-PLT. Whilst FV and FVIII remained on average ≥ 0.4 IU/mL.

Contact Activation

Contact activation of units was assessed using S-2302. In total, 4/16 LP units and 2/16 WB-PLT units showed increase rates of S-2302 turnover by day 35 of storage, compared to day 1. In LP, contact activation was seen at day 28 onwards, whilst for WB-PLT it was at day 14 in one unit, and day 35 in another. Units with increases in S-2302 turnover demonstrated increases in FVII activity and PT ratio throughout storage (Figure 1), as described above. There was no increase in contact activation for WB+PLT units throughout the storage period.

Whole Blood Thromboelastography

Thromboelastography was performed on WB arms post-LD (day 1) and throughout storage. As shown in Figure 3, the most obvious difference between the two components was seen in the MA. WB-PLT, showed a low stable MA throughout storage. R is slightly shortened, over the first 14 or so days of storage, and in the WB+PLT units compared to WB-PLT. The angle did not demonstrate major changes over time, being generally higher in WB-PLT than WB+PLT, presumably due to the platelet contribution of clot formation. K, determined for WB+PLT only, increased during storage.

Thrombin Generation

Thrombin generation of the plasma separated from units was carried out at 5 pM tissue factor. As detailed in Fig. 4, lag time was increased in WB+PLT compared with LP and WB-PLT. Overall, the endogenous thrombin potential (ETP) was stable in all components throughout storage. Peak and time to peak (ttpeak) were similar in all components, but the effect over time differed between components.

Discussion

The viability of red cells at 2–6 °C for up to 35 days is well understood, but the consequences for platelets and plasma, particularly when held together under these conditions, are not well established. With increasing interest in the use of whole blood (WB) for resuscitation of patients, more detailed understanding of plasma and platelet function is required to inform a suitable shelf life for WB component. We assessed the *in vitro* haemostatic potential of plasma held alone, or with red cells, with or without platelets concurrently in the same study, up to 35 days after venepuncture, in order to re-establish the shelf life for WB-PLT in the first instance.

With respect to red cells, our data showed that levels of ATP were higher in RBC/(SAGM) than WB+/-PLT, as would be expected due to the lack of adenine in CPD plasma in WB. ATP levels were similar at day 14 and 21 in WB+/-PLT compared to day 35 in RBC/(SAGM) which is the current end of shelf-life of red cells in additive solution in the UK. In WB+/-PLT ATP levels were >2.3 µmol/g Hb in ≥75% of units at day 21, and all units at day 14, the minimum threshold for acceptable post-transfusion survival suggesting that for red cell quality a day 21 shelf life would be acceptable.[13] These data are consistent with the previous observed satisfactory *in vivo* recovery of red cells in CPD to 21 days, and that the current shelf-life of red cells suspended in CPD anticoagulated plasma in the UK is shorter than that of red cells stored in additive solutions/anticoagulants of plasma containing adenine, 28 days compared to 35 respectively.[4,5] Haemolysis in WB was greater in the presence of platelets at all time points, but all units were ≤ 0.8 % at day 21.

Thrombin generation remained stable throughout storage in all components, although there were differences seen between components. Generally, thrombin generation at 5 pM tissue factor is considered to be most affected by changes in extrinsic pathway factors. Therefore, the differences seen here in LP, WB-PLT and WB+PLT are likely to be attributable to changes in factors other than FV or FVII, to which it is relatively insensitive.[14]

In thromboelastography, the biggest differences between the two WB components were seen in the MA, which was understandably higher in the WB+PLT component where platelets contribute approx. 80% of clot strength. A higher angle seen with WB-PLT compared to WB+PLT, is also likely due to the absence of platelets, whilst a shorted R time, as seen in WB+PLT, has been linked to increases in red cell and platelet microparticles which provide a procoagulant surface.[15-17]

The coagulation factor content of WB+/-PLT decreases in relation to time stored at 2–6 °C. Whilst certain coagulation factors remain unaffected by storage with platelets and/or red cells (FVIII, fibrinogen and α_2-antiplasmin), others varied more dramatically between components. Most notably, the loss of FV and FVII activity in plasma activity is greater during storage of WB+PLT compared with WB-PLT. The reduction in FV correlates with loss in platelet count in WB, as platelets become activated and release microparticles. It is possible that FV is either being internalised to platelets, cleaved by proteases, or becomes bound/consumed on the platelet surface as these become activated during storage of whole blood. The latter seems likely given that there is no associated major decrease in clot formation in WB by thromboelastography or thrombin generation, but this remains to be elucidated. Importantly, in WB components levels of α_2-antiplasmin, the key fibrinolytic inhibitor, is maintained throughout storage. This is probably important in the context of major haemorrhage associated with trauma where one of the pathways associated with trauma-induced coagulopathy is enhanced fibrinolysis.[18]

In addition to the loss of FV and FVII activity in plasma, the other factor that decreased during storage and showed differences between the different arms of the study was protein S. Free protein S activity decreased to the same extent in all components, and by day 35, activity is below the level of detection in the majority of units. However free PS antigen remained stable in LP, and decreased equivalently throughout storage in the WB arms, although to a lesser extent than activity. The difference between free protein S antigen assay and protein S activity is probably caused by inactivation of PS by thrombin, alternatively, PS may be binding to red cells. The recommended assay for diagnosis of protein S deficiency is the free antigen assay since clotting based assays vary considerably in the aspects of PS activity they measure, most assess the co-factor role of PS in inhibition of FVa and FVIIIa, but PS also has direct activity against FXa and is also a co-factor for TFPI functions that are not necessarily measured by these assays.[19] Previously low levels of protein S were associated with thrombotic events following large volume transfusion of a type of solvent-detergent treated plasma (SD+, Vitex) resulting in the FDA in the USA insisting on a change in indications/labelling for the product and ultimately it was withdrawn from the market.[20] However, the product also had very low levels of α_2-antiplasmin, the main inhibitor of fibrinolysis in plasma. In trauma, the risk of thrombosis is high and therefore a key consideration is whether low levels of PS in WB (or LP) stored for extended

periods could augment this risk. However, thromboprophylaxis is a standard protocol in trauma patients once bleeding has been controlled, and additionally this risk could be reduced by limiting the maximum number of WB units before transitioning to standard component-based therapy. Further, at day 14 of storage of WB, PS activity remains above that recommended in the current SD FFP product available in Europe and the USA, and at day 21 is on average 26 % and 30 % in WB-PLT and WB+PLT respectively. (Table 2).

For current plasma products in the UK, i.e. FFP and TP, quality monitoring and specifications stipulate a minimum level of FVIII activity in all clinical products (75% must have ≥ 0.7 IU/mL after thawing). With respect to coagulation factor levels in whole blood intended for use in trauma, it is difficult to determine what an adequate level of any factor may be, in part because we do not know what levels are required for the haemostatic effect of plasma and this may alter dependent upon the indication for transfusion. Additionally, haemostasis is a complex and multifactorial process, and thus considering any one factor in isolation is not meaningful. What factors should we consider in setting an appropriate shelf-life of WB in relation to its plasma content, and how should we decide where to set the bar? In the setting of major haemorrhage patients may be transfused either thawed plasma that has been stored for an extended period, or liquid plasma. Thus, comparison to FFP, the gold standard, seems inappropriate. With the exception of protein S, factors measured were similar at day 14 in WB compared to TP at day 5 (Table 2). Since TP is the standard of care for such patients, it seems reasonable to expect a similar content in WB for the same indication.

Another consideration in determining shelf-life is the avoidance of cold activation of plasma which occurs when plasma is stored at 4 °C whether on its own, or in WB. Generally, there appears to be a greater propensity for units to undergo contact activation when stored as plasma alone (LP), or plasma with red cells (WB-PLT), than with WB containing platelets (WB+PLT). As with FV it may be that contact activation does occur, but the kallikrein generated is either localised to the platelet surface, internalised by platelets, or cleaved by proteases, so not measurable in the assay system used here. Cold activation can be avoided by storing WB or LP for no more than two weeks, and is part of the rationale that the UK and others consider a shelf-life beyond this for LP as inappropriate.[6,21] The consequences of contact activation in biological products transfused to patients are not fully known, particularly in major haemorrhage. However, thrombogenic effects in patients

receiving IVIg products have been linked to increased activated coagulation factor content, particularly activated FXI.[22]

In considering the shelf-life of WB we also need to take into account other variables such as the logistics of supply and potential wastage of the product. Internationally, the resurgence of interest in the use of whole blood for the management of major haemorrhage has seen several countries adopting the practice, using Terumo filters that were assessed in this study. Notably, Norway, New Zealand and some areas in United States employ the use of whole blood in the pre-hospital setting, with Norway and the United States storing the units for 21 days and 14 days respectively.[23] The United States group started with 21 days, but reduced it to 14 days to allow the remanufacture of red cells in order to minimise wastage.

Further for WB+PLT a key consideration is the maintenance of platelets function during storage. Previous data has suggested that this is maintained to 14 days, but studies directly comparing the function of platelets to day 14 in WB and platelet concentrates stored at 4°C or room temperature are lacking.[24,25]

Taken together, international experience, data on plasma content of whole blood from this study and platelet function from other studies suggest that the current approved shelf-life of CPD whole blood should be between 14 to 21 days, and beyond 21 days is probably too long if we want to transfuse functional platelets and plasma. The relative merits and contribution of WB compared to component therapy needs to be addressed with well-designed clinical studies.

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Illustrations

1. **Coagulation profile of WB-PLT, WB+PLT and LP**

A) Activated partial thromboplastin time (APTT) **B)** FVIII **C)** FVIII levels in Group O **D)** fibrinogen **E)** Prothrombin time (PT) **F)** FV **G)** FVII and **H)** a_2-antiplasmin in components stored at 2-6 °C for 35 days. N = 16 (N=11,12,11 for WB-PLT, WB+PLT and LP, respectively, for Group O only FVIII activity). Error bars indicate mean±SD. * indicates WB+PLT statistically difference (p-value <0.05) from LP. ● indicates WB-PLT statistically different (p-value <0.05) from LP.

2. **Free Protein S in WB-PLT and WB+PLT**

Free Protein S antigen and Free Protein S activity in components stored at 2-6 °C for up to 35 days. N=16. Error bars indicate mean±SD. * indicates WB+PLT statistically difference (p-value <0.05) from LP. ● indicates WB-PLT statistically different (p-value <0.05) from LP.

3. **Thromboelastography (whole blood)**

Thromboelastography was performed on WB-PLT and WB+PLT throughout storage with kaolin. N=16. Error bars indicate mean±SD.

4. **Figure 4 Thrombin Generation**

Lag time, endogenous thrombin potential (ETP), peak and time to peak (ttpeak) in plasma separated from WB-PLT, WB+PLT and LP throughout storage at 2–6 ºC, in the presence of 5 pM tissue factor. N=16. Error bars indicate mean±SD.

Table 1 Red Cell quality parameters and platelet count of RCC(SAGM), WB-PLT and WB+PLT during storage.

	Day 1			Day 5			Day 9			Day 14			Day 21			Day 28			Day 35		
	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT	RCC (SAGM)	WB-PLT	WB+PLT
Red Cell ATP (µmol/g Hb ± SD)	5.29 ± 0.50	4.14 ± 0.61	4.10 ± 0.52	5.38 ± 0.54	3.81 ± 0.47	3.75 ± 0.59	5.11 ± 0.54	3.62 ± 0.65	3.62 ± 0.71	4.32 ± 1.26	3.29 ± 0.57	3.25 ± 0.82	4.07 ± 0.65	2.81 ± 0.57	3.04 ± 0.91	3.69 ± 1.02	2.41 ± 0.62	2.52 ± 1.07	2.92 ± 0.39	1.96 ± 0.70	1.98 ± 0.82
2,3 DPG (µmol/g Hb ± SD)	3.72 ± 1.09	4.41 ± 2.12	3.88 ± 1.55	2.53 ± 1.40	3.30 ± 2.14	3.01 ± 1.70	1.33 ± 0.82	2.50 ± 1.79	2.08 ± 1.38	0.57 ± 0.46	1.56 ± 1.06	1.12 ± 0.84	0.30 ± 0.20	0.98 ± 0.58	0.79 ± 0.45	0.41 ± 0.70	0.60 ± 0.31	0.51 ± 0.25	0.29 ± 0.36	N/D	N/D
Supernatant Potassium (mmol/unit ± SD)	0.45 ± 0.09	2.06 ± 0.15	2.10 ± 0.19	3.49 ± 0.44	4.25 ± 0.43	4.34 ± 0.51	5.80 ± 0.70	6.04 ± 0.78	6.21 ± 0.60	8.18 ± 0.98	7.83 ± 1.00	8.03 ± 0.87	10.92 ± 1.22	10.17 ± 1.13	10.21 ± 1.14	13.16 ± 1.45	12.01 ± 1.38	12.49 ± 1.50	14.94 ± 1.58	13.70 ± 1.51	13.79 ± 1.33
Haemolysis (% ± SD)	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.04	0.12 ± 0.06	0.11 ± 0.04	0.13 ± 0.07	0.16 ± 0.08	0.18 ± 0.10	0.20 ± 0.12	0.24 ± 0.11	0.25 ± 0.12	0.30 ± 0.22	0.31 ± 0.15	0.42 ± 0.21	0.52 ± 0.44	0.38 ± 0.19	0.62 ± 0.38	0.79 ± 0.74
Platelet count (x 10^9/L)	n/a	n/a	169 ± 24	n/a	n/a	165 ± 24	n/a	n/a	161 ± 51	n/a	n/a	123 ± 23	n/a	n/a	102 ± 20	n/a	n/a	86 ± 14	n/a	n/a	89 ± 14

Table 2 Coagulation factors in UK licensed plasma components

	Published data for plasma components			Liquid Plasma		WB-PLT		WB+PLT	
	Thawed FFP Day 5[6]	Octaplas – LG[26]	MB-FFP 24 hour following thawing[27]	Day 9	Day 14	Day 9	Day 14	Day 9	Day 14
APTT	1.26 (± 0.05)	34.5 s	36.4 s (± 3.7)	1.40 (± 0.08)	1.43 (± 0.09)	1.33 (± 0.14)	1.33 (± 0.15)	1.29 (± 0.13)	1.32 (± 0.13)
FVIII (IU/mL)	0.57 (± 0.14)	Not measured.	0.62 (± 0.11)	0.48 (± 0.12)	0.42 (± 0.11)	0.58 (± 0.15)	0.56 (± 0.14)	0.51 (± 0.22)	0.49 (± 0.24)
PT	1.13 (± 0.05)	11.3 s	13.5 s (± 1.4)	1.12 (± 0.08)	1.15 (± 0.08)	1.11 (± 0.10)	1.13 (± 0.11)	1.21 (± 0.10)	1.26 (± 0.10)
FV (IU/mL)	0.74 (± 0.11)	0.96	0.80 (± 0.15)	0.80 (± 0.11)	0.75 (± 0.10)	0.75 (± 0.10)	0.72 (± 0.10)	0.69 (± 0.12)	0.59 (± 0.14)
FVII (IU/mL)	0.79 (± 0.13)	1.09	1.04 (± 0.16)	0.77 (± 0.15)	0.73 (± 0.14)	0.75 (± 0.20)	0.75 (± 0.26)	0.65 (± 0.12)	0.63 (± 0.12)
Fibrinogen (g/L)	2.61 (± 0.39)	2.31	2.07 (± 0.34)	3.04 (± 0.68)	3.09 (± 0.70)	2.75 (± 0.42)	2.65 (± 0.44)	2.82 (± 0.50)	2.80 (± 0.47)
Free Protein S antigen (%)	94 (± 13.9)	86	92.9 (± 16.45)	84 (± 15.1)	83 (± 15.4)	74 (± 12.3)	67 (± 10.9)	78 (± 10.8)	71 (± 11.7)
Free Protein S activity (%)	72.5 (± 11.7)	61	Not measured.	63 (± 10.7)	50 (± 11.8)	54 (± 10.0)	41 (± 11.9)	65 (± 11.6)	48 (± 9.8)
a_2-antiplasmin (%)	Not measured.	Not measured.	Not measured.	105 (± 6.4)	105 (± 7.8)	100 (± 7.3)	100 (± 8.2)	103 (± 6.7)	105 (± 8.1)

Values reported as mean (Std) unless otherwise stated.

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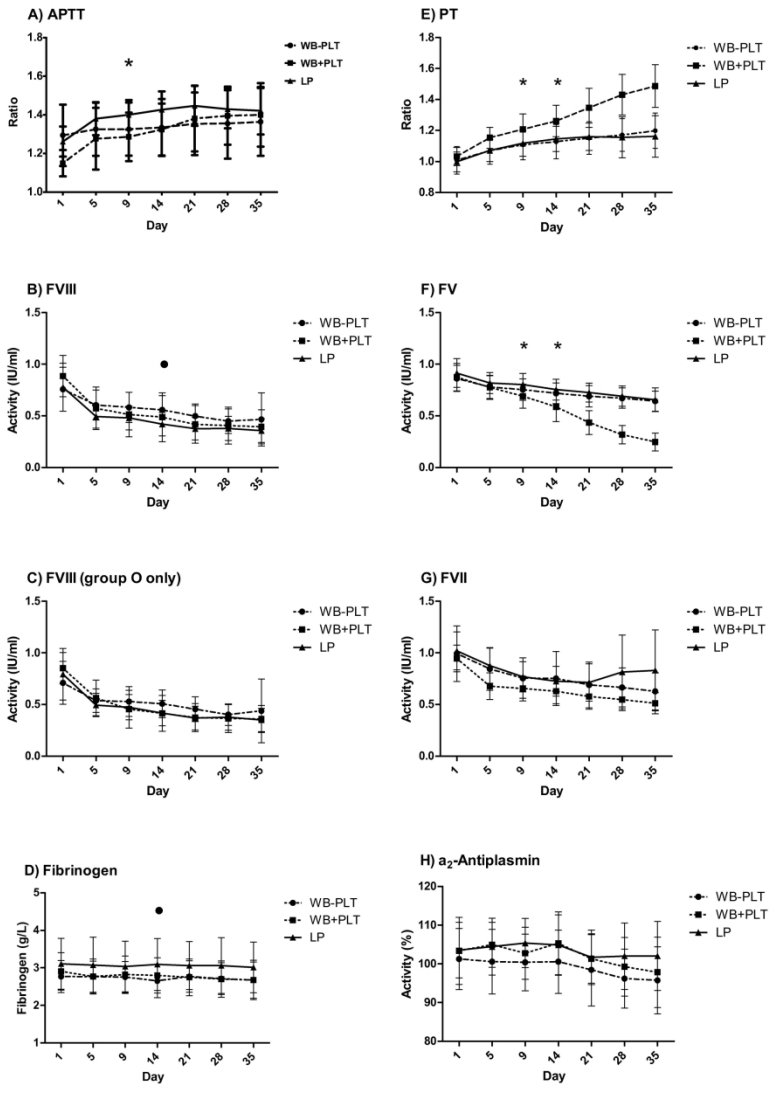

Figure 1. Coagulation profile of WB-PLT, WB+PLT and LP. A) Activated partial thromboplastin time (APTT) B) FVIII C) FVIII levels in Group O D) fibrinogen E) Prothrombin time (PT) F) FV G) FVII and H) a2-antiplasmin in components stored at 2-6 °C for 35 days. N = 16 (N=11,12,11 for WB-PLT, WB+PLT and LP, respectively, for Group O only FVIII activity). Error bars indicate mean±SD. * indicates WB+PLT statistically difference (p-value <0.05) from LP. ● indicates WB-PLT statistically different (p-value <0.05) from LP.

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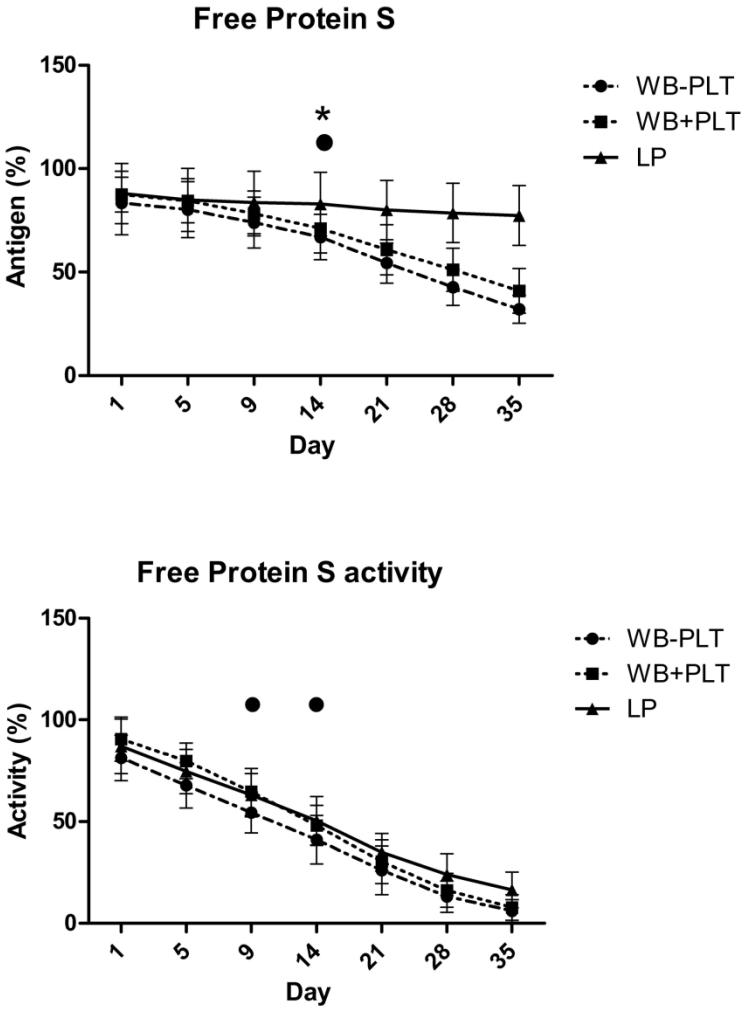

Figure 2. Free Protein S in WB-PLT and WB+PLT. Free Protein S antigen and Free Protein S activity in components stored at 2-6 °C for up to 35 days. N=16. Error bars indicate mean±SD. * indicates WB+PLT statistically difference (p-value <0.05) from LP. • indicates WB-PLT statistically different (p-value <0.05) from LP.

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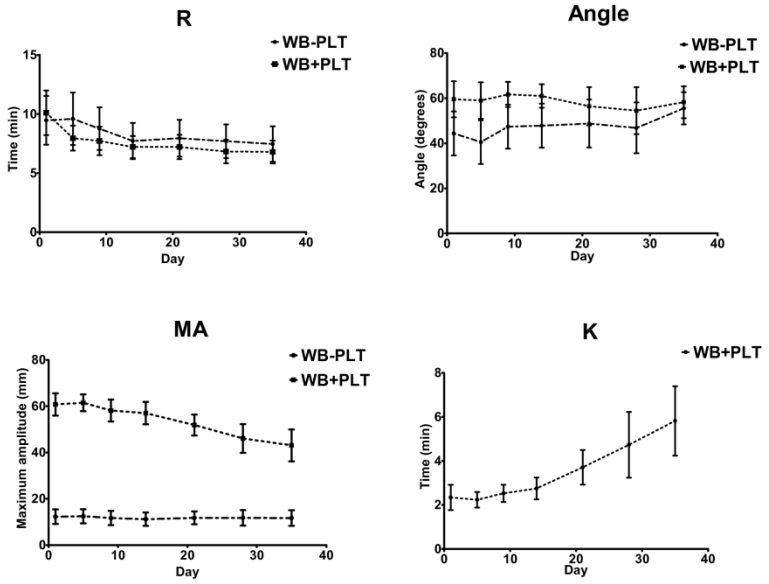

Figure 3. Thromboelastography (whole blood). Thromboelastography was performed on WB-PLT and WB+PLT throughout storage with kaolin. N=16. Error bars indicate mean±SD.

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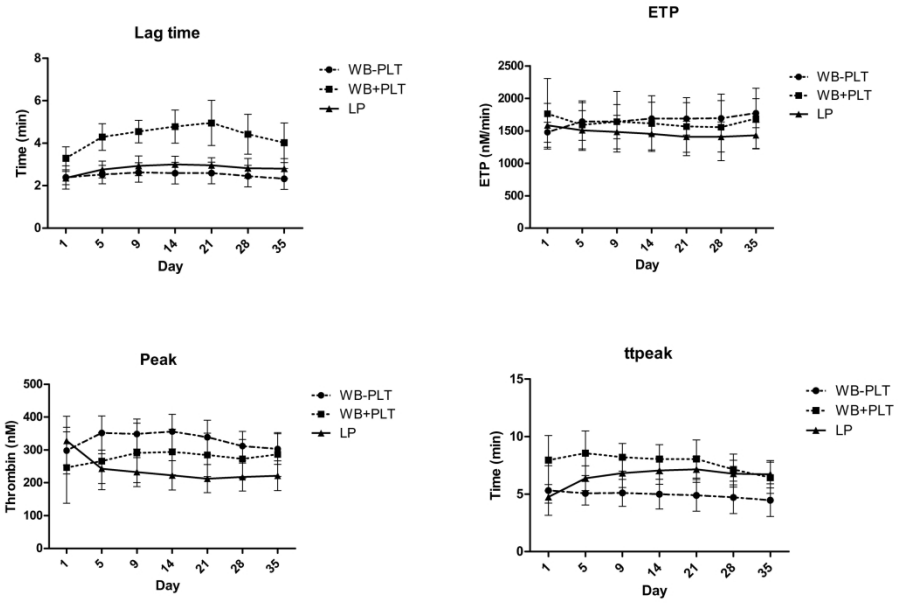

Figure 4. Thrombin Generation. Lag time, endogenous thrombin potential (ETP), peak and time to peak (ttpeak) in plasma separated from WB-PLT, WB+PLT and LP throughout storage at 2–6 ºC, in the presence of 5 pM tissue factor. N=16. Error bars indicate mean±SD.

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