OPTIMAL PREPARATION OF BLOOD COMPONENTS REQUIRES
AN OVERNIGHT HOLD OF WHOLE BLOOD AT 20°C

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INTRODUCTION

The concept of overnight storage of whole blood at 20°C was introduced by the Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross (CLB) as part of the measures which were needed to use all donations of blood in The Netherlands, both for the production of plasma proteins and for the preparation of red cells and platelets in the routine practice of blood banks (see Fig. 1).

Therefore, this concept was based on the requirements for the optimal storage conditions for platelets, the optimal recovery of plasma factor VIII and the optimal conditions for the preservation of the function and viability of red blood cells.

The overnight hold at 20°C was part of the quality management starting with the selection of donors and ending with the release of specified blood components after blood processing. It will be demonstrated that donor selection, blood collection, transport and holding of donations and blood processing are all favorably influenced by this concept.

DONOR SELECTION

The availability of donors

Self-sufficiency for all blood components is one way to obtain a low incidence of transmission of blood-derived diseases by transfusion; the use of non-paid volunteer donors as the only source of blood is another one. The availability of a sufficient number of volunteer non-paid donors is crucial for this purpose.

Donors registered at the CLB are recruited in villages and small towns from all over The Netherlands by the local chapters of the Red Cross; these donors are called for donation of blood in the direct vicinity of their residences in the evening. Donor selection, blood collection, storage and transport of whole blood are performed by mobile teams of the CLB with the aid of volunteer helpers of the local Red Cross. Volunteer help is only available in the evening, as the people involved have normal day-time jobs. Volunteer non-paid donors prefer to give blood in the evening near their homes, as this avoids long-lasting absenteeism from work and home. They like to be helped by the local Red Cross staff and they like to be acknowledged by their fellow-citizens and family during the collection of blood.
Overnight storage of blood at 20°C is a prerequisite of blood collection in the evening, as it avoids subsequent blood processing in night shifts. Therefore, overnight storage of blood at 20°C promotes the availability of donors and Red Cross volunteer help for blood collection (see Table 1).

**BLOOD COLLECTION**

*Prevention of bacterial contamination*

The collection of already contaminated blood or contamination with skin bacteria
during venepuncture\textsuperscript{4} were the only possibilities which remained after the replacement of bottles by plastic blood bags. Endogeneous contamination can neither be avoided by additional questioning of prospective donors nor by additional tests during the donor selection procedure\textsuperscript{5}. The bactericidal activity of whole blood is the only preventive barrier for this type of infection. Contamination with skin bacteria can be partially avoided by proper cleansing of the arm at the venepuncture site\textsuperscript{6}; however, subcutaneously located bacteria will escape and may enter the blood donation when they are hit by the venepuncture needle. Frequent donations by the same donor, as often practised in platelet pheresis, will result in scarring of the venepuncture site; scarred sites may host concentrations of skin bacteria\textsuperscript{7} and thus facilitate contamination. The use of products derived from whole blood donations instead of pheresis products reduces the number of venepuncture per donor to maximally 4 times per year and will reduce the induction of scarred venepuncture sites.

The increased use of platelets derived by pheresis was induced by an increased number of patients with a refractory reaction to platelets derived from whole blood donations; these refractory reactions were mainly due to leukocyte-contaminated platelet preparations\textsuperscript{7}. Therefore, a return from pheresis platelets to platelets derived from whole blood will only be possible when leukocyte-depleted platelet concentrates are prepared\textsuperscript{1} and a sufficient number of donors is available. Both conditions are favored by the overnight
hold of whole blood at 20°C.

Contamination by subcutaneously located bacteria can only be eliminated by the bactericidal activity of the leukocytes in whole blood; a hold of 5-24 hours at 20°C, followed by removal of the leukocytes from red cells and platelets with the buffy-coat method\(^1\), is sufficient to take care of the highest possible contamination with both the skin flora and endogenous bacteria concerned\(^8\).

**Prevention of activation.**

The recovery of platelets and the bactericidal activity of granulocytes will be diminished when blood is activated during collection. Inadequate mixing of blood and anticoagulant\(^6\), an improper ratio of blood and anticoagulant at the end of the collection\(^9\), overtime bleeding\(^11\) and stripping back of clotting blood from the line into the blood bag\(^12\) will all enhance this activation. This activation can be limited by the following measures\(^13\):

a) by mixing blood and anticoagulant every minute during collection;
b) by control of the blood-flow rate by the donor itself with the aid of an electronic flow monitor at each bed (COMPOGUARD\(^\text{®}\));
c) by monitoring the amount of blood collected with a tripping scale which stops the bleeding automatically when the amount required is reached (FENWAL\(^\text{®}\));
d) by ending each donation which lasts longer than 12.5 minutes and by marking these units as not being suitable for platelet and factor-VIII preparation;
e) by replacing clotting blood located in the blood line with anticoagulated-blood from the bag by transfer of the needle from the vein into the rubber stopper of a sterilly evacuated tube at the end of the donation, and
f) by reducing the temperature of the blood to 20°C within 2 hours after the collection and by keeping it at this temperature during the hold.

The combined effect of these measures resulted in a minor activation as can be judged from the factor-VIII recovery in the plasma (78 ± 8%) after a hold of 16-20 hours at 20°C\(^2\).
TRANSPORT AND HOLDING OF DONATIONS

A uniform temperature history

The actual temperature history of a donation after blood collection may vary considerably depending on the exchange of heat with its surroundings and the amount of energy which is available to reach and maintain the temperature required. It was found that in an insulated box with melting ice in separate cooling compartments, it took more than 6 hours before some of the stored blood bags reached a temperature of 8°C, whereas others in the core of the pile of bags were still at 20°C. The decrease in temperature of blood after collection does affect the plasma proteins, the platelets, the granulocytes and the red blood cells.

A uniform temperature history is reached when, after collection, each donation is put on a metal container filled with solid butane-1,4-diol; 200 g of butane-1,4-diol per unit of blood is required to cool the blood from 37°C to 20°C within 2 hours and to keep it at that temperature for 16 hours thereafter. Butane-1,4-diol is a non-toxic wax, melting at 20°C with a melting heat of 36 Kcal per kg; it is hygroscopic and uptake of water lowers the melting point. Therefore, the container should be vapor-tight; aluminum containers with 2 kg butane-1,4-diol, suitable for cooling 8 donations, are commercially available (COMPOCOOL). There is no other compound known with a melting point at 20°C and a melting heat high enough to cope with the amount of energy required to obtain a sufficient cooling rate and cooling capacity for each donation.

DETROUTEFAL EFFECTS OF AN OVERNIGHT HOLD AT 2-6°C: COMPARISON WITH 20°C HOLD

Changes of plasma clotting factors

Cold activation of factor VII: cooling of blood below 4°C may lead to spontaneous activation of clotting factor VII, in particular of women using oestrogen contraceptives. Enhanced levels of kallikrein and cold inactivation of C1-esterase inhibitor promote its occurrence; this phenomenon requires at least 2 hours of storage, thus overnight storage at 4°C in an additional promoting factor. Storage at 20°C prevents cold activation of factor VII.
Degradation of von Willebrand factor VIII at 4°C

Storage of blood at 4°C leads to considerable losses of von Willebrand factor VIII and deteriorates its multimeric structure as well as the functional properties of the von Willebrand factor-VIII complex. This loss is partly due to hydrolases released by degrading leukocytes. Storage of blood at 22°C instead of 4°C was shown to prevent these losses.

Loss of factor VIIIc by cold precipitation

Cooling of blood below 8°C leads to cold precipitation of factor VIIIc. After overnight storage of whole blood at 4°C, subsequent separation of plasma and blood cells by centrifugation led to more than 50% loss of factor VIIIc from the plasma as it precipitated together with the blood cells. Storage at 20°C over the same period prevented this.

Granulocyte function, bactericidal activity and storage at 4°C

The bactericidal activity of whole blood after donation is based on the presence of granulocytes. The function of granulocytes to move to a bacterium in the bloodstream, to recognize its antibody-coated membrane, to engulf it, to attack its membrane with oxygen radicals and to destroy the bacterial constituents is based on the delicate structure of the granulocyte. Upon chilling to 4°C, granulocytes lose their membrane integrity, their mobility, their ability to produce oxygen radicals and their granules filled with hydrolases, they obtain the morphological appearance of a damaged cell. All these changes start in the first 24 hours of storage at 4°C after blood collection; these changes can be avoided by holding the blood at 20-24°C. This hold is also the optimal condition for the endogenous irradiation of the bacteria which might have entered the blood during venepuncture: to complete the phagocytosis of these bacteria, the granulocytes need a 5-to-24-hour hold at 22°C. Thereafter, the granulocytes loaded with bacteria have to be removed from the plasma, packed cells and platelet concentrates as some species (S. aureus) may resist the digestion by granulocytes and grow out later on during the storage of the previously mentioned products.
Platelet viability after chilling

Electronmicroscopic studies revealed that the circumferential band of microtubules in platelets depolymerizes immediately upon chilling to 4°C, resulting in a morphological change from disc to sphere. This change is only partly reversible and it induces a loss of viability of stored platelets after transfusion.

The decrease in temperature during storage of platelets is rather critical as this loss starts immediately below 20°C; these changes can only be prevented as the temperature during the hold of whole blood prior to platelet preparation is carefully maintained above 20°C.

Red-cell storage at 4°C: effect on metabolites

Originally the chilling of whole blood during storage was introduced to preserve the viability of red blood cells; the low temperature, in combination with the acid pH caused by the citrate, inhibited the use of energy-rich phosphates to such an extent that the remaining glycolytic activity could cope with the use of ATP for a storage period of 3 weeks. The introduction of CPD as anticoagulant prolonged this storage for another week as a result of the effect of phosphate on the level of ATP.

Analysis of the metabolites of red cells revealed that in particular 2,3-bisphosphoglycerate (2,3-DPG) decreased rapidly during storage; 2,3-DPG is consumed to maintain the level of ATP and the latter compound hardly changes during the first 2 weeks of storage. The decrease of 2,3-DPG is heavily dependent on the temperature history (see Table 2).

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>37</th>
<th>30</th>
<th>25</th>
<th>19</th>
<th>15</th>
<th>10</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of 2,3-DPG (mmoles/liter red cells/h)</td>
<td>0.63</td>
<td>0.32</td>
<td>0.18</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The level of 2,3-DPG is known to have an effect on the oxygen dissociation of hemoglobin but has no effect on the survival of red cells. After transfusion of 2,3-DPG-depleted red cells, the original level of this compound is rapidly restored.

The choice for 20°C for an overnight hold was based on the fact that at this temperature the rate of 2,3-DPG was already greatly diminished (9-fold), whereas the level of adenylates was constant provided that the temperature history of each donation was uniformly imposed with the aid of melting butane-1,4-diol. In this way, the level of adenylates was maintained at 100% (ATP + ADP = 2.2 ± 0.4 millimoles per liter of red cells), whereas the level of 2,3-DPG decreased to 80% of its original level (3.9 millimoles 2,3-DPG per liter of red cells) after an overnight hold (16 hours) at 20°C. Therefore, function and viability of red cells is better preserved than after a poorly defined chilling to 4°C as was so far routine in our blood banks.

**BLOOD PROCESSING**

*The preparation of pure red-cell and pure platelet concentrates*

The ultimate goal of blood processing is to obtain concentrates of red cells and platelets next to plasma in the highest possible yield and quality. This quality of red-cell and platelet concentrates with respect to viability, tenability and side-effects is determined by the contamination with other blood cells.

The presence of platelets and leukocytes in red-cell concentrates induces, during storage at 4°C, the formation of microaggregates; microaggregates clogg the transfusion equipment and the microvascular system of the lungs.

The degradation of granulocytes during storage causes the release of hydrolases which enhance the hemolysis; the remaining lymphocytes and monocytes consume part of the glucose and acidify the medium by lactate production. Non-hemolytic fibrile transfusion reactions are induced by the leukocytes present in red-cell concentrates, which is a major problem for multiple transfused patients.

In platelet concentrates, the presence of leukocytes induces an increased lysis of platelets during storage at 22°C, as leukocytes consume a part of the available glucose and acidify the medium. Patients become refractory to platelet transfusion by the contaminating leukocytes, as these cells induce HLA immunization.
These ontowards effects of the impurity of both red-cell and platelet concentrates can be prevented by the removal of buffy-coat cells from the packed red cells and by the use of the buffy-coat as a source for the preparation of platelets. As the leukocytes provide the bactericidal protection of whole blood, the buffy-coat should be removed after an overnight hold at 20°C.

The removal of the "buffy-coat" cells from the red cells is based on the difference in specific gravity of blood cells, whereas the separation of platelets from the buffy-coat is based on the difference in size of the various blood cells.

Whole blood is spun first until buoyant density equilibrium is reached by its constituents, then plasma and buffy-coat cells are extracted successively and the packed red cells are resuspended in additive solution. The buffy-coat, consisting of the bulk of the leukocytes and platelets, some plasma and red cells, is then spun softly until leukocytes and red cells are sedimented and the platelets, concentrated in the supernatant plasma, can be extracted.

The separation is not only governed by size and density of the cells; a temperature below 20°C will decrease the deformability of red cells and influence the characteristics of the membranes of leukocytes and platelets, leading to trapping of leukocytes and platelets in the layer of red cells. A similar effect is induced by an excessive force during centrifugation.

As can be derived from Fig. 2, the amount of leukocytes and platelets remaining in the packed cells after removal of the buffy-coat is minimal, as the product of centrifugal force and duration of the centrifugation is 23,400 g.min. The rather complicated manipulation with four-bag systems during the removal of buffy-coats is standardized, speeded up and facilitated by the use of an automated device (COMPOMAT). In the routine practised of component preparation, automation is a prerequisite for the quality of operation as can be derived from Table 3.

CONCLUSION

The combination of a strict maintenance of the temperature at 20°C, the overnight hold and the removal of leukocytes from the cellular components, which have to be stored in the liquid phase, is a prerequisite for the optimal exploitation of the bactericidal capacity
of blood after collection. After the extensive measures, taken to prevent the transmission of viruses by blood transfusion, we have to rely on this bactericidal activity of blood as the only way to prevent post-transfusion septicemia. So far, the growth characteristics of skin flora and environmental bacteria have governed the preventive measures in this field and the bactericidal activity of whole blood was never studied properly. In the time of open systems and blood bottles, there was a technical excuse for this negligence, but since sterile connection devices and multiple bag systems are available, this excuse will no longer hold. Moreover, there is now an overwhelming amount of data available about the blood cells that are responsible for the bactericidal activity of blood; so far, this knowledge has not been taken into account for the prevention of post-transfusion septicemia; it is about time to do so.
### TABLE 3

**SPECIFICATION OF THE COMPOSITION AND CONDITIONS USED TO OBTAIN RED-CELL AND PLATELET CONCENTRATES WITH AN AUTOMATE (COMPOMAT*)**

<table>
<thead>
<tr>
<th>Centrifugation conditions for whole blood: centrifugal force</th>
<th>24,700 g.min (radius to cup bottom = 28 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature</td>
<td>20°C</td>
</tr>
<tr>
<td>acceleration time</td>
<td>70 sec.</td>
</tr>
<tr>
<td>deceleration time</td>
<td>270 sec.</td>
</tr>
<tr>
<td>preparation time for plasma, buffy-coat and red-cell concentrate in additive solution (SAGM)</td>
<td>115 ± 5 sec. per donation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Centrifugation conditions for platelet preparation from buffy-coat: centrifugal force</th>
<th>1700 g.min (radius to cup bottom = 28 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature</td>
<td>20°C</td>
</tr>
<tr>
<td>acceleration time</td>
<td>55 sec.</td>
</tr>
<tr>
<td>deceleration time</td>
<td>180 sec.</td>
</tr>
<tr>
<td>preparation time</td>
<td>77 ± 8 sec. per donation</td>
</tr>
</tbody>
</table>

| Composition of red-cell concentrate in additive solution (SAGM)                       | (296 ± 14) ml                               |
| volume                                                                                 | (62 ± 2) %                                  |
| cell-volume ratio                                                                     | (609 ± 458)x10⁶ = (20 ± 11)% of the original leukocytes |
| leukocyte count                                                                       | 0 (51 ± 5) gram = (92 ± 5)% of the original amount |
| hemoglobin-content                                                                    |                                            |

| Composition of platelet concentrate in plasma                                        | (62 ± 3) ml                                 |
| volume                                                                                 | (62 ± 16)x10⁸ = (66 ± 8)% of the original platelets |
| platelet count                                                                        | (2 ± 1)x10⁹ = (0.05 ± 0.03)% of the original leukocytes |
| leukocyte count                                                                       |                                            |
REFERENCES


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