Optimization of blood component preparation

Processing and donor influence

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CHAPTER 3

Critical re-appraisal of blood component quality after overnight hold of whole blood outside current room temperature limits

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Pieter F van der Meer
Dirk de Korte
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ABSTRACT

Background and Objectives
According to European guidelines, the temperature of whole blood (WB) has to be maintained at 20-24°C until processing within 24h, but in blood bank practice, WB is frequently held at temperatures between 18-25°C. We aimed to assess the impact of these small temperature deviations on the quality of the blood components.

Materials and Methods
After rapid cooling, 7 WB units were held overnight at 18°C and 8 units at 25°C, reflecting worst case holding conditions, and separated into a red cell concentrate (RCC), plasma and buffy coat (BC). RCCs were filtered at test temperature and stored for 42 days at 2-6°C. BCs were processed to single-BC platelet concentrates (sPC) and stored up to day 8 at 20-24°C.

Results
After overnight hold at 18°C, 2,3-DPG in WB decreased by 34±9% while at 25°C the decrease was 82±6%. Accordingly, the 2,3-DPG levels in the RCCs in the 25°C group were significantly lower than in the 18°C group (2.2±1.4 vs. 10.4±2.9 µmol/g Hb). RCCs and sPCs in the 25°C group showed higher initial lactate levels and lower pH compared to the 18°C group, but these differences leveled off at the end of storage. RCCs showed small differences in ATP levels and hemolysis. Plasma in both groups showed comparable Factor VIII:C levels.

Conclusion
The temperature of WB during overnight hold strongly affects initial 2,3-DPG levels of RCCs and supports the maintenance of temperature limits between 20-24°C. Other in vitro effects of the temperature deviations were small and of no practical relevance.
INTRODUCTION

Overnight hold of whole blood (WB) at room temperature (RT) until processing within 24 hours after phlebotomy has many advantages, like a better quality and recovery of platelets, possible bacterial removal by leukocytes, an easier logistic process and the possibility to process components during day shifts. The main known disadvantages of the long holding time are a decrease of 2,3-DPG content of red cells and a slight decrease of Factor VIII:C. This subject has been studied extensively and was recently reviewed [1].

An important part of this strategy is active and rapid cooling of the collected WB units to <25°C using butane-diol plates or climate cabinets to ensure the temperature history of WB units. Also, strict temperature control contributes to a better quality of the red cell concentrates (RCC) [2]. According to European guidelines, the temperature of the WB units must be maintained at 20°C - 24°C until the moment of processing [3].

When the WB units arrive at one of our blood centers, they are taken from the butane-diol plates and placed on a desk. Climate control and continuous temperature registration of the processing rooms was introduced to fulfill the requirements for 20°C - 24°C hold. However, in practice, temperatures may be less well controlled, and RT variations from 18°C to 25°C have to be taken into account. Also, temperature excursions may occur during transport, during handling, or if delays in transport occur. Especially during winter or summer, WB temperatures have been suspected to come outside the range of 20°C to 24°C, in spite of using butane-diol plates that have a protective effect against changes in environmental temperature. The question arose, both from a scientific as from a quality assurance point of view, how the in vitro quality of blood components was affected by small deviations (18 to 25°C) from the required temperature limits (20-24°C).

We performed an unpaired study to investigate quality of all blood components – RCC, plasma and platelet concentrates – from WB units that were held at worst case situations of both lowest (18°C) or highest (25°C) room temperature limits, combined with the maximum allowable holding time (22 to 23 h). To differentiate the effects of overnight hold temperature on RCCs, we also performed a small paired study afterwards. On the one hand, we aimed to gain knowledge about the impact of small deviations of the required temperature limits of 20-24°C on the quality of the blood components, and on the other hand we aimed to obtain evidence to support somewhat less restrictive temperature limits.
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MATERIALS AND METHODS

Collection, overnight hold and separation of WB
Fifteen units WB (target 500 mL) were collected after written consent from the donors, in quadruple top-and-bottom systems with an in-line leukoreduction filter (C3941, Fresenius, Emmer-Compascuum, the Netherlands) and with 70 mL citrate-phosphate-dextrose (CPD) as anti-coagulant. The units were cooled for 1:10-2:20 hours using butane-1,4-diol plates (Compocool, Fresenius), which were cooled at the collection site for >24 h at 4°C and warmed to room temperature for at least half an hour before use. Cooling of WB was sufficiently long to lower temperature to <25°C [4]. Immediately after receipt, a sample pouch (P4159, Fresenius) was connected (TSCD II, Terumo, Tokyo, Japan) to the units and a small sample (<10 ml) was collected for in vitro measurements. Subsequently, the units were held overnight for on average 21 hours at 18°C (n=7) or 25°C (n=8) in a temperature-controlled cabinet, while temperature was registered with temperature loggers (CellaLog, Keller, Ibbenbüren-Lagenbeck, Germany). Total WB holding times were 22 to 23 h.

After taking another sample, the WB units were centrifuged at 4,790 x g up to an accumulated centrifugal effect of 9.00x10^7 (Sorvall RC12BP, Thermo Fisher Scientific, Asheville, NC, USA) at test temperature. Units were separated into RCCs, plasmas and buffy coats (BCs) using a semi-automatic device (CompoMat G5, Fresenius) and time between collection and completion of component preparation, including filtration of RCCs, never exceeded 24 h.

Preparation and sampling of components
Red blood cells were suspended in 110 mL saline-adenine-glucose-mannitol (SAGM) additive solution and filtered at test temperature in the temperature-controlled cabinet. The leukoreduced RCCs were sampled aseptically using a swan-lock adapter and syringe on Day 1, and thereafter stored at 2-6°C; sampling was repeated on Day 7, 28, 35 and 42. Plasma units were sampled with a sample pouch connected with a sterile connection device, and thereafter partly used for preparation of platelet concentrates. BCs were processed to single-BC platelet concentrates (sPC) as described in detail by Van der Meer [2]. In brief, 60 g of plasma and the content of the corresponding BC were transferred into a small container (P4206, Fresenius) which was centrifuged at a soft spin (254 x g, 5 min, Sorvall RC12BP). The platelet rich supernatant was expressed manually by using a plasma clamp into a 600-mL PVC-di(2-ethylhexyl) phthalate (DEHP) container (P4204, Fresenius), suitable for platelet storage [5]. The sPCs were
Whole blood temperature and component quality

Aseptically sampled on Day 1 using a swan-lock adapter and syringe, and the units were then stored at 20-24°C on a flat-bed shaker. Sampling was repeated on Day 6 and 8.

To address additional study questions regarding red cell storage, a paired experiment was performed where 7 units WB were collected and sampled, then split into halves and stored overnight at 18°C or 25°C for 22 h. Subsequently, the split WB units were sampled again, and leukocytes and platelets were removed by filtration (PQ31451, Fresenius). After separation into leukoreduced RCCs and plasma (which was discarded), the RCCs were resuspended in 55 mL SAGM, stored at 4°C in a 600 mL container (PVC-DEHP) and sampled on Day 1, 4, 7, 14, 35 and 42.

**In vitro measurements**

The units were weighed to determine the volume, using the specific gravity (1.060 g/mL for WB, RCC and BC; 1.026 g/mL for plasma and sPC). Complete blood counts were determined using a Sysmex XT2000i (TOA, Tokyo, Japan) hematology analyzer. Low level leukocytes in RCCs were counted with the LeucoCount kit (ref 340523, BD Biosciences, San Jose, CA, USA) on a FACSCalibur (BD Biosciences).

Measuring pH at 37°C, blood gases, glucose, lactate and potassium was done using a blood gas analyzer (ABL705, Radiometer, Copenhagen, Denmark). Adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) content in RBC containing samples were determined as described before [2]. To determine hemolysis, free hemoglobin was measured with a Anthos HT3 microtiter plate reader (Day 1-7, Anthos-Labtec, Heerhugowaard, the Netherlands) or a LowHb analyzer (Day 28-42, HemoCue, Angelholm, Sweden), not appropriate for accurate measurement of very low hemoglobin levels.

Morphology of platelets (PLT) was judged according to Kunicki’s method [6], and the swirling effect was scored on a scale from 0 to 3. CD62P expression and annexin V binding were determined as described elsewhere [7,8]. On Day 8 a bacterial culturing test (Bact/Alert, BioMerieux, Boxtel, The Netherlands) was performed if pH$_{37°C}$ was below 6.5, to exclude bacterial contamination as cause of low pH.

Factor FVIII:C (FVIII) was determined both from WB samples and plasma units using the chromogenic method on a coagulation analyzer (Blomek 2000, Beckman Coulter, Fullerton, California, USA). FVIII recovery was calculated as: 100% * FVIII (plasma)/FVIII (WB day 0). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using standard coagulation methods (Sysmex CA-7000, Siemens Healthcare Diagnostics, Breda, the Netherlands) with Innovin and Actin FSL reagents (Dade Behring, Marburg, Germany).
respectively. Activation of the coagulation system was assessed by measuring prothrombin fragment 1+2 (F 1+2) and thrombin-antithrombin (TAT) using standard enzyme linked immunosorbent assays (Enzygnost, Siemens Healthcare) and by measuring Factor XIIa-C1 esterase inhibitor complex with a radio immuno assay as described elsewhere [9].

Statistical analyses
Sample size calculations were not performed because the study character was exploratory, and no special parameter was examined. Grubb’s test for detecting outliers was performed when values were suspected of being an outlier. Differences between the two groups were analyzed using an unpaired t-test or a paired t-test, in case of the additional paired experiment (Microsoft Excel, version 2013). A repeated-measures analysis of variance followed by Dunnett’s post-test (Instat, Version 3.06, GraphPad, San Diego, CA) was performed to analyze differences due to storage effects. A p-value of less than 0.05 was considered significant.

RESULTS

Whole blood
WB units fulfilled European guidelines for composition in both groups (Table 1). Registrations showed that actual temperatures during overnight hold were in accordance with the intended temperature of 18°C (18.1±0.3°C) and only slightly below the intended temperature of 25°C (24.4±0.4°C, mean±SD of 190 measurement time points). During overnight hold, some swelling of red blood cells was observed, with a larger increase of the mean corpuscular volume (MCV) after 25°C compared to 18°C hold, on average 5% versus 2% (p<0.001, Figure 1). The 2,3-DPG content decreased 34±9% in the 18°C group and 82±6% in the 25°C group (p<0.001), while ATP levels in both groups were similar. Both groups in this unpaired study showed a comparable slight decrease in FVIII content of 8% on average (Table 1).

Leukoreduced red cell concentrates
Filtration times of the RCCs were 42±5 min for the 18°C group and 37±5 min for the 25°C group (not significant), and the number of residual leukocytes was similar in both groups (Table 1). However, RCC prepared from 25°C-held WB showed a slightly higher recovery of red blood cells (82±1 versus 79±3%, p<0.05) and a lower number of residual platelets, which reveals a small difference in centrifugation and/or filtration conditions between the groups. Composition of all RCCs fulfilled European guidelines [3].
Table 1: Composition of WB before and after overnight hold at 18°C (n=7) or 25°C (n=8) and subsequently prepared components (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>18°C</th>
<th>25°C</th>
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</thead>
<tbody>
<tr>
<td><strong>WB Day 0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>554 ± 1</td>
<td>553 ± 1</td>
</tr>
<tr>
<td>Hemoglobin, g</td>
<td>67 ± 4</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>Factor VIII:C, IU/mL</td>
<td>0.96 ± 0.16</td>
<td>0.92 ± 0.21</td>
</tr>
<tr>
<td><strong>WB Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>547 ± 1</td>
<td>546 ± 2</td>
</tr>
<tr>
<td>Hemoglobin, g</td>
<td>66 ± 4</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>Factor VIII:C, IU/mL</td>
<td>0.89 ± 0.16</td>
<td>0.90 ± 0.21</td>
</tr>
<tr>
<td>Platelets, x10^9/L</td>
<td>213 ± 30</td>
<td>189 ± 37</td>
</tr>
<tr>
<td><strong>RCC Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>278 ± 10</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Hemoglobin, g</td>
<td>52 ± 4</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>Leukocytes, x10^6</td>
<td>&lt; 0.05</td>
<td>&lt; 0.06</td>
</tr>
<tr>
<td>Platelets, x10^9/L</td>
<td>13 ± 1</td>
<td>5 ± 1*</td>
</tr>
<tr>
<td><strong>Buffy coat Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>47 ± 1</td>
<td>46 ± 1</td>
</tr>
<tr>
<td><strong>Plasma Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>309 ± 10</td>
<td>312 ± 21</td>
</tr>
<tr>
<td>Factor VIII:C, IU/mL</td>
<td>0.87 ± 0.13</td>
<td>0.83 ± 0.25</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Fragment 1+2, pmol/L</td>
<td>156 ± 49</td>
<td>100 ± 32b</td>
</tr>
<tr>
<td>Thrombin-anti-thrombin, µg/L</td>
<td>3.0 ± 0.5</td>
<td>3.7 ± 1.4</td>
</tr>
<tr>
<td>FXIIa-C1 inh, mU/mL</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>PT, s</td>
<td>11.5 ± 0.5</td>
<td>11.8 ± 0.7</td>
</tr>
<tr>
<td>APTT, s</td>
<td>29 ± 2</td>
<td>30 ± 2</td>
</tr>
</tbody>
</table>

*p<0.001, b p<0.05

The RCCs clearly reflected MCV and 2,3-DPG levels of the corresponding WB, while no difference was seen in ATP content in both groups (Figure 1). On Day 7 and thereafter, mean ATP levels were slightly higher in RCCs prepared from 18°C held WB, but the difference was not significant (Figure 1). At the end of our current shelf life (Day 35) all units showed ATP levels >2.7 µmol/g Hb, the target value proposed by Van der Meer and Pietersz [4]. On Day 42, both groups contained one unit with ATP <2.7 µmol/g Hb.

RCCs in the 25°C group showed slightly lower pH values on Day 1 (Figure 1), lower glucose concentrations (18°C: 30.4±0.5 vs 25°C: 28.5±1.2 mM, p<0.01) and higher lactate concentrations reflecting a higher metabolic rate at a higher temperature. These differences leveled off during the 4°C storage period caused by a higher lactate production rate in the 18°C group, which was significantly higher between Day 1 and 7 (18°C: 0.15±0.02 vs 25°C: 0.13±0.02 mmol/10^12 RBC/day, p<0.05). Hemolysis (Figure 1) and potassium leakage (data not shown) in both groups were comparable throughout storage. Hemolysis during storage
remained well below the limit of 0.8% according to European guidelines, except one unit in the
25°C group which showed very high hemolysis (0.97%) on Day 42. This was the same unit as
the one with the low ATP level, and could be a donor-related effect [10].

Plasma
Plasma units in both groups revealed no differences and the FVIII yield was comparable (Table 1,
recovery on average 90% in both groups) and resulted in mean values >0.7 IU/mL, in accordance
with European guidelines [3]. FVIII levels below 0.7 IU/mL were only observed in three units (18°C
group: one of blood group O; 25°C group: one of blood group O, one of blood group B) prepared
from WB containing <0.7 IU/mL FVIII already at the day of collection. Fibrinogen concentration,
clotting times and activation markers of the clotting cascade TAT and FXIIa-C1 inhibitor were
similar in both groups, but slightly higher levels of F 1+2 were detected in the 18°C group (Table 1).
Single-BC platelet concentrates

One of eight sPC prepared from 25°C-held WB was excluded because it contained a very high number of platelets (104x10^9), exceeding the storage capacity of the container (which is around 90 x10^9 platelets). As a result of the high platelet number, several storage parameters of this sPC were marked as outliers. The sPC prepared from 18°C-held WB (n=7) had a volume 59±10 mL and contained 71±12 x10^9/L platelets, sPC prepared from 25°C-held WB (n=7) had a volume 68±2 mL and contained 63±7 x10^9/L platelets. Differences in volume and platelet concentration (1.24±0.29 vs 0.93±0.11 x10^9/mL) were small but significant (p<0.05), while the difference in platelet content was not. Each BC yielded more than 50x10^9 PLT in the sPC, the target number per BC for routine production of platelet concentrates from 5 BC. The sPC were not leukodepleted, and a small difference in residual leukocytes was detected between the groups (18°C: 8±2 x10^6, 25°C: 2±1 x10^6, p<0.001).

On Day 1, sPC in the 25°C group had lower pH values and glucose concentrations and higher lactate concentrations (Figure 2), reflecting the higher metabolic activity of cells stored at higher temperatures. On the other hand, morphology scores on Day 1 in this group were significantly better.

During storage at 22°C, the initial differences in pH, glucose and lactate disappeared, resulting in a comparable glucose consumption (18°C:0.09±0.03 vs 25°C:0.08±0.03 mmol/10^11 PLT/day) and lactate production rate (18°C:0.12±0.04 vs 25°C:0.11±0.03 mmol/10^11 PLT/day) calculated over the entire storage period. Accordingly, bicarbonate levels showed the same trends as pH, and blood gas levels showed no differences between the groups (data not shown). Morphology scores in the 25°C group remained almost the same throughout storage, whereas morphology in the 18°C group increased to the same high scores on Day 6 as the 25°C group but decreased afterwards. Average values of CD62P expression and annexin V binding remained lower in the 25°C group (not significant). On Day 8, all sPC except one (likely due to the high platelet count, this unit was found negative in the BacT/Alert screening) had a pH_{sPC} >6.5 and a swirling score ≥2. In summary, only small differences in composition and storage parameters were seen in sPC prepared from 18°C or 25°C held WB, but the latter showed slightly better in vitro properties.
Paired experiment

Following this unpaired experiment, the question remained whether a significant difference in ATP levels in RCCs could be demonstrated, as suggested from the higher mean values in the 18°C group. Therefore, a paired experiment with 7 split WB units was performed.

Again, similar to the unpaired experiment, a large effect of overnight hold temperature on the 2,3-DPG content of WB was observed, which resulted in a 2,3-DPG decrease of 39±9% and 89±4% in the 18°C and 25°C group, respectively (p<0.001). ATP levels in WB after overnight hold were comparable in both groups (18°C: 4.0±0.9; 25°C: 3.9±0.9 µmol/g Hb).

In the RCCs a substantial increase of ATP was measured on Day 1 in the 25°C group, after finishing preparation by addition of SAGM and sampling (Figure 3). ATP in this group decreased from Day 1 onwards, whereas ATP content in the 18°C group increased to significantly higher levels on Day 14, resulting in higher levels at the end of storage. Therefore,
these additional paired experiments confirmed what was earlier observed in our unpaired study. Using pooled data of both experiments, a good correlation was found between the initial pH of the RCC and the overall decrease of ATP between Day 1 and 42, in favor of the 18°C groups that has a higher initial pH (Figure 3, inset). Metabolic differences were also more pronounced in the paired study: up to Day 14 a significantly lower pH was seen in the 25°C group, and up to Day 35, a lower lactate production rate was observed (18°C: 0.10±0.01 vs 25°C: 0.08±0.01 mmol/day/10¹² RBC, p<0.001). In each group, 6 of the 7 units had a hemolysis that was <0.5% at Day 42, thereby fulfilling guidelines, but one split unit showed high hemolysis when held at 18°C (Day 35:0.65%, Day 42:0.83%) and excessive hemolysis when held at 25°C (Day 35: 1.78%, Day 42: 2.64%). This again probably demonstrates a donor-related effect, with a higher risk for hemolysis in RCCs after holding WB at 25°C.

Figure 3: ATP content in (paired) split WBs before and after overnight hold at 18°C (◊) or 25°C (▲) and in subsequently prepared RCCs stored at 2-6°C (n=7, mean±SD, **p<0.01). Inset: overall decrease of ATP in RCCs during storage for 42 days as a function of initial pH (○, unpaired experiments, 18°C; ◊, paired experiments, 18°C; ■, unpaired experiments, 25°C; ▲, paired experiments, 25°C)
CHAPTER 3

DISCUSSION

This study shows that the overnight ambient hold of WB units at the dual worst case conditions of temperature (18°C or 25°C) and time (22 to 23 hour) resulted in different initial levels of 2,3-DPG in RCCs as the most prominent effect. Other effects on in vitro quality of all components were small. All red cell and plasma units fulfilled European guidelines with respect to composition in both groups. Irrespective of the overnight holding temperature, RCCs could be stored up to 42 days and sPCs up to 7 days with good in vitro quality.

The finding that small temperature deviations of overnight holding temperature results in sufficient in vitro quality of all components, supports our earlier results. A comparison of the in vitro quality of RCCs in the current 2 study groups with those of a formerly prepared reference group [2], strictly held at 19.5-21.5°C for 20.5-22.5 h showed no major differences. The number of contaminating leukocytes and platelets differed slightly, but were all well below the limits of <1x10^6 and <50x10^9/L, respectively, in all groups. This means that possible differences in centrifugation and/or filtration conditions caused by RT variations, as indicated by the different RBC recovery and residual platelets in RCCs between the study groups, are of no concern.

Parameters sensitive for temperature history of the WB units are those reflecting glycolytic rate (pH, glucose, lactate) and metabolites (ATP, 2,3-DPG). As shown in our experiments, the influence of temperature for these parameters was in favor of the RCCs in the 18°C group. This is in agreement with studies without active cooling of WB [2,11]. It is known that overnight hold of WB results in a substantial decrease of 2,3-DPG [12,13] due to lower activity of the enzyme catalyzing synthesis of 2,3-DPG from 1,3-DPG. This enzyme, DPG mutase, remains active at intracellular pH_{22°C} >7.2 [14]. DPG mutase activity gradually decreases as pH decreases, as a result of collection in acidic CPD anti-coagulant and subsequent metabolic activity producing lactic acid. Differences in depletion of 2,3-DPG after overnight hold clearly reflected the effect of holding temperature on this mechanism, causing slow (18°C group), moderate (19.5-21.5°C, reference group [2]) or fast depletion (25°C group). Although 2,3-DPG is rapidly regenerated after transfusion [15], it is of importance in cases where immediate delivery of oxygen is required, like pediatric transfusions. ATP in RCCs is of importance for maintaining in vitro quality and on recovery in vivo [16,17]. ATP on Day 35 and 42 was significantly higher by about 14% in the 18°C group of the paired study (supported by the unpaired experiment, although that difference was not statistically significant due to the low number of tested units), indicating a more favorable starting situation. This observation is
in agreement with the higher ATP levels in RCCs from actively cooled WB [2]. The higher pH on Day 1 may account for the higher glycolytic rate in the 18°C group during one or more weeks of storage at 4°C, presumably due to more activity of the 2,3-DPG mutase. The 4-6 times higher 2,3-DPG levels may account for an extra metabolic source for ATP generation, considering that conversion of 2,3-DPG into lactate takes place downstream in the glycolytic pathway and does not consume but only generates ATP (2 moles ATP per 1 mole 2,3-DPG). Hemolysis in the reference group [2] was comparable with those in both study groups. Caution has to be taken for temperatures of 25°C because of a higher donor-dependent risk for hemolysis, and more studies in this area are needed. In this study a seemingly high frequency of RCCs with donor-related high hemolysis was found (9%: 1/15 in the unpaired and 1/7 in the paired study), but the unit in the unpaired study showed a hemolysis that was marginally too high (>0.8 but <1.0%) and - not unlikely - partly caused by the abnormally high storage temperature of the WB. The high value detected in the paired study is probably a matter of chance, resulting in a high frequency due to the small size of the group. One study that analyzed large national quality control databases revealed a frequency of not more than 2% of leukoreduced RCCs showing high hemolysis [18].

An effect of temperature on cellular and protein content of plasma was not found. A small effect on Factor VIII:C was expected but not found, which is in agreement with a paired study of WB, cooled actively or non-actively from 34°C to 20°C [2], as well as a recently published paired study of plasma held overnight at 22°C or 4°C [19]. Our experiments therefore confirm that FVIII content is not very sensitive to temperature history during overnight hold of WB, and that the decline during overnight hold is simply time-dependent.

The sPC showed small differences in composition and storage parameters. Platelets are sensitive to cold and a negative effect of 18°C hold of WB was expected [20,21]. The most pronounced negative effect was seen in the lower morphology score immediately after preparation. Also, it is likely that the statistically insignificant but higher starting levels of the activation marker CD62P and phosphatidyl serine (as measured with Annexin V), which persisted throughout storage, were also caused by the low holding temperature of the WB. Nonetheless, these differences were small and probably of no clinical consequence. The lower morphology score recuperated once the sPCs were placed on an agitator at 20-24°C.

Regarding RCCs, WB overnight temperatures above 24°C resulted in rapid depletion of 2,3-DPG as a main effect and moreover in suboptimal ATP levels and higher risk of hemolysis. So, raising the upper temperature limit to 25°C as a standard is not recommended.
Regarding sPCs, it is better to prevent WB overnight temperatures beneath 20°C. This study revealed only morphologic changes after 18°C-hold of WB, but Gotschall et al [20] reported lower survival and recovery of platelets after three-day storage at 19.5°C, indicating that significant clinically relevant changes are induced already slightly below 20°C. Therefore, a decision to lower the acceptable temperature limit to 18°C as a standard is also not recommended and effects of temperatures slightly below 20°C in combination with the duration on the quality of platelets have to be studied in more detail.

On the other hand, no dramatic drop in component quality was found when the holding temperature was slightly beyond the current limits during almost a full day, and thus short temperature excursions seem to be tolerated. Hence, for our routine transport, collected blood is placed under butane-1,4-diol plates and transported within a maximum of 4 hours to the processing site at ambient temperature.

In conclusion, a balance needs to be found between RCC and PC quality, and therefore, we recommend avoiding temperatures below 20°C and above 24°C and maintaining current limits to guarantee the best conditions for both platelets and red cells, although small temperature deviations are tolerated.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

IB designed the study, performed the experiments, analysed the results and wrote the article. PvdM and DdK designed the study and wrote the article.

CONFLICT OF INTEREST

There are no conflicts of interest.
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