

Comparison of three commercially available buffy coat pooling sets for the preparation of platelet concentrates

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Vox Sanguinis

Background A disposable set for platelet concentrate (PC) preparation by the buffy coat method allows pooling of buffy coats, centrifugation and cell separation with in-line leucocyte filtration. This study compares three commercially available pooling sets in combination with INTERCEPT pathogen inactivation (PI).

Materials and methods Sets for pooling of buffy coats were from Fresenius Kabi (FRE), Macopharma (MAC) and Terumo BCT (TER). Platelet yield, recovery and concentration were compared before and after PI ($n = 20$). Platelet quality was assessed by annexin V binding, P-selectin expression and PAC1 binding.

Results The TER pooling set had the highest platelet yield ($5.39 \pm 0.44 \times 10^{11}$) compared with MAC (4.53 ± 0.77) and FRE (4.56 ± 0.51) prior to PI. This was the result of a significantly higher platelet concentration in the TER storage bag ($1.41 \pm 0.12 \times 10^6/\mu\text{L}$) compared with MAC (1.18 ± 0.19) and FRE (1.28 ± 0.15). However, the TER platelet content decreased by 15.6% after PI, yielding $4.55 \pm 0.47 \times 10^{11}$ platelets compared with smaller reductions at 9.5% for MAC (4.10 ± 0.69) and 4.4% for FRE (4.36 ± 0.52). None of the individual PC contained $>10^6$ leucocytes. The pH in TER PC was lower compared with MAC and FRE caused by a higher lactic acid production rate. Consequently, PAC1 binding after TRAP activation was lowest for TER PC on day 6. P-selectin and annexin V were not different between suppliers.

Conclusion This study demonstrates the added value of evaluating the entire component production process when introducing a new consumable. This study helped to inform a decision on what pooling set is ideally suited for routine implementation taking into account PI.

Key words: filter, pathogen inactivation, platelets.

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Introduction

The Belgian Red Cross-Flanders Blood Service issues approximately 39,000 platelet concentrates (PC) per year to hospitals in the Northern part of Belgium (Flanders). About 40% of these are prepared by apheresis, the major number is prepared by manual pooling of buffy coats derived from whole blood donations.

Following donation, whole blood is stored overnight in sealed temperature-controlled cases [1] awaiting component preparation the next morning. Manual pooling is by six buffy coats per concentrate. This high number is chosen because a minimal platelet content of 3.0×10^{11} platelets has been set by the Belgian competent authority [2]. During pooling of buffy coats, platelet additive solution is added and after centrifugation and transfer to the final storage bag of the set, all PCs are transferred to a new disposable set for treatment with amotosalen and UV-A light pathogen inactivation (PI) (INTERCEPT Blood System, Cerus Corp, CA) [3, 4]. Until August 2017,

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pooling sets were purchased from Fenwal (part of Fresenius SE & Co, Bad Homburg, Germany) but their production line ceased its activities and a new supplier had to be chosen.

Three competing manufacturers of pooling sets were entered in an independent comparative study. Platelet yield and platelet quality were determined at different time-points during and after PC preparation. The primary criteria for PC prepared by each of these pooling sets were (1) effective leucocyte depletion ($<10^6$ leucocytes per unit), (2) compatibility with the available equipment including separators, centrifuges and PI sets and (3) fulfil all criteria for transfusable PI-treated platelets as set by the Blood Service.

Materials and methods

Study design

Pooling sets from three different manufacturers were selected for comparative analysis based on a product requirements list. The three pooling sets were PT52600/6 from Fresenius Kabi (FRE), TRV8006XU from Macopharma (MAC) (Tourcoing, France) and TF*RP0610M1 from TerumoBCT (TER) (Lakewood, CO). Twenty platelet concentrates were prepared ($n = 20$) with each pooling set. The platelet yield was determined relative to the number of platelets present in the primary buffy coats used for each individual product. Figure 1 shows a model of the study design and PC preparation process. Platelets were counted using an automated blood cell counter of the types XN-10 or XS1000i (Sysmex, Kobe, Japan). Platelet quality was determined immediately after PI on day 1 and following 5 days of storage in a standard platelet incubator with agitation. To assess the pooling sets in routine use, a larger cohort of platelet concentrates ($n = 50$) was produced. In this series only platelet content after PI was determined, in the final platelet concentrate bag.

Platelet concentrate preparation

All pooling sets were of the octopus type consisting of (i) multiple tubing ends for connecting buffy coats and additive solution to (ii) an intermediary pooling bag, (iii) a leucocyte reduction filter and a (iv) storage bag (Fig. 1). The buffy coats were from voluntary whole blood donations. Six buffy coats were manually pooled as described [4, 5]. A 280 ml fixed volume of PAS-E additive solution (SSP+, Macopharma) was added. Acceptance levels for plasma carryover were minimum 32% and maximum 47%. Next, the bag was centrifuged at 542 g for 450 s at 22°C to separate red and white cells from platelets. The buoyant platelet suspension was then separated on an

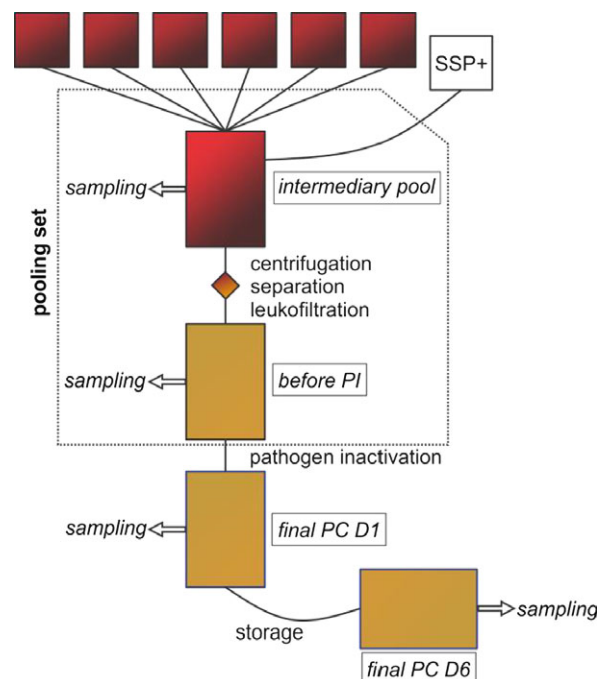


Fig. 1 Study design. Each pooling set (dotted line) was designed to pool six buffy coats into one intermediary pooling bag with additive solution (SSP+). Soft spin centrifugation and automated separation over a leucoreduction filter yielded a standard PC (before PI). These were then treated with PI and stored on the agitator from day 1 on (final PC D1). All concentrates were stored for 5 days. A final sample was taken on day 6 (final PC D6). Samples were taken where indicated (sampling). Platelet yield and quality were determined in these samples.

automated separator (Macopress Smart, Macopharma) and transferred to the storage bag whilst passing over a leucocyte reduction filter and a detection system for haemoglobin to prevent transfer of red cells. Next, the PCs were treated with INTERCEPT PI as described [6]. After the final adsorption step to remove residual amotosalen and its photoproducts, PC was transferred to the storage bag included in the PI disposable set and then stored in a temperature-controlled cabinet with continuous agitation. Samples were taken from (i) the intermediary buffy coat pool before centrifugation, from (ii) the platelet concentrate before PI, (iii) after PI and (iv) after storage (Fig. 1).

Laboratory methods

Platelet concentrate volume was determined by weighing at specified moments during processing. Volumetric mass density used was 1.028 g/ml. Plasma carryover was determined in the intermediary pool (Fig. 1) by weighing and haematocrit (Hct %) determination. The equation used was $\frac{(V_{tot} - (V_{tot} \times Hct\%) - 280)}{V_{tot} - (V_{tot} \times Hct\%)}$, with V_{tot} the total volume determined by weighing and 280 ml the additive solution volume. Platelets were counted in a fourfold dilution in

saline using an automated haematology analyser (Sysmex XS-1000i, Sysmex Corp, Japan). Recoveries were calculated relative to the sum of all platelets measured in each of the six composing buffy coats (set as a 100%). Quality control included determination of pH, glucose and lactic acid levels using a point-of-care blood gas analyser (RAPIDPoint, Siemens Healthineers, Erlangen, Germany). Leucocytes were counted by flow cytometry on a FACS Canto II using the LeukoCOUNT Combo Control kit (both (BD Biosciences, Erembodegem, Belgium) according to the manufacturer's instructions.

Flow cytometry was performed essentially as described [4]. In brief, expression of P-selectin (anti-CD62P~phycoerythrin, BD Biosciences), activated integrin $\alpha_{IIb}\beta_3$ (PAC1~fluorescein, BD Biosciences) and phosphatidylserine (annexin V~peridinin chlorophyll-Cy5-5; BD Biosciences) was determined using an acoustic focusing flow cytometer (Attune, Life Technologies, Carlsbad, CA, USA). Isotype negative control antibodies were fluorescein labelled IgM, clone G155-228 and phycoerythrin labelled IgG, clone X40 (BD Biosciences). Platelets were incubated with labelled antibodies or ligand for 10 min at room temperature in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.4 with 0.9% (w/v) NaCl (HBS), 1 mM $MgSO_4$ and 5 mM KCl, then diluted a thousand fold immediately before readout as described before [7]. For annexin V measurements, buffers were supplemented with 2 mM $CaCl_2$. As a negative control, a buffer without $CaCl_2$ was used. For

measurements of integrin $\alpha_{IIb}\beta_3$ activation on stimulated platelets, the PAR1 agonist thrombin-related activating hexapeptide SFLLRN (TRAP, Sigma-Aldrich, St Louis, MO, USA) was added at three different concentrations 4, 8 and 40 μM . The signals of the isotype antibody controls were used to set threshold gates including 0.5% of 10,000 negative events. Percentage positive events were determined of 10,000 cells stained for the platelet marker CD61 (anti-CD61~allophycocyanin, Life Technologies).

Statistics

Sample means were compared by *t*-test or Mann–Whitney test for parametric and nonparametric datasets, respectively. For analysis with multiple variables, two-way ANOVA with multiple comparisons was performed. Results were considered significant if *P* values were smaller than 0.05. Computational analysis was with Prism (GraphPad Software Inc, La Jolla, CA, USA).

Results

The platelet content and recovery in the intermediary bag after buffy coat pooling were not different between the three suppliers (Fig. 2a,b and Table 1). In the storage bag prior to PI, the TER set harvested significantly more platelets than those from the other two suppliers (Fig. 2a,b and Table 1). This could be explained by a better platelet recovery per transferred volume in the TER pooling set,

Fig. 2 Platelet content and recovery. (a) Platelet content at three different moments during production: (○) intermediary pool, (□) before pathogen inactivation, (△) after pathogen inactivation. (b) Platelet recovery relative to the amount of platelets in the composing buffy coats, intermediary pool (open bars), before (blue bars) and after (red bars) pathogen inactivation. Bars are median, and whiskers are range. (c) Platelet concentration at three different moments during production: (○) intermediary pool, (□) before pathogen inactivation, (△) after pathogen inactivation. (d) Plasma carryover for all three sets. Median is the horizontal line, $n \geq 20$. The horizontal dotted lines indicate the acceptance levels.

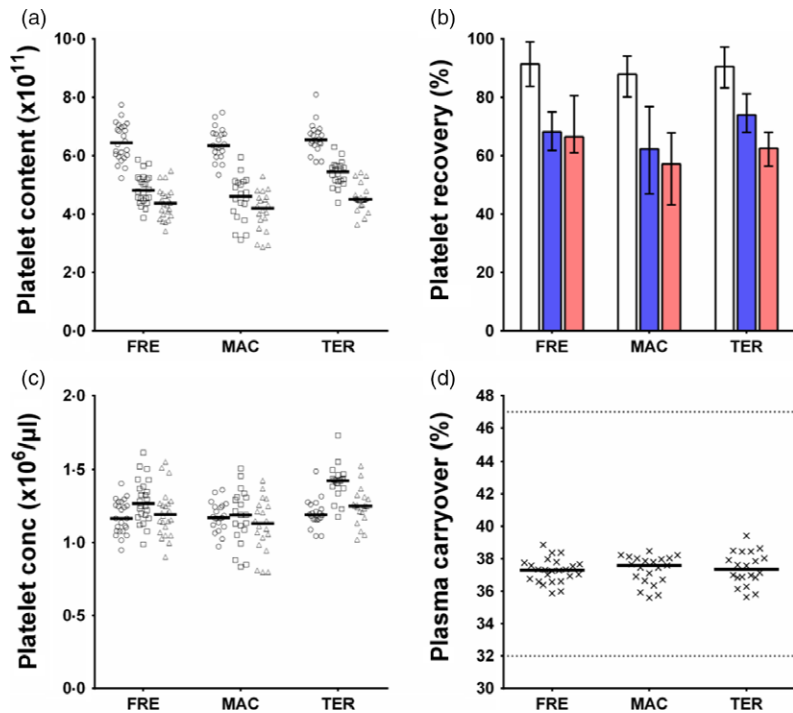


Table 1 Raw data to Figure 2a–c

| | | FRE | MAC | TER | Statistic |
|---|--------------|-------------|-------------|-------------|---|
| Platelet content ($\times 10^{11}$) | Intermediary | 6.47 (0.64) | 6.42 (0.54) | 6.61 (0.52) | |
| | Before PI | 4.56 (0.51) | 4.53 (0.77) | 5.39 (0.44) | (F vs. T)** (M vs. T)**** |
| Recovery (%) | After PI | 4.36 (0.52) | 4.10 (0.69) | 4.55 (0.47) | (M vs. T)* |
| | Intermediary | 91.0 (3.5) | 88.2 (3.0) | 90.5 (4.1) | |
| | Before PI | 68.3 (3.4) | 61.9 (7.7) | 73.8 (3.5) | (F vs. T)**** (M vs. T)**** (F vs. M)**** |
| | After PI | 67.5 (4.5) | 56.0 (6.7) | 62.2 (3.6) | (F vs. T)**** (M vs. T)**** (F vs. M)**** |
| Concentration ($\times 10^6/\mu\text{L}$) | Intermediary | 1.17 (0.12) | 1.18 (0.10) | 1.20 (0.10) | |
| | Before PI | 1.28 (0.15) | 1.18 (0.19) | 1.41 (0.12) | (F vs. T)** (M vs. T)**** (F vs. M)* |
| | After PI | 1.20 (0.16) | 1.12 (0.18) | 1.25 (0.12) | (M vs. T)*** |

Data are given as mean with SD between brackets ($n = 20$). Two-way ANOVA results are given by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and **** $P < 0.0001$.

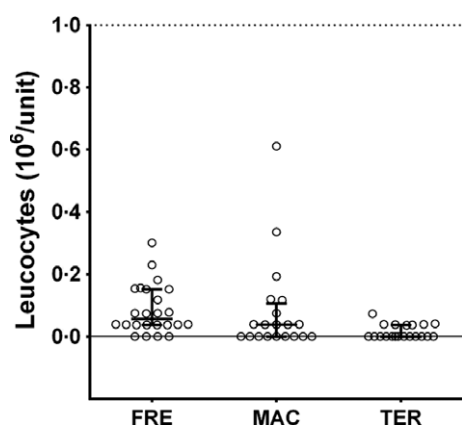


Fig. 3 Efficiency of leucodepletion. Leucocytes were counted by flow cytometry in a sample taken before PI, but after the pooling set routine. Median and interquartile range are shown as horizontal lines and whiskers, respectively. Individual data are shown as open circles. The solid horizontal line indicates that leucocytes were not observed in the selected sample volume. The dotted horizontal line indicates the maximal acceptable leucocyte count (Council of Europe Guidelines).

resulting in a higher platelet concentration compared with MAC and FRE (Fig. 2c and Table 1). However, after PI, this difference largely disappeared because 15.6% of platelets were lost from the TER bag compared with 9.5% and 4.4% for MAC and FRE, respectively. Still, the TER pooling set could yield significantly more platelets than the MAC bag set (Fig. 2a–c and Table 1). Plasma carry-over was not different between sets (Fig. 2d), and none of the individual products contained $>10^6$ leucocytes (Fig. 3). A follow-up study to assess routine production of PC ($n = 50$) confirmed that the TER pooling set resulted

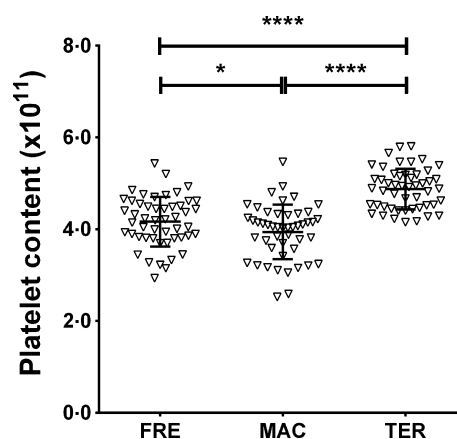
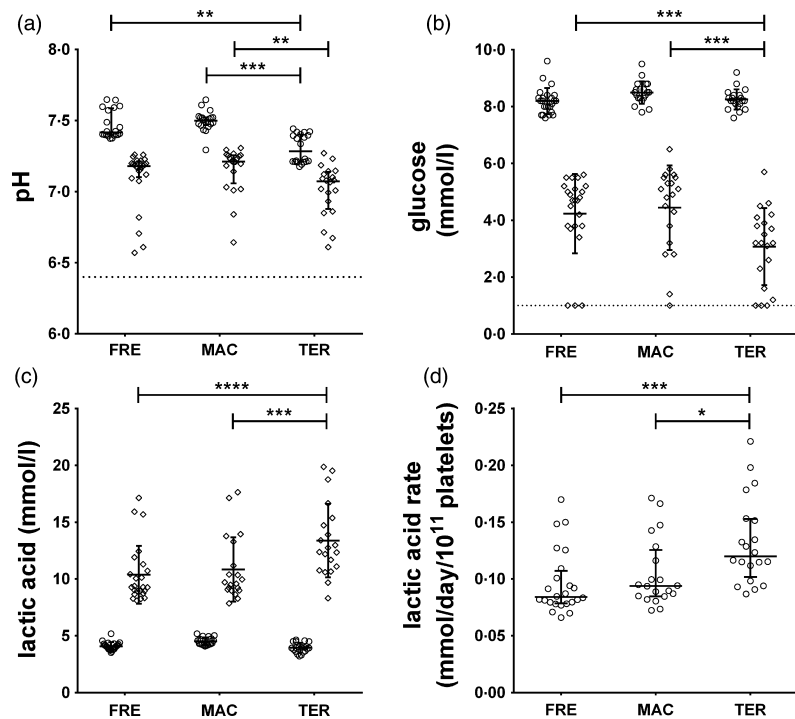


Fig. 4 Platelet content in a follow-up larger series. To assess routine use, a larger series ($n = 50$) of platelet concentrates was produced using the three different sets. Platelet content in the final platelet concentrate bag after PI was determined. All data are shown, bars represent median, and whiskers represent range. ANOVA with multiple comparisons was performed, statistic is indicated on top of each panel; * $P < 0.05$; **** $P < 0.0001$.

in higher yields compared with those from MAC and FRE after PI (Fig. 4).

The pH was lower on day 1 for PC prepared by the TER pooling set compared with MAC and FRE (Fig. 5a, circles). As expected, product pH declined in function of storage but none of the PC had levels below 6.4 on day 6 (Fig. 5a, diamonds). The lowest average pH on day 6 was found in TER PC, reaching significance only in comparison with MAC sets. For all three suppliers, a number of PC had depleted glucose levels (Fig. 5b). Glucose levels

Fig. 5 Platelet metabolism. (a) Platelet concentrate pH. The dashed line indicates the lower acceptance criterion (Council of Europe Guidelines). Median and interquartile range are shown (b) The glucose concentration. The dashed line indicates the lower limit of detection of the blood gas analyser. Mean and standard deviation are shown. (c) The lactic acid concentration. Mean and standard deviation are shown. (d) The lactic acid production rate determined over 5 days of storage and per 10^{11} platelets. Median and interquartile range are shown. All data are shown as individual symbols for day 1 (○) and for day 6 (◇) of storage. Median and interquartile range are given.



were the lowest for PC prepared by the TER pooling set. This corresponded with lactic acid production rates which were highest for TER platelets (Fig. 5c,d).

Platelet function was assessed by integrin $\alpha_{IIb}\beta_3$ activation in response to increasing concentrations of TRAP. Integrin activation was lowest for the MAC platelets on day 1 (Fig. 6a) compared with TER and FRE. After 5 day storage, all platelets responded poorly to low and intermediate TRAP concentrations. At high concentrations of agonist, TER platelets responded the least compared with MAC and FRE sets (Fig. 6b). Annexin V binding was the highest for MAC platelets on day 1, reaching significance in comparison with TER, not FRE (Fig. 7a). After storage, annexin V binding was similar for all sets. Little difference could be noted in P-selectin expression (Fig. 7b) which was lowest on day 1 for TER platelets, reaching significance in comparison with FRE.

Discussion

There are several ways to prepare PC from blood donations [5]. Many blood institutions in the EU use the buffy coat method among others to provide PC to patients. The buffy coat method is considered gentle because it minimally activates platelets [8]. The number of buffy coats composing one PC depends on the blood institution and national guidelines or legislation. In Belgium, a minimal platelet content per adult transfusion is required since in 2011 the competent authority issued a circular that for

PI-treated PC the platelet content should be at least 3×10^{11} . Therefore, six blood group matched buffy coats are mixed and combined with additive solution to prepare one PC.

The current study shows that MAC and FRE pooling sets yield similar platelet quantities. In comparison, the TER set yields significantly more platelets. This pooling set has a polyurethane in-line leucocyte filter (Imugard III S PL) in a soft housing but overall retained within a hard case. The FRE leucocyte filter is just in a soft housing which is designed to empty its content 'automatically' by the elasticity of the housing material. The MAC leucocyte filters are in a hard housing. Both FRE and MAC filters have a polyester base, which differs from that of TER. The latter supplier claims that the Imugard filter does not retain leucocytes by adhesion, but only mechanically through sieving. The polyester-based FRE leucocyte filter has a net neutral charge as well, but probably combines adhesive as well as mechanical retention of leucocytes (personal communication from Fresenius Kabi). Taken together, differences in physicochemical composition as well as in housing may explain the observations. Of note, different types of leucocyte filters have been compared before in older studies, including the Imugard filter. Differences in yield [9, 10] as well as in post hoc platelet activation or quality [11] have been described.

The TER set consistently yielded more platelets than the other two suppliers, but the PI process caused a significant loss of that surplus platelet yield. Based on the data before

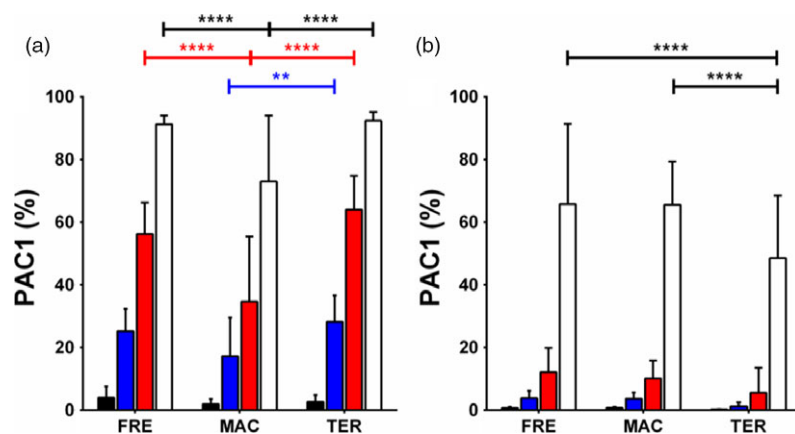


Fig. 6 Binding of PAC1 in response to TRAP on day 1 and day 6. The percentage of PAC1 positive platelets in response to increasing concentrations of TRAP, 0 μM (black), 4 μM (blue), 8 μM (red) and 40 μM (white) is shown. Bars represent mean and whiskers standard deviation for (a) day 1 and (b) day 6 of storage. Statistics by two-way ANOVA is shown on top of the panels, when not significant there is no indication; ** $P < 0.01$ and **** $P < 0.0001$.

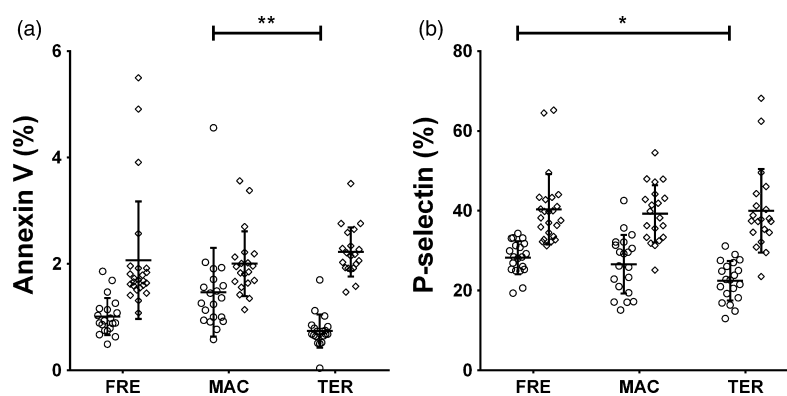


Fig. 7 Exposure of negatively charged phospholipids and P-selectin. (a) The percentage of platelets staining positive for annexin V. (b) The percentage of platelets staining positive for P-selectin. All data are shown as individual symbols for day 1 (○) and for day 6 (◇) of storage. Mean is shown as a horizontal line, and whiskers indicate standard deviation. Statistics by two-way ANOVA is shown on top of the panels, when not significant there is no indication; * $P < 0.05$ and ** $P < 0.01$.

and after PI, 15.6% of platelets were lost from the TER PC in comparison with just 9.5% and 4.4% from the MAC and FRE PC, respectively. This shows that it is important to take into account PI when evaluating the overall yield of different pooling sets, as the PI process may act differently on products produced by different pooling sets. It is unclear what this might be in particular.

Small but significant differences in platelet metabolism were found. It is not clear why TER PC had a significantly lower pH on day 1 compared with both MAC and FRE. This could not be explained by metabolism as such, because lactic acid concentrations were not different on day 1. Platelets produced by the TER pooling sets did have an increased lactic acid production rate. This may follow the platelet concentration and/or content because we previously showed that PI treatment can increase storage lesion when the platelet number is high [2, 12]. Whether this underlies the difference in lactic acid found in this study is not clear.

The PAC1 binding in response to TRAP was lowest for MAC and highest for TER platelets on day 1, indicating

that TER platelets sense the agonist better at that time. Over 5 days of storage however, all PC significantly lost sensitivity for the intermediate TRAP concentrations which is indicative of storage lesion. Only at high concentrations of TRAP, PAC1 binding was evident with the least binding to TER platelets. This is in line with the metabolic data showing that TER platelets had increased lactic acid production rates. Of note, this increased metabolic rate did not cause increased apoptosis as determined by annexin V binding, nor increased alpha-degranulation as determined by P-selectin expression. This indicates that the differences between the three pooling sets in terms of storage lesion effects are small.

All three suppliers fulfilled the criteria as set by the Blood Institution. The choice for a particular pooling set depends on many factors, including but not restricted to platelet yield and platelet quality over storage time. The combination with PI adds to the complexity of the production process and the interpretation of validation data. Therefore, although a high platelet yield can be preferred

in many instances, the combination with PI can impose a risk for premature dropout caused by storage lesion. The data show that thorough comparison of platelet yield and quality is important to direct decisions in a setting with PI.

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References

- 1 Vanaudenaerde D, Verdegem L, Hintjens P, *et al.*: Poster Abstracts. *Vox Sang* 2013; 105:65–299
- 2 Feys HB, Devloo R, Sabot B, *et al.*: High platelet content can increase storage lesion rates following Intercept pathogen inactivation primarily in platelet concentrates prepared by apheresis. *Vox Sang* 2017; 112:751–758
- 3 Van Aelst B, Devloo R, Zachee P, *et al.*: Psoralen and ultraviolet a light treatment directly affects phosphatidylinositol 3-kinase signal transduction by altering plasma membrane packing. *J Biol Chem* 2016; 291:24364–24376
- 4 Van Aelst B, Feys HB, Devloo R, *et al.*: Riboflavin and amotosalen photochemical treatments of platelet concentrates reduce thrombus formation kinetics *in vitro*. *Vox Sang* 2015; 108:328–339
- 5 Hardwick J: Blood processing. *ISBT Sci Ser* 2008; 3:148–176
- 6 Irsch J, Lin L: Pathogen inactivation of platelet and plasma blood components for transfusion using the INTERCEPT blood system. *Transfus Med Hemother* 2011; 38:19–31
- 7 Goodall AH, Appleby J: Flow-cytometric analysis of platelet-membrane glycoprotein expression and platelet activation. *Methods Mol Biol* 2004; 272:225–253
- 8 Metcalfe P, Williamson LM, Reutlingsperger CP, *et al.*: Activation during preparation of therapeutic platelets affects deterioration during storage: a comparative flow cytometric study of different production methods. *Br J Haematol* 1997; 98:86–95
- 9 Hild M, Soderstrom T, Egberg N, *et al.*: Kinetics of bradykinin levels during and after leukocyte filtration of platelet concentrates. *Vox Sang* 1998; 75:18–25
- 10 van der Meer P, Pietersz R, Reesink H: Leukoreduced platelet concentrates in additive solution: an evaluation of filters and storage containers. *Vox Sang* 2001; 81:102–107
- 11 Krailadsiri P, Seghatchian J, Williamson LM: Platelet storage lesion of WBC-reduced, pooled, buffy coat-derived platelet concentrates prepared in three in-process filter/storage bag combinations. *Transfusion* 2001; 41:243–250
- 12 Drawz SM, Marschner S, Yanez M, *et al.*: Observational study of corrected count increments after transfusion of platelets treated with riboflavin pathogen reduction technology in additive solutions. *Transfusion* 2015; 55:1745–1751

Conflict of interest

The authors have no conflict of interest to declare.

Authorship contributions

HBF, JC and VC designed the research. RD and BS performed experiments. HBF wrote the manuscript. JC and VC supervised the study. All authors critically reviewed and amended the manuscript.