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ASSESSMENT OF PLATELET CONCENTRATE PREPARED FROM FRESH AND OVERNIGHT HELD WHOLE BLOOD

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ABSTRACT

Separation of platelet rich plasma within 8 hrs after holding whole blood at ambient temperature is the current practice in the preparation of platelet concentrate (PC). However, the ability to hold whole blood for up to 24 hrs before PC preparation would allow every unit of whole blood to be used regardless of the distance from the collection site to the processing center. Therefore, the aim of this study is to assess and to compare the platelet quality and activation of the PC prepared from fresh whole blood (within 8 hrs from collection) and overnight hold of whole blood at room temperature. In this study, 23 units of PC were prepared freshly from whole blood (i.e., within 8 hrs of collection) and another 23 units were prepared after 24 hrs of storage at 20-24°C. The following parameters of each unit of PC were assessed: pH, total white blood cell (TWBC) count, platelet count, presence of swirling, and platelet activation. When the parameters were compared between groups, no significant difference in platelet activation rate was found on sampling days 1, 3, and 5. pH and TWBC count for both groups were within the quality requirements of the National Blood Centre (>75% units tested fall within the standard), but not all of the samples complied with the standard requirement for platelet count. All units of PC prepared after 24 hrs showed the presence of swirling, whereas one unit of PC in the fresh group did not show swirling activity after 3 days of storage. Delaying whole blood processing for up to 24 hrs does not significantly affect certain *in vitro* quality or activation parameters as compared with freshly prepared PC.

Keywords: Platelet concentrate, Platelet rich plasma, Blood, Platelet count, Total white blood cell count.

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INTRODUCTION

Platelet concentrate (PC) is a clinically significant blood product that is prepared from whole blood collected from voluntary donors. It is defined as the component derived from fresh whole blood that contains the majority of the original platelet content in a therapeutically effective form [1]. To prepare platelet rich plasma (PRP), the respective unit of whole blood is centrifuged so that an optimal number of platelets remain in the plasma and the numbers of leukocytes and red cells are reduced to a defined level [1]. PC is considered to be freshly prepared when PRP is separated within 4 hrs after completion of phlebotomy or within the time frame specified by the American Association of Blood Banks for blood collection, processing, and storage, which typically is 8 hrs [2]. PC derived from overnight held whole blood refers to samples prepared after whole blood has been stored at ambient temperature for up to 24 hrs.

Ensuring the quality of each unit of PC is crucial, and documenting the temperature and storage history of whole blood before component manufacturing is a necessary part of quality control [3]. Initially, whole blood storage at 20-24°C before PC preparation was limited to 4 hrs after collection. However, the whole blood collection often takes place at mobile clinics, which makes such a short time limit logistically problematic [4]. Thus, the drive to extend the storage time to 8 hrs reflected an increased demand for PC and the need to allow more time to transport whole blood from distant collection sites to the component production laboratories [5]. In certain countries, the permissible time for whole blood storage at ambient temperature is limited to 8 hrs due to the concerns about bacterial contamination and loss of 2,3-diphosphoglycerate in red blood cells (RBCs) as well as the preservation the factor VIII content of plasma. This 8 hrs holding time usually requires processing staff to work irregular hours throughout the day and night [6]. If blood must be stored for longer than 8 hrs before processing, the units must be cooled to 4°C, rendering them unsuitable for platelet preparation [6]. Furthermore, PCs prepared from units processed after 8 hrs are considered to be of inferior quality [3].

An overnight held of whole blood for up to 24 hrs before PC preparation would provide several advantages as it would allow every unit of whole blood to be used regardless of the distance from the collection site to the processing center, operating cost will be reduced and potentially lower bacterial contamination due to ingestion of bacteria by white blood cell [6]. Therefore, the aim of this study was to evaluate the impact of an overnight hold (24 hrs) of whole blood on several parameters of PC processed using the PRP method. Quality and platelet activation parameters were compared between PC prepared from fresh (within 8 hrs) and overnight held (after 24 hrs) whole blood.

METHODS

Blood samples preparation

The PCs used in this study were processed following the PRP method. Whole blood samples were collected in Terumo (Tokyo, Japan) triple bag with volume of 450 ± 50 ml with citrate phosphate dextrose adenine anticoagulant and single donor unit PRP were prepared in two ways. In the fresh group, PRP was prepared immediately after collection of whole blood (i.e., within 8 hrs from the collection time); in the overnight group, whole blood was held for 24 hrs at room temperature and PRP was prepared the next day.

In both groups, PRP was obtained by soft spin centrifugation equipped with a swing-out bucket rotor (Thermo Scientific, MN, USA) (2500 g/5 minutes at 20°C) of whole blood units. For each sample, PRP was transferred into an empty satellite bag using plasma

expresser. The PRP then was spun again using hard spin centrifugation (4000 g/10 minutes at 20°C). The plasma was expressed into another empty bag (Terumo, Japan) as single donor unit, leaving at least 50 ± 10 ml of plasma with the platelet pellet. The platelet pellet (i.e., the PC) was suspended and stored under continuous horizontal agitation in a platelet incubator for several hours. All centrifugation and storage steps were carried out at 20-24°C. Samples from each PC unit were drawn aseptically in a closed system using a Terumo transfer bag on days 1, 3, and 5. These samples were used to analyze the quality and activation parameters of the PC units.

Sample size

The study was conducted using PCs provided by the National Blood Centre (NBC, Malaysia). The study was originally designed to include 60 units of PC obtained mostly from mobile collection sites or in-house collection at the NBC and the Hospital Tuanku Ja'afar, Seremban, Negeri Sembilan. The whole blood collected was processed within 6-8 hrs for the first group (mean of 7 hrs) and after 24-28 hrs (mean of 26 hrs) for the second group. All units of PC that met the inclusion criteria were randomized into two groups: (i) Freshly prepared PCs and (ii) PCs derived from overnight held whole blood. An informed consent was obtained from the donors. Ethical clearance for this study was obtained from the Human Research Ethics Committee USM (registration number: FWA 00007718).

The criterion for significance (α) was set at 0.050, and a two-tailed test was used (i.e., an effect in either direction would be interpreted). With the proposed sample size of 30 for each of the two groups, this study would have power exceeding 90% to yield a statistically significant result [7]. Unfortunately, 30 samples of PC for each group were not available and only 23 units of PC for each group were used in this study. However, this sample size still provided power exceeding 85% to yield a statistically significant result.

Inclusion and exclusion criteria

Inclusion criteria for PC sampled were as follows: PC produced from whole blood, PC prepared using the PRP method, and PC with a volume >40 ml. Units of PC that had leakage from any part of the container, red cell contamination, abnormal color, and/or visible clots were excluded from this study.

Quality and activation parameters of the PC

About 23 units of PC for each group were obtained from the production and component unit of the NBC and tested for platelet count, total white blood cell (TWBC) count, pH, and presence of swirling on days 1, 3, and 5 of storage. In addition, a platelet activation test was conducted.

Preparation of PC for analysis

The tubing of the PC bag was connected to the tubing of the Terumo transfer bag using a Terumo sterile connecting device. The content of PCs was well mixed before the tubing of PCs bag was stripped using a hand tube stripper. This step was repeated five times before the end of tubing of Terumo transfer bag was sealed using a desktop tube sealer. The sample was taken from the segment after the content was properly mixed and stripped. The length of tubing was estimated to contain 3-4 ml of PC. The remaining PC was returned to the platelet incubator for storage. This process was performed for each PC sample on days 3 and 5. A part of the content of the sealed tubing for each unit of PC was transferred into 5 ml plain tubes for cell count analysis and flow cytometry testing, and another portion from the same tubing was transferred into 10 ml plain tubes for pH testing.

Analysis of quality parameters

Platelet count and TWBC count

A fully automated blood count analyzer (LH 750 Beckman Coulter, MN, USA) hematology analyzer was used to analyze the platelet and TWBC counts in the PC samples. This analyzer has five different modes of real-time random access testing, and the mode used was complete blood count.

pH

pH analysis of the samples was performed using a pH meter (Mettler Toledo Delta 320, MA, USA). Before every sample testing, two calibration measurements were made using reference buffer solutions with known pH values.

Swirling

The swirling test is an *in vitro* test that is considered to be optimal for predicting the survival and activation of stored platelets. It is the only non-invasive test that can predict good morphology of platelets, and therefore, their survival. On each day of sampling (i.e. days 1, 3, and 5), the presence of swirling was observed under the light for each unit of PC [8].

Platelet activation test

Test procedure

Flow cytometer (FACS Calibur, MN, USA) was used to analyze the platelet activation. In this procedure, multicolor staining was used for direct immunofluorescence staining. Staining and fixation of platelets were performed within 10 minutes of sampling. For the analysis, a 12 mm × 75 mm tube was labeled for each PC sample. Next, 20 µl of activation-independent platelet and specific antibody (CD61) were pipetted into each labeled tube, then 20 µl of platelet activation-dependent monoclonal antibody (CD62p) were added. Using a fresh micropipette tip for each sample, 5 µl of unstimulated PC (diluted in 1:5 v/v) was carefully added to the bottom of each labeled tube. For the negative control, 5 µl of negative control (Mouse IgG) was extra added to the bottom of each labeled tube. The tubes were gently swirled to mix the contents, and then all of the tubes were incubated for 15-20 minutes at room temperature in the dark. Next, 1 ml of cold 1% paraformaldehyde solution was added to each tube, and the contents were vortexed. Stained and fixed cells were stored at 2-8°C in the dark for at least 30 minutes but not for more than 24 hrs. Stained and fixed cells were then analyzed using the flow cytometer. A minimum of 10,000 cells within the gated region were analyzed.

Statistical analysis

Statistical analysis of data was performed using Statistical Package for Social Science (SPSS) software version 18. Mann Whitney test was used to compare the differences in the outcome variables between groups while Wilcoxon Signed Rank was used to compare the differences in the outcome variables within the groups across the test period. $P < 0.05$ was considered statistically significant.

RESULTS

Quality parameters of stored PC

Compliance with the standard for pH, TWBC count, platelet count, and swirling

Testing of quality parameters to all the blood products is one of the important tools to ensure the blood products produced met the standard requirement. NBC has established a standard guideline of testing quality parameters as an approach to monitor, validate and rectify problems that may affect the quality of blood products. Therefore, a high-quality blood product will be continuously produced to meet the demands. This is relevant to samples from PCs, as platelets can easily undergo shape changes and time/temperature-dependent changes in their aggregation states. In this study, the adherence to the standard guidelines as set by NBC, i.e., >75% of unit tested must fall within the value indicated for each parameter (NBC, 2008), was also observed. For compliance with the NBC's quality standards, at least 75% of units tested must fall within the standards. Results for our samples are shown in Table 1. The pH and TWBC counts were acceptable for both groups. However, the platelet counts were below the indicated quality requirement, as they were only acceptable on day 1 for the fresh group and the overnight group did not comply with the requirement at any time point. Among all of the samples, only one unit in the fresh group

did not show the presence of swirling on days 3 and 5; swirling was present in all other samples at all-time points. The PC without swirl had a pH <6.5.

Comparison of median values of pH, TWBC count, and platelet count between groups

For the pH, TWBC count, and platelet count data, the frequency distributions were not normally distributed; therefore, the non-parametric statistical test for two independent samples (i.e., the Mann–Whitney test) was used to compare the median values between groups for each day of sampling. Median values were not significantly different between the fresh and overnight groups for all parameters at all-time points ($p>0.05$) except for pH on day 1 and TWBC count on day 1 ($p<0.05$) (Table 2).

Comparison of median values of pH, TWBC count, and platelet count within groups

For the pH, TWBC count, and platelet count data, the frequency distributions were not normally distributed; therefore, the non-parametric statistical test for two related samples (i.e., the Wilcoxon Signed-Ranks test) was used to compare median values within groups for each day of sampling. Significant differences in median values of pH between days 1, 3, and 5 in the overnight group and between days 3 and 5 in the fresh group were detected (Table 3). For both groups at all-time points, the only significant difference in TWBC count was detected between days 1 and 5 in the fresh group. No significant differences in median values of platelet count were detected for either group.

Platelet activation

Comparison of median values between groups

The frequency distributions of the platelet activation data were not normally distributed; therefore, the non-parametric statistical test for two independent samples (i.e., the Mann–Whitney test) was used to compare median values of platelet activation between groups for each day of sampling. No differences in the median value of platelet activation rate between the fresh and overnight groups were detected throughout the storage period ($p>0.05$) (Table 4). A trend is seen for lower platelet activation for overnight PCs, at least at day 5 ($p=0.067$).

Comparison of median values within groups

The frequency distributions of the platelet activation data were not normally distributed; therefore, the non-parametric statistical test for two related samples (i.e., the Wilcoxon Signed-Rank test) was used to compare the median values of platelet activation within groups for each day of sampling. Table 5 shows that there was a significant difference in the median value of platelet activation rate between days 1 and 5 and between days 3 and 5 for both groups ($p<0.05$).

DISCUSSION

In general, pH was well maintained during 5 days of storage of PC (>95% complying the standards) (i.e., >75% pH 6.5) (Table 1). Only one unit in the fresh group did not meet the standard (pH of 5.90 on day 3 and 5.52 on day 5). The NBC guidelines do not give an upper limit for pH. On day 1, the pH value was significantly lower in the overnight group than in the fresh group (Table 2). However, the difference disappeared during the storage period ($p>0.05$). In the overnight group, the median pH was lower than the fresh group on day 1 due to the build-up of lactic acid produced by the large quantity of RBCs in the stored whole blood. However, as carbon dioxide tensions equilibrated once the PC was stored in a gas-permeable container, the pH rapidly changed to become similar to that of the freshly prepared PC (Tables 2 and 3) [6].

Using the buffy coat method [9] found that the platelet counts in freshly prepared PC were 32% and 40% lower than in those in PC prepared after overnight storage of buffy coat or whole blood, respectively. They concluded that the lower platelet count in the fresh group was due to the relatively short rest period of the whole blood and to the formation of aggregates, which are removed during centrifugation. Pietersz (2011) reported that storage of whole blood at ambient temperature for up to 24 hrs before component preparation allowed higher yields of platelets in the buffy coat and better buoyant density separation of the blood components after high-speed centrifugation, resulting in a higher plasma yield [10].

van der Meer *et al*. (2011) reported that the platelet count was 33% higher in PC prepared from overnight held whole blood, even when it was prepared using the PRP method [6]. In an earlier study, Holme *et al*. (1989) found that platelet yield in PC prepared by the PRP method

Table 1: Compliance with the NBC's standards for pH, TWBC count, platelet count, and swirling for both groups

Standard	Group	n	Compliance with standard (%)		
			Day 1	Day 3	Day 5
pH>6.5	Fresh	23	23 (100)	22 (95.7)	22 (95.7)
	Overnight	23	23 (100)	23 (100)	23 (100)
TWBC <0.2×10^9/unit	Fresh	23	23 (100)	23 (100)	23 (100)
	Overnight	23	23 (100)	23 (100)	23 (100)
Platelet count >60×10^9/unit*	Fresh	23	18 (78.3)	17 (73.9)	16 (69.6)
	Overnight	23	13 (56.5)	13 (56.5)	13 (56.5)
Presence of swirling	Fresh	23	23 (100)	22 (95.7)	22 (95.7)
	Overnight	23	23 (100)	23 (100)	23 (100)

*Calculations based on the PC volume of day 1 before sampling. NBC: National Blood Centre, TWBC: Total white blood cell

Table 2: Comparison of median values of pH, TWBC count, and platelet count between groups

Parameters	Sampling day	Fresh group median (IQR)	Overnight group median (IQR)	Median differences	p value
pH	Day 1	7.4 (0.13)	7.3 (0.05)	0.1	<0.001
	Day 3	7.4 (0.21)	7.5 (0.18)	0.1	0.18
	Day 5	7.3 (0.21)	7.4 (0.15)	0.1	0.51
TWBC count (10^9/L)*	Day 1	0.04 (0.03)	0.02 (0.02)	0.02	<0.001
	Day 3	0.03 (0.03)	0.03 (0.02)	0	0.13
	Day 5	0.03 (0.02)	0.03 (0.01)	0	0.35
Platelet count (10^9/L)*	Day 1	71 (18)	63 (33)	8	0.23
	Day 3	71 (25)	66 (25)	5	0.38
	Day 5	71 (34)	63 (36)	8	0.23

*Based on the PC volume. IQR: Interquartile range, TWBC: Total white blood cell

Table 3: Comparison of median values of pH, TWBC count, and platelet within groups

Parameters	Sampling day	Fresh group		Overnight group	
		Z value	p value	Z value	p value
pH	Day 1 versus Day 3	−1.756[a]	0.07	−4.198[a]	<0.001
	Day 1 versus Day 5	−1.476[b]	0.14	−3.832[a]	<0.001
	Day 3 versus Day 5	−4.200[b]	<0.001	−4.200[b]	<0.001
TWBC	Day 1 versus Day 3	−1.394[b]	0.16	−0.911[a]	0.36
	Day 1 versus Day 5	−2.049[b]	0.04	−1.018[a]	0.30
	Day 3 versus Day 5	−0.989[b]	0.32	−0.133[a]	0.89
Platelet count	Day 1 versus Day 3	−0.393[b]	0.69	−1.047[a]	0.29
	Day 1 versus Day 5	−0.766[b]	0.44	−1.120[b]	0.26
	Day 3 versus Day 5	−0.852[b]	0.39	−1.423[b]	0.15

[a]Based on negative ranks, [b]Based on positive ranks, [c]Wilcoxon Signed-Ranks test. TWBC: Total white blood cell

Table 4: Comparison of median values of platelet activation rate between groups

Sampling day	Fresh group	Overnight group	Median differences	p value
Day 1	21.58 (14.72)	13.81 (26.64)	7.77	0.156
Day 3	21.17 (16.73)	20.62 (8.37)	0.55	0.621
Day 5	31.28 (17.60)	25.67 (16.40)	5.61	0.067

Table 5: Comparison of median values of platelet activation rate within groups

Sampling day	Fresh group		Overnight group	
	Z value	p value	Z value	p value
Day 1 versus Day 3	−0.243[a]	0.808	−1.703[a]	0.082
Day 1 versus Day 5	−3.711[a]	<0.001	−3.011[a]	0.003
Day 3 versus Day 5	−4.015[a]	<0.001	−3.103[a]	0.002

[a]Based on negative ranks, [b]Wilcoxon Signed-Ranks test

from whole blood with an 8 hrs holding period was higher (but not significantly higher) than that prepared from whole blood held for 1-2 hrs or 6 hrs [11]. In freshly prepared PC, aggregation of activated platelets with WBCs can occur and can lead to a lower platelet count in fresh PC compared to PC derived from overnight held blood [12]. However, the opposite result was found in the current study (Table 2); the median platelet count in the overnight group was lower than that in the fresh group at days 1, 3, and 5, but the difference was not significant ($p>0.05$). In addition, the platelet count in the PCs did not fulfill the NBC's requirement that at least 75% of the units tested have a platelet count $>60 \times 10^9$/unit. By the end of the storage period, only 56.5% and 69.6% of the units in the overnight and fresh groups, respectively, fulfilled the requirement (Table 1) [3,4,13]. The swirling phenomenon is routinely used to evaluate the quality of PC [14], as the ability to swirl indicates the presence of healthy PCs [12]. The presence of swirling is highly effective in predicting that the pH value of the PC is within an adequate range [14]. In the current study, the swirling effect did not differ much between the fresh and overnight groups, indicating that the quality of both was acceptable.

Although the median value of the platelet activation rate was lower in the overnight group than in the fresh group throughout the storage period, the differences were not statistically significant (p=0.621 on day 3 and p=0.067 on day 5) (Table 4). Similarly, van der Meer *et al*. (2011) reported that CD62p expression did not differ significantly between PC prepared using the PRP method from fresh and overnight held whole blood, although the level of activation was lower in the overnight group [6]. Thibault *et al*. (2006) conducted a similar study of PRP prepared PC and found that the PC in the overnight group was less activated that in the fresh group, but the level of CD62p activation on day 1 was similar for both groups (37 ± 11% and 38 ± 16%, respectively) [4]. In another study, platelets prepared using the PRP method were not more activated following an overnight hold than when obtained 6 hrs after blood collection [15]. For PCs prepared using the buffy coat method, CD62p expression was lower in the overnight group compared to the fresh group during the storage period, and the difference was significant on day 5 [10].

Sanz *et al*. (1997) concluded that the second day of storage after preparing PC using the PRP method is the appropriate time to notice any supplementary activation attributable to the overnight hold method [15]. In the current study, platelet activation began to increase significantly on day 3 (Table 5). The median values differed significantly between days 3 and 5 for both groups (p=0.002 for the overnight group and p<0.001for the fresh group), whereas they did not differ significantly between days 1 and 3. Evaluation of the median value of platelet activation within each group between days 1 and 5 also was significant (p=0.003 for the overnight group and p<0.001for the fresh group).

When whole blood is held overnight at room temperature, the prolonged incubation of platelets with metabolically active granulocytes can result in higher levels of platelet activation [15]. However, this did not seem to occur in the current study and it is possible that RBCs play a protective role in granulocyte-dependent platelet activation in blood components by hampering the physical contact between platelets and granulocytes [15].

CONCLUSION

The quality parameters (pH, TWBC count, and platelet count) of the overnight group during the storage period were comparable with those of the freshly prepared PC samples. All parameters except platelet count met the NBC's standard requirement. This study also observed a lower platelet count in the overnight group but was unable to conclude that the overnight held affected the quality of PCs and resulted in lower platelet count. There are variable factors such donor's factor, adequacy of mixing of blood during collection, time difference between phlebotomy and processing may exist, and these could affect the concentration of platelet count. In general, this study is able to show that the delay in whole blood processing up to 24 hrs has no significant effect on certain *in vitro* quality parameters and function compared with freshly prepared PCs. However, due to insufficient evidence and certain limitations during the study period, it is premature to support the findings of PCs prepared from overnight held whole blood has a better quality than freshly derived PCs. Therefore, additional experiments are necessary to improve the platelet count.

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REFERENCES

1. Council of Europe. Guide to the Preparation, use and Quality Assurance of Blood Components. 12th ed. Europe: Council of Europe; 2006.
2. AABB. Collection, preparation, storage and distribution of components from whole blood donations, In: Brecher ME, Leger RM, Linden JV, Roseff SD, editors. AABB Technical Manual, 15th ed. Bethesda: AABB; 2005. p. 175-99.
3. Pietersz RN, de Korte D, Reesink HW, Dekker WJ, van den Ende A, Loos JA. Storage of whole blood for up to 24 hours at ambient temperature prior to component preparation. Vox Sang 1989;56(3):145-50.
4. Thibault L, Beauséjour A, de Grandmont MJ, Lemieux R, Leblanc JF. Characterization of blood components prepared from whole - blood

donations after a 24-hour hold with the platelet-rich plasma method. Transfusion 2006;46(8):1292-9.
5. Moroff G, AuBuchon JP, Pickard C, Whitley PH, Heaton WA, Holme S. Evaluation of the properties of components prepared and stored after holding of whole blood units for 8 and 24 hours at ambient temperature. Transfusion 2011;51 Suppl 1:7S-14.
6. van der Meer PF, Cancelas JA, Vassallo RR, Rugg N, Einarson M, Hess JR; BEST Collaborative. Evaluation of the overnight hold of whole blood at room temperature, before component processing: Platelets (PLTs) from PLT-rich plasma. Transfusion 2011;51 Suppl 1:45S-9.
7. SPSS. Available from: http://www-01.ibm.com/software/analytics/spss/. [Last accessed on 2013 Sep 07].
8. Fratantoni JC, Poindexter BJ, Bonner RF. Quantitative assessment of platelet morphology by light scattering: A potential method for the evaluation of platelets for transfusion. J Lab Clin Med 1984;103(4):620-31.
9. Dijkstra-Tiekstra MJ, Kuipers W, Setroikromo AC, de Wildt-Eggen J. Overnight or fresh buffy coat - derived platelet concentrates prepared with various platelet pooling systems. Transfusion 2008;48(4):723-30.
10. Pietersz RN. Storage of whole blood for up to 24 hours at ambient temperature before component preparation: Implementation in the Netherlands. Transfusion 2011;51 Suppl 1:3S-6.
11. Holme S, Moroff G, Whitley P, Hallinen D, Heaton A. Properties of platelet concentrates prepared after extended whole blood holding time. Transfusion 1989;29:689-92.
12. Dijkstra-Tiekstra MJ, van der Meer PF, Cardigan R, Devine D, Prowse C, Sandgren P, *et al.* Platelet concentrates from fresh or overnight - stored blood, an international study. Transfusion 2011;51 Suppl 1:38S-44.
13. Rácz Z, Baróti C. Storage of platelet concentrates from overnight-stored blood and overnight-stored buffy coat: *In vitro* studies. Vox Sang 1995;68(3):160-3.
14. Tynngård N. Preparation, storage and quality control of platelet concentrates. Transfus Apher Sci 2009;41(2):97-104.
15. Sanz C, Pereira A, Faúndez AI, Ordinas A. Prolonged holding of whole blood at 22 degrees C does not increase activation in platelet concentrates. Vox Sang 1997;72(4):225-8.