



## EUROPEAN EXPERIENCE WITH EXTENDED STORAGE OF PLATELET POOLS

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### Background

Separation of whole blood into plasma, a buffy coat and red cells following hard spin centrifugation have been practised in Europe since the late 1960ties. The buffy coat is used as a source for platelet concentrate preparation, first from single donor units, later from buffy coat pools. Pooling was first introduced in Scandinavia by Högman and colleagues, who applied platelet additive solution (PAS) to save plasma.

In The Netherlands storage of buffy coat platelets was improved in the mid 1980ties by the introduction of 4-bag systems. In these systems it became possible to prepare a platelet concentrate from a buffy coat in a closed system [1]. The platelet concentrates were for that time (1986) leukocyte-poor, i.e.  $< 5 \times 10^7$  residual leukocytes and could be stored for 5 days at 20-24°C. Later, discussions about the residual number of leukocytes made us decide to fix the number of residual leukocytes to  $< 5 \times 10^6$ . This number could only reliably be achieved when a leuko-reduction filter was used. Due to the high cost of the filters, the availability of the sterile connection device and the availability of large platelet storage bags with good gas permeability routine manufacturing of leuko-reduced platelet pools from buffy coats was implemented in 1995 [2]. Storage time of platelets in plasma being 7 days.

In 1998 Sanquin the national foundation for the blood supply has been founded in The Netherlands consisting of 9 blood bank divisions and a division plasma products, division research and division diagnostics from the former CLB. In 2002 further merging led to 4 blood bank divisions. Sanquin develops national guidelines amongst others a guideline with specifications for blood components 6<sup>th</sup> edition January 2003. Important Sanquin decisions for the blood components were:

- ?? Bacterial screening of all platelet concentrates: derived from buffy coat pools and from apheresis as of November 2001.
- ?? Universal leuko-reduction of cellular blood components as of January 2002; residual number of leukocytes  $< 1 \times 10^6$  in 90% of the units to warrant with 95% confidence a residual number of leukocytes below  $5 \times 10^6$ .
- ?? Leuko-reduced plasma (donor re-tested) to be collected as of July 2002, to be distributed to hospitals as of April 2003.

### Methods

#### *Method of Preparation of leuko-reduced platelet pools (LR-PP)*

Blood is collected in Bottom and Top quadruple bag systems with an integrated leuko-reduction filter, CPD as anticoagulant and SAGM as additive solution for the red cells. Whole blood 500 mL  $\pm$  10% is collected on mixers, checking flow and collection time. For platelet preparation a maximum collection time of 12 min is allowed. The whole blood units are immediately cooled with butane-1,4-diol cooling plates to  $\pm 22^\circ\text{C}$  and subsequently stored at ambient temperature for a minimum of 4 hours or overnight with a storage time of 12 to 22 hours [3]. Next the whole blood is centrifuged for  $\pm 30,000$  gmin at 20-24°C. Separation into plasma, buffy coat and red cells is done with automated equipment: Compomat™ (Fresenius Hemocare) [4]. The red cells suspended in SAGM are subsequently filtered, within 24 hours of collection.

The separation automates are programmed to deliver buffy coats of 50 mL, with a hematocrit of 40%. Following separation the buffy coats should rest for at least 2 h.

For the platelet preparation 5 ABO identical buffy coats and the plasma of one of the donors are selected. For Rh-D neg *all* units should be Rh-D neg, one Rh-D pos makes the pool Rh-D pos.

An integrated set consisting of a pool bag with 6 leads (pig tails), a leuko-reduction filter, a platelet storage bag and a sample bag with a special adapter for culturing the platelets is used. These systems are available from various manufacturers. At the moment we use Terumo.

The buffy coats and the plasma are sterile connected to the leads, next the original donation numbers are linked to a pool number in the computer. The buffy coats are drained by gravity into the pool bag and so is the plasma. The centrifugation is at about 1,000 g, a total of  $\pm 2,200$  gmin. The platelet-rich-supernatant is then transferred through the filter into the platelet storage bag, again using the automated equipment, programmed to do so. The Compomat is programmed to terminate expression when red cells are detected. The content of the filter is drained by gravity. Next a sample ( $\pm 25$  mL) is

taken from the well mixed LR-PP in the integrated sample bag for culturing and QC. The LR-PP are stored up to 7 days on a flat bed shaker in a climat cabinet at 20-24°C.

*Method for Bacterial screening in the BacT/Alert™ (Biomérieux)*

The Sanquin guideline includes the following:

- ?? Culture bottles were inoculated via the special adapter on the sample bag of the LR-PP with aseptic techniques in a laminar air flow cabinet; within 2 h of production (apheresis PC within 12 h of production)
- ?? The anaerobic bottle was first inoculated, then the aerobic bottle; sample tube for QC last.
- ?? Volume was between 5 and 10 mL (in Amsterdam average 10 mL).
- ?? Incubation in the BacT/Alert at 35°C until positive signal or for 7 days.

When a PC had a positive signal:

PC were withdrawn from the inventory as well as the red cell concentrates (RCC) from the same donations; hospitals were informed and request for recall of PC and RCC; if already transfused a request for information about transfusion history was asked for.

Confirmation was performed in a microbiology laboratory of the implicated culture bottles, the sample bag, and the original PC (if available). RCC were cultured in the BacT/Alert, if positive the same routing was followed as for the PC. Only if the RCC was positive, the plasma was recalled and also cultured in the BacT/Alert. Conventional cultures existed of inoculation in broth, on agar plates; determination of the species, an antibiogram and typing if necessary.

**Note:**

a monitor connected to the computer of the BacT/Alert system is placed at the distribution department, prior to issue each PC is checked by scanning the pool number. All PC are issued 'negative to date' (and with swirling effect present). In case a PC becomes positive it is shown on the monitor. Staff is trained according to SOPs.

*Method for Clinical evaluation*

Out patients with hemato-oncological diseases and a platelet count < 20 x 10<sup>9</sup>/L were selected. Exclusion criteria were major bleeding (WHO grade 3 or higher) and refractoriness defined as 2 consecutive non-successful platelet transfusions. Successful transfusions were defined as a CCl<sub>1h</sub>>10 or a CCl<sub>1h</sub> > 7.5.

**Results and Discussion**

*Platelet pool preparation and in vitro data of storage up to 7 days*

As shown in Table1 the QC data of the LR-PP were consistent with earlier results of LR-PP published in 1999 [ref 2 and 4, respectively]. The number of platelets is well above the limit of 250 x 10<sup>9</sup> per pool in >95% of the pools. Anderson et al compared PRP derived pools with buffy coat derived pools and showed a significant higher platelet yield in buffy coat pools.

Table 1  
QC data of routine production of LR-PP in 2001

	unit	n	Mean±SD	Requirement
Volume	mL	10,193	312 ±15	150-400
Platelets	x10 <sup>9</sup>	3,467	364 ±44	>250
Leukocytes	x10 <sup>6</sup>	440	<0.03	<1
Red cells	x10 <sup>9</sup>	95	0.18±0.13	<2
pH (day8)		257	6.95±0.13	6.8-7.4

In our institute various studies have been performed to evaluate storage bag material and size. Van der Meer et al. showed in a paired experiment that there was no significant difference in pH up when 1,3 L PL2410, 1,0 L polyolefin, 1,5 L polyolefin, 1,5 L CLX and 1,0 DnDP-PVC bags were compared, except for the CLX bag [5]. The pH remained well above 6.8 up to 9 days storage except in the CLX bag that showed a quick drop in pH from day 5 on. The swirling pattern frequently associated with disc-like morphology of the platelets, showed the same patters: in the CLX bag swirling deteriorated after day 5.

Another critical point in storage of platelet pools is the platelet concentration. When the storage medium is plasma the concentration may be 1.4 x10<sup>9</sup>/mL to maintain a pH of 6.8 or higher. However, when platelets are stored in additive solution (PAS-II) this pH can only be maintained with a platelet concentration of 1.15 or lower.

In summary there are various studies to support storage of platelet pools up to 7 days provided an appropriate storage container is used. For logistics the expiry of 5 day old platelets averaged 20 to 25%, whereas for 7-day storage a drop of the expiry to below 10% was observed.

#### *Evaluation of one year bacterial screening of PC [personal communication Pietersz + 6]*

Of 8,778 PC 81 were initially positive and 76 (0.9%) confirmed positive. Predominantly skin flora was found. The species cultured were *Propioni species* (49%), *Stapylococcus species* (33%), *Coryne bacterium species* (5%), *Bacillus species* (4%), *Peptostreptococcus* (4%) and *Micro coccus species* (1%). About 50% of the BacT/Alert cultures became positive in the first 24-48 h, whereas the other 50% at day 4-5 (predominantly *Propioni species*).

In conclusion this method of bacterial screening of PC contributes to the safety of blood transfusion and allows extending storage of PC up to 7 days provided the quality of the platelets is warranted.

#### *Clinical evaluation of LR-PP stored for up to 7 days [7]*

From all evaluable transfusions 341/ 349 (98%) showed a successful  $CI_{1h} > 10$ ; and 170/179 (95%) a successful  $CCI_{1h} > 7.5$ . When comparing the number of unsuccessful transfusions of 5 vs 7 day stored platelets there was no difference in  $CI_{1h}$  2/79 for 5 days old vs 2/77 for 7 days old. For the  $CCI_{1h}$  the number of unsuccessful transfusions was the same for both storage times 2/37. Comparison of  $CI_{1h}$  after transfusion on different times to the same patient of 5 or 7-day stored LR-PP revealed a mean SD of  $28 \pm 12$  (n=48) for 5 day old and  $24 \pm 10$  (n=59) for 7 day old. The p value was 0.04 (with a power of 53%). For the  $CCI_{1h}$  there was no difference for 5-day old  $14 \pm 5$  (n=25) and 7-day-old  $12 \pm 4$  (n=31), respectively.

Although the  $CI_{1h}$  and  $CCI_{1h}$  of 5 and 7 days have a tendency to be slightly lower than the values after transfusion of 2-day old platelets, the results are promising, especially because storage up to 7 days does not mean that only 7-day old platelets will be transfused. This study will be continued to obtain more data to draw conclusions.

#### *European experience*

In Europe the LR-PP are used in all Scandinavian countries, Danmark, the UK, Ireland, The Netherlands, Germany, France, Switzerland, Austria, and Spain. Sweden and Norway store for 7 days with bacterial screening, the UK is considering it.

#### References

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