Impact of Different Preparation Methods on the In Vitro Quality of 8 Days Storage Platelet Concentrates

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Abstract: Background: Adequate prolongation of platelet (PLT) shelf life can achieve improved availability, logistical management and decreased wastage. The coupling of reliable methods of bacterial detection and optimum methods of platelet preparation can preserve the quality of platelets with extended storage. **Objectives:** This study aimed at evaluating the applicability of extending platelet shelf life up to 8 days, using different methods of platelet preparations. **Subjects and methods:** Thirty six platelet concentrates (PCs) were collected and divided into 3 equal groups, according to preparation procedure: Group (1): Non-leucofiltered random-donor PLTs (RDPs); Group (2): Leucofiltered RDPs; and Group (3): Single-donor aphaeresis PCs. All units were stored at 22-24°C on a flatbed agitator for 8 days. PLT characteristics and metabolic variables, CD62p and CD63 expression and RANTES levels, were assessed on days 1, 5 and 8 of storage. Besides, automated bacterial screening was performed on days 1 and 8 using BACTEC blood culture system with aerobic medium. **Results:** Until the end of shelf life, the mean PLT recovery, mean PLT volume (MPV), PLT distribution width (PDW), swirling scores, glucose and lactate dehydrogenase (LDH) levels showed best suitable values among the aphaeresis units, compared to the other 2 groups. pH was maintained > 6.8 in all groups. Also, the lowest expression of CD62p and CD63 was found among group 3, on day 8, compared to the other groups. However, RANTES results showed highly significant lower levels in groups 2 and 3 compared to group 1 on all days. No bacteriological growth was detected in all PC units, till day 8 of storage. **Conclusion:** Aphaeresis units could provide the highest quality with 8 days storage, particularly when assisted by a good and rapid bacterial detection system. Thus, the choice between different methods of preparing PCs with extended shelf life should depend on a critical balance between safety, quality and cost.

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1. Introduction

Platelet transfusion is often considered a life-saving measure, being essential for the prevention and treatment of bleeding in patients who have quantitative and/or functional platelet disorders [(1)]. Nowadays, in many western countries, the demand for platelet concentrates (PCs) is obviously growing, almost up to 80% increase, compared to a decline in the use of packed red blood cells [(2)].

The development of potentially curable chemo/radiotherapeutic regimens, which lead to prolonged periods of severe myelosuppression and which in turn placed a considerable pressure on the logistics of platelets supply, necessitated intensive research into the biology of platelets, methods and devices needed for their collection and storage, and platelet transfusion practices [(3, 4)].

Studies conducted with PCs revealed that these cells lose their viability very quickly during the storage period, implying the need for continuous renewal of stock [(5, 6)]. So, in order to reduce the bulk of outdated and wasted products, many studies have been directed towards extending the platelet storage time for more than 5 days and assessing their acceptability in vivo performance. If this can be successfully achieved, it will confer additional advantage and flexibility to blood banks and transfusion services [(7)].

In fact, PLTs undergo a number of operations during collection, processing and storage that adversely affect their structure, resulting in reduced post-transfusion recovery and functionality. The aim of this study is to evaluate the applicability of extending platelet shelf life up to 8 days using different standard methods of platelet preparation namely, whole blood derived-PCs (WBD-PCs) either filtered or non-filtered and aphaeresis PCs and comparing their in vitro viability during the extended storage period. This will be an important step in transfusion services if platelets can be rendered available in a timely manner while diminishing the wastage of time-expired platelets.

2. Materials and Methods:

Platelet collection and storage:

Thirty-six PC units freshly collected in triple blood bags containing 63 ml of CPDA-1 anticoagulant, (***JMS Singapore Pte Ltd***), using blood collection monitor **HemoMatic(TM),** from healthy

blood donors who visited the blood bank of Theodor Bilharz Research Institute, were enrolled in this study. They were classified equally into 3 groups according to the preparation method applied: (a) Group 1, included 12 non-filtered random donor PCs (RDPs) derived from whole blood by platelet-rich plasma (PRP) method, (b) Group 2, as well, included 12 random donor PCs, yet leucofiltered using pre-storage platelet leucoreduction filter (**Fresenius Kabi BIO P Plus Filter**) and (c) Group 3 in which single donor PCs were prepared from the remaining 12 donors using aphaeresis device (**COBE Spectra ™v. 7.0 LRS Turbo**).

The study was approved by the local ethics board (Institutional Board Review) and an informed consent was obtained from all subjects.

Following preparation, PCs were left for 1 hour without agitation at room temperature for resting highly activated platelets during preparation. Subsequently, all the 36 units were kept on a flatbed platelet agitator **(Helmer, Inc, USA)** then stored with continuous gentle agitation to prevent clumping and facilitate gas exchange at 22-24°C for a total of 8 days.

Analysis of PLT characteristics and metabolic variables during storage:

Samples were drawn aseptically under a laminar flow hood from all units at days 1, 5 and 8 of storage. For each sample, PLT count, and indices (MPV, PDW), together with the white blood cell (WBC) count of the non-filtered units were estimated using the automated cell counter (**Beckman Coulter Act Diff III**). However, residual WBCs count in leucoreduced PCs either the leucofiltered RD-PLTs or aphaeresis units, was assessed by flowcytometric enumeration using (**BD Leucocount™ kit, Flowcytometer Epics®Elite "Coulter" system**) [(8)].

Swirling phenomenon was evaluated by examining the gently rotated PC units against the light. The normal discoid platelets refract light and produce swirling pattern, which can be identified and scored (0-3) by visual inspection of trained personnel in blood bank [(9)].

pH of all samples was assessed immediately after sampling at a temperature of 22°C using hand-held pH meter (**HANNA Instruments HI 98103 Checker pH Tester, Italy**). Glucose and LDH enzyme as indicators of platelet metabolism were determined according to the standard methods by using the semi-automated, single-beam filter photometer (**RIELE 5010**).

To detect platelet activation markers, PRP was separated and freshly tested for CD62p and CD63 expression using fluorescein isothiocyanate conjugated (FITC) monoclonal antibodies (moAbs), Mouse Anti-Human CD62p and CD63 antibodies (**BD Biosciences.Com, Pharmingen™**). A non-specific Isotype Control was used with each sample. All antibodies were of the **IgG1κ Isotype** and **Flowcytometer Epics®Elite "Coulter"** system was used for the analysis [(10)]. Results were expressed as specific CD62p and CD63 percentage of positive platelets, calculated by subtracting the nonspecific fluorescence of the isotype control from the specific fluorescence of the moAbs. For subtraction, the manufacturer's software was used.

Platelet poor plasma (PPP) samples were also separated and stored frozen at -70°C for testing of the platelet derived cytokine, regulated on activation normal T expressed and secreted (**RANTES; CC chemokine ligand 5**) using enzyme-linked immunosorbent assay (ELIZA) (**Quantikine**) [(11)].

Bacteriological screening of PC units:

The study had a standardized testing protocol that used aerobic culture bottles (**BACTEC Plus Aerobic/F bottles**) inoculated with 6ml of PLT samples, and **BACTEC 9050 System (BD Microbiology, Cockeysville, MD)**. These cultures were carried out for each PC unit on day 1 (24 hours post collection) and day 8. Continuous monitoring blood culture system in the incubator (37°C) for 8 days after inoculation was performed for the detection on bacterial contaminants in PLT preparations. Even though, an automated system was used, the cultures were also controlled visually for signs of growth, cloudiness or a color change and gas bubbles or clumps of bacteria, in the broth.

Statistical methods:

Results were expressed as mean ± standard deviation (SD) or number (%). Comparison between the mean values at different dates within the same group was performed using paired t-test. Comparison between the mean values of different parameters between the different groups were performed using one way analysis of variance (ANOVA) of the mean percent change of each parameter with post hoc using the least significant difference. Correlation between parameters was performed using Spearman's rank correlation coefficient. SPSS computer program (version 18 windows) was used for data analysis. P-value ≤ 0.05 was considered significant and p-value <0.01 was considered highly significant. The percent change of each parameter was calculated by subtracting the baseline (day 1) result from the final result (day 8), then dividing the result of this subtraction by the baseline result, and finally multiplying by 100.

3. Results:

The mean volume of aphaeresis units on day 1 was about 228.33 ± 20.74 ml, which was obviously

much higher than the other 2 groups (non filtered and filtered PRP-PC, 59.58 ± 8.39 and 61.00 ± 8.19, respectively). However, the volume of all units was decreased gradually during storage due to the sampling (10 ml) each time, on days 1, 5 and 8, in order to monitor the studied parameters during storage period.

Table (1): Storage changes as regards; PLT count, indices, residual WBC counts, swirling and metabolic parameters at different studied storage dates.

	Day 1	Day 5	Day 8
PLT Count ($x10^{10}$/unit)			
Non-filtered PRP	6.30 ± 1.43	4.98 ± 0.96^{aa}	4.03 ± 0.72$^{aa\ bb}$
Filtered PRP	5.05 ± 0.88	4.38 ± 0.59^{aa}	4.23 ± 0.73^{aa}
Apharesis PC	50.83 ± 6.55	48.33 ± 6.51^{aa}	47.42 ± 7.69^{a}
MPV (fl)			
Non-filtered PRP	5.50 ± 0.86	6.85 ± 0.68^{aa}	7.65 ± 0.97$^{aa\ bb}$
Filtered PRP	6.18 ± 0.65	6.81 ± 0.64^{aa}	7.57 ± 0.86$^{aa\ bb}$
Apharesis PC	6.81 ± 0.84	7.56 ± 0.87^{aa}	7.94 ± 0.79$^{aa\ bb}$
PDW (fl)			
Non-filtered PRP	19.50 ± 0.85**	20.35 ± 0.97^{aa}	20.97 ± 0.63$^{aa\ b}$
Filtered PRP	19.23 ± 0.90**	20.35 ± 0.97^{aa}	20.69 ± 0.64^{aa}
Apharesis PC	18.77 ± 0.79**	19.66 ± 0.68^{aa}	20.04 ± 0.50$^{aa\ b}$
WBCs ($x10^{6}$/Unit)			
Non-filtered PRP	185.50±67.44	144.43±49.51^{aa}	113.87±39.66$^{aa\ bb}$
Filtered PRP	0.67±0.41	0.57±0.37^{aa}	0.43±0.31$^{aa\ bb}$
Apharesis PC	0.44±0.19	0.28±0.13^{aa}	0.17±0.07$^{aa\ bb}$
pH			
Non-filtered PRP	7.39 ± 0.15	7.38 ± 0.11	7.17 ± 0.20$^{aa\ bb}$
Filtered PRP	7.27 ± 0.33	7.18 ± 0.29	6.95 ± 0.29$^{aa\ bb}$
Apharesis PC	7.33 ± 0.14	7.26 ± 0.17	7.13 ± 0.17^{aa}
Glucose (mg/dl)			
Non-filtered PRP	405.17 ± 3.69	392.33 ± 7.69^{aa}	361.25 ± 26.79$^{aa\ bb}$
Filtered PRP	409.42 ± 32.40	399.67 ± 44.68	379.92 ± 54.62
Apharesis PC	344.53 ± 29.93	299.67 ± 39.07^{aa}	248.08 ± 45.02$^{aa\ bb}$
LDH (U/L)			
Non-filtered PRP	287.47 ± 35.34	316.18 ± 61.34	346.69 ± 78.82$^{a\ b}$
Filtered PRP	387.23 ± 85.47	419.90 ± 95.13	442.15 ± 108.45^{a}
Apharesis PC	171.69 ± 61.42	179.31 ± 58.58	188.35 ± 68.58^{a}
Swirling score			
Non-filtered PRP	3.00 ± 0.00	2.83 ± 0.39	2.42 ± 0.67$^{a\ b}$
Filtered PRP	3.00 ± 0.00	2.08 ± 0.29^{aa}	1.50 ± 0.52$^{aa\ bb}$
Apharesis PC	3.00 ± 0.00	3.00 ± 0.00	2.92 ± 0.29

$^{a}p \le 0.05$; $^{aa}\ p < 0.01$ (relative to day 1).
$^{b}p \le 0.05$; $^{bb}\ p < 0.01$ (relative to day 5).
^{a}, ^{b} = Significant difference. ^{aa}, ^{bb} = Highly significant difference

Table (2): Mean CD62p and CD63 platelet surface expression and RANTES levels among donors and at different studied storage dates.

	Donor	Day 1	Day 5	Day 8
CD62p (%)				
Non-filtered PRP	3.36 ± 2.86	24.55 ± 9.65**	37.78 ± 12.45^{**aa}	51.38 ± 12.47^{**aabb}
Filtered PRP	3.73 ± 1.76	33.96 ± 12.49**	43.78 ± 9.50^{**aa}	46.73 ± 13.16^{**aa}
Apharesis PC	2.54 ± 1.08	24.75 ± 6.09**	27.57 ± 8.63^{**a}	29.58 ± 7.83^{**aa}
CD63 (%)				
Non-filtered PRP	6.08 ± 3.53	9.82 ± 4.83**	15.01 ± 6.97^{**aa}	24.64 ± 8.69^{**aa}
Filtered PRP	4.97 ± 2.59	15.38 ± 12.33**	21.10 ± 15.01^{**a}	25.10 ± 15.63^{**aa}
Apharesis PC	6.81 ± 2.61	13.47 ± 3.87**	13.73 ± 3.81**	15.30 ± 4.69^{**ab}
RANTES (ng/ml)				
Non-filtered PRP	1.60 ± 20.50	196.8 ± 48.88**	241.4 ± 53.32^{**aa}	314.73 ± 100.86^{**aabb}
Filtered PRP	1.20 ± 0.45	96.59 ± 42.23**	117.9 ± 49.73^{**aa}	168.54 ± 58.85^{**aabb}
Apharesis PC	1.73 ± 0.41	11.88 ± 1.98**	130.2 ± 60.43^{**aa}	205.92 ± 44.02^{**aabb}

$^{*}p \le 0.05$; $^{**}p < 0.01$ (relative to donor). $^{a}p \le 0.05$; $^{aa}\ p < 0.01$ (relative to day 1).
$^{b}p \le 0.05$; $^{bb}\ p < 0.01$ (relative to day 5). ^{a}, ^{b}, * = Significant difference. ^{aa}, ^{bb}, * *= Highly significant difference

Table (3): Mean percent change of evaluated parameters between different studied groups.

	Non-filtered PRP-PCs (n= 12)	Filtered PRP-PCs (n=12)	Aphaeresis PCs (n=12)	F- value	p-value
Platelet count	-35.980	-16.34 [aa]	-11.150 [aa]	12.590	0.000
Swirling	-19.440	-50.000 [aa]	-2.780 [a bb]	23.140	0.000
MPV	39.090	22.370 [aa]	16.590 [aa]	9.532	0.001
PDW	7.520	7.630	6.790	0.199	0.820
Ph	-3.020	-4.470	-4.120	1.276	0.292
LDH	20.600	20.720	17.710	0.173	0.842
Glucose	-10.840	-7.210	-27.990 [aa bb]	12.621	0.000
Residual	-38.620	-35.720	-62.050 [aa b]	5.259	0.010
RANTES	59.860	74.490	1995.200 [aa bb]	122.821	0.000
CD62	109.300	37.600 [aa]	48.120 [aa]	7.774	0.002
CD63	151.020	63.230 [a]	27.830 [aa]	8.585	0.001

Data are expressed as mean percent change [a] p< 0.05; [aa] p< 0.01 relative to non-filtered PRP.
[b] p< 0.05; [bb] p< 0.01 relative to filtered PRP
[a], [b] = Significant difference. [aa], [bb] = Highly significant difference

Table (4): Correlation between WBCs counts /Unit versus PLT count, swirling, MPV, RANTES, CD62 and CD63 in different platelet products on day 8 storage.

	Non Filtered PC		Filtered PC		Apharesis PC	
	R	P value	r	P value	r	P value
Plt conc.	-0.634	0.027*	-0.601	0.039*	0.375	0.230 [NS]
Swirling	0.198	0.536 [NS]	-0.411	0.185 [NS]	0.322	0.307 [NS]
MPV	0.611	0.035*	0.442	0.150 [NS]	0.705	0.010*
RANTES	0.258	0.418 [NS]	0.196	0.542 [NS]	0.050	0.878 [NS]
CD62	0.111	0.731 [NS]	0.239	0.454 [NS]	0.683	0.014*
CD63	0.237	0.458 [NS]	0.702	0.011*	0.095	0.769 [NS]

r= correlation coefficient. p= p value. NS= Not significant (p> 0.05). # = Correlation was invalid.
*= Correlation is significant at the 0.05 level (2-tailed) (Significant correlation).

Table (5): Correlation between pH versus Platelet count, swirling, MPV, LDH, Glucose, CD62, CD63 in different platelet products on day 8 storage.

	Non Filtered PC		Filtered PC		Apharesis PC	
	r	*P value*	*r*	*P value*	*r*	*P value*
Plt conc	0.254	0.426 [NS]	-0.201	0.530 [NS]	0.123	0.704 [NS]
Swirling	-0.407	0.189 [NS]	#	#	0.251	0.430 [NS]
MPV	-0.582	0.047*	0.103	0.750 [NS]	-0.511	0.090 [NS]
LDH	-0.355	0.258 [NS]	-0.182	0.570 [NS]	-0.400	0.198 [NS]
Glucose	-0.253	0.427 [NS]	-0.106	0.743 [NS]	-0.414	0.181 [NS]
CD62	-0.082	0.801 [NS]	-0.145	0.654 [NS]	-0.670	0.017*
CD63	-0.195	0.544 [NS]	-0.480	0.114 [NS]	-0.285	0.368 [NS]

r= correlation coefficient. p= p value. NS= Not significant (p> 0.05). # = Invalid correlation.
*= Correlation is significant at the 0.05 level (2-tailed) (Significant correlation).

In **Table (1),** intergroup comparison revealed highly significant difference regarding mean PLT count/unit among the aphaeresis group compared to the other 2 groups (p<0.01) during all studied storage times. Meanwhile, group 2 showed significant lower PLT count than group 1 only on days 1 and 5 (p<0.01 and p<0.05, respectively). As regards the PLT indices, PDW was significantly lower among group 3 compared to the first 2 groups on all storage days (p<0.01) and among group 2 compared to group 1 on day 1 (p<0.01). On the contrary, the MPV was significantly high among group 3 in comparison to group 1 on day 1 (p<0.01) and in comparison to group 2 on day 5 (p<0.05).

Mean WBC counts were significantly low among both leucoreduced groups (groups 2 and 3), in relation to group 1 on all studied storage times (p< 0.01) and also among group 3 in relation to group 2 on days 5 and 8 (p<0.05 and p<0.01, respectively).

The metabolic characteristics of the studied PCs during storage revealed that, the pH level was maintained above > 6.8, with no significant difference between all groups all over the storage period. However, glucose and LDH results showed significantly lower levels among the aphaeresis group

compared to the other groups ($p < 0.01$) on all storage days. Also, significant high LDH levels were found among group 2 compared to group 1 on days 1, 5 ($p< 0.01$) and 8 ($p< 0.05$).

Swirling scoring showed a non-significant difference between all groups on day 1, however, a highly significant lower score was reported in group 2 compared to the others groups on day 5 ($p< 0.01$). On day 8, there was a significant high score in aphaeresis group compared to the first group and there was a significant lower score in group 2 compared to groups 1 and 3 ($p< 0.01$).

Regarding PLT activation markers illustrated in **table (2)**, intergroup comparison revealed significantly lower CD62p expression among the aphaeresis group compared to group 2 on days 1, 5 and 8 ($p< 0.05$, $p<0.01$ and $p<0.01$, respectively) and compared to group 1, only on day 8 ($p<0.01$). However, CD63 showed only significantly lower expression among the aphaeresis group compared to group 1 on day 8 ($p<0.01$). In addition, analysis of the results revealed significantly lower RANTES levels among the aphaeresis group compared to group 1 on all days and compared to group 2 on days 1 and 8 only ($p<0.01$).

Mean percent change of evaluated parameters among the 3 studied groups were shown in **table (3).** Correlation studies between WBC count versus PLT count, swirling, MPV, RANTES, CD62 and CD63 in different platelet products on day 8 of storage were illustrated in **table (4),** while between pH versus PLT count, swirling, MPV, LDH, Glucose, CD62, CD63 in different platelet products on day 8 of storage were shown in **table (5).**

No bacteriological growth was observed in all units within the studied groups neither in cultures performed on day 1 nor on day 8 which is actually considered as confirmatory culture to day 1.

4. Discussion:

It has been reported that three fundamental quality standard parameters, namely PLT counts, PLT activation and metabolic alterations, must be considered for a proper evaluation of the effect of prolonging PLT shelf-life [12].

The significant reduction in PLT counts at the studied time-points of storage, in the 3 groups, indicates an increase in platelet elimination with storage, which could be attributed to platelet senescence, as the platelets' life span is 7-10 days [13]. Fortunately, despite this decline in PLT counts in all groups, only the studied aphaeresis units till day 8, were fulfilling the quality criteria of aphaeresis units ($>24.0 \times 10^{10}$/unit) as mentioned by **Vasconcelos et al.** [14] **.**

Concurrently, on performing the intergroup comparison, we have estimated the mean percent change of studied parameters, assuming that it would eliminate the changeable irrelevant factors such as, variability of donor's criteria, baseline levels and discrepancy in units' volume among the studied groups. The observed higher mean percent reduction of PLT count in the non-filtered compared to other groups could be referred to their much higher leucocytic counts which in turn showed highly significant reduction particularly on day 8 compared to day 1. It seems likely, that lyses of WBCs probably resulted in release of their cytokines and proteolytic enzymes that affect platelet viability.

This is in accordance with the findings of ***Kaufman*** and his colleagues [15] who noticed that the quality of stored platelets could be improved by leucoreduction. It seems likely that stored platelets are exposed to proteolysis by enzymes released from leucocytes and from activated platelet themselves, such as metalloproteases [16].

Nevertheless, on comparing both leucoreduced groups (filtered WBD-PCs versus aphaeresis PCs), it has been noticed that filtration had a negative effect on platelet yield. Herein, the mean platelet count had dropped from $7.55 \pm 0.65 \times 10^{10}$/unit before filtration to $5.05 \pm 0.88 \times 10^{10}$/unit after filtration (i.e. presenting $66.2 \pm 9.8\%$ of the pre-filtration value). On the other hand, in the aphaeresis PCs, the leucoreduction was performed automatically during the collection and had no effect on platelet yield and was completely dependent on the previously programmed centrifugal separation of the aphaeresis device protocol.

PLT indices namely MPV and PDW, evaluated in conjunction with PLT counts, constitute further indicative parameters in assessment of the PC quality [12]**.** In our study, as part of PLT count analysis, MPV and PDW were recorded and showed significant increase on comparing days 5 and 8 versus day 1. These findings were the same in all groups, denoting that the effect of storage under the blood bank conditions was constant for all studied units. These changes in MPV and PDW values during storage were accounted for by the gradual change in platelet shape from discoid to a spherical shape [14]**.** Similarly, platelets derived from whole blood and aphaeresis procedures come in contact with various artificial surfaces that may promote changes in membrane lipids aggregation, microvesiculation and contact activation during collection, processing and storage [17]**.**

Derived data demonstrated that the aphaeresis group showed the least mean percent increase in MPV followed by the filtered group. It has to be mentioned that a mixture of small and large

platelets may give a normal MPV but a high PDW, this being indicative of active platelet release and consequent unsuitability of the product. Taken together MPV and PDW can thus provide a more reliable description of the platelet volume distribution than if MPV is considered alone.

The pH measurement is considered a global indicator of the platelet environment, demonstrating the balance between platelet metabolism, bacterial contamination if present and the buffer capacity of the medium, with an acceptable range of 6.4 – 7.4 at 22°C in Europe and > 6.2 in USA in order to retain platelet function **[18].**

The current study recorded a significant decrease in pH level in both non-filtered and filtered-PRP-PCs, on day 8 versus days 1 and 5, while for the aphaeresis group, on day 8 versus day 1 only. This decrease in pH, which was within the acceptable range, could be attributed to the production of lactic acid and carbon dioxide by platelet metabolism during storage. However, the difference in pH levels between all 3 groups was insignificant even at all studied days of storage and the mean percent decrease in pH as well, was insignificantly different between them. This limitation in pH decline could be explained by the absence of bacterial contamination as demonstrated by negative culture in all studied PCs and by the fact that the quality of the storage containers allowed proper exchange of oxygen and CO_2 between the outside air and the suspended platelets **[19]**.

In accordance to the prior study done by **Singh** and his colleagues in **2009 [20],** the higher leucocyte contamination in the non-filtered PCs group included in our work, resulted in significant glucose consumption over time and consequently its concentration showed significant decrease on day 8 versus days 1 and 5. However, on comparing the 3 PCs groups, the aphaeresis PCs showed the lowest mean glucose concentration and within this same group, its mean concentration showed significant decrease on days 5 and 8 versus day 1 and in day 8 versus day 1. This gradual drop in glucose concentration could be attributed to its high cellular platelet compartment, which entails relatively higher glucose consumption during metabolism over the storage time. On the other hand and in concomitance with a prior report **[21]**, the glucose concentration showed insignificant drop among the filtered PCs group all over the eight days. This is possibly due to the lower platelet count in comparison with the aphaeresis group, and lower white blood cell count in comparison with the non-filtered PCs group.

Intergroup comparison of the mean LDH level revealed highly significant lower results among the aphaeresis PCs compared to the other 2 groups, meanwhile significant higher levels among the filtered-PRP-PCs compared to the non-filtered. These findings may be attributed to the process of filtration resulting in subsequent platelet damage and evidenced by lower post-filtration platelet yield. Measurement of LDH helps in evaluating the extent of cell damage for both platelets and leucocytes. Nevertheless, it has to be mentioned that the LDH cannot be considered an indicative marker of platelet status in the case of non-filtered-PRP-PCs, as the contaminant leucocytes have an effect, which cannot be neglected, and definitely contribute to remarkable LDH increase **[22]**.

Aphaeresis collected PCs included in this study, showed the best swirling score and the lowest mean percent change decrease during the storage period, in contrast with the single filtered-PRP-PCs, which showed the highest mean percent decline. However, it has to be mentioned that swirling phenomena in all groups were within the acceptable range till day 8 of storage. From these findings, we deduce that aphaeresis process had minimal effect on platelet viability, whereas, the filtration process had a negative impact on platelet viability; but it is yet to be confirmed whether it is reversible or irreversible in vivo.

It has to be mentioned that, loss of swirling is associated with major pH derangement, poor morphology and loss of platelet viability; it may also be considered a gross measure of apoptosis in the vast majority of platelets in PCs. Accordingly, it is a reliable index of subsequent poor platelet survival and function. However, it may be very sensitive when the irreversible damage affects fewer numbers of platelets in PCs **[9].**

It has been demonstrated that, the extent of platelet activation depends mainly on methods of collection, processing and to a lesser extent on the duration of storage and the storage medium of PCs **[23].** Upon activation, granule membrane proteins such as CD62p and CD63 are expressed on the external membrane of the platelet **[24].** Our findings revealed a significantly lower CD62p expression among the aphaeresis PCs, most probably, because those aphaeresis units were collected, separated and leucoreduced with less handling procedures than the whole blood derived PCs. Also, on day 8 there was a highly significantly lower CD63 expression among the same aphaeresis PCs, which was consistent with the findings reported by **Vassallo** and **Murphy [25]**.

The current findings appear to be compatible with previous in vivo studies which have observed superior radiolabel recovery and post-transfusion increments for platelets derived from aphaeresis compared with platelet-rich plasma whole blood-derived platelets **[26, 25].** Moreover, CD62 expression

has shown to be inversely correlated with the platelet count increment and recovery of platelets, so it may serve as a useful quality control measurement [27].

As regards the platelet derived cytokine (RANTES) and in accordance with prior studies [28, 29], analysis of the results revealed highly significant low levels among the leucoreduced PCs compared to the non-filtered-PRP PCs at all studied storage days, together with a gradual significant increase in its levels over time among all collected PC units. It has been suggested in previous studies that under normal storage conditions mononuclear cells in PCs in particular monocytes, which are a major constituent of the leucocyte population, have the ability to synthesize and secrete cytokines including RANTES for at least 5 days [30]. More and above, it has been noticed that the substantial accumulation of storage time dependent platelet-derived bioactive substances takes place in all PCs, presumably as a consequence of platelet activation or disintegration [31].

Data derived from this study revealed that the 36 enrolled PCs, showed negative results for bacterial culture on the 1st and 8th day of storage. In fact, culture testing on day 8 can be considered as confirmatory to that of day 1, added to the acceptable limited metabolic biomarker changes, namely pH and glucose, and the maintained swirling pattern in all units. These findings support the concluded facts of 2 previous studies concerning sensitivity and rapidity of BACTEC system which supported the feasibility of its performance for bacterial testing in PCs [32, 33].

To sum up, during the 8 days storage period, aphaeresis collected PC units were superior to the whole blood derived platelets whether filtered or non-filtered as evidenced by: highest platelet count per unit, better viability of platelets with highest swirling score, least metabolic changes of the plasma media, and least expression of platelet activation markers namely CD62p and CD63. Taken in consideration, that PLT counts in both WBD-PCs groups were not fulfilling the standard quality criteria at the end of 8 days storage. However, despite the definite superiority in quality, aphaeresis units were costly. The procedure of donation using the aphaeresis device is safe, yet we found difficulties in recruitment of voluntary thrombocytaphaeresis donor as the time needed to complete the donation was long, and the dual needle technique adds to the donor fears of the process of donation.

Our choice between different methods of preparing PCs should actually depend on a critical balance between safety, quality and cost. To minimize outdating of PCs that are licensed now for a maximum of 5 days, we can conclude that PCs obtained by aphaeresis could provide the highest quality possible, when coupled with a good and rapid bacterial detection system to assure the sterility of PCs, kept at 22 – 24°C for 8 days. Yet, studies in therapeutic efficacy in PLT products should be made to promote appropriate transfusion practice.

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