Effect of Whole Blood Storage on Factor VIII Recovery in Fresh Frozen Plasma and Cryoprecipitate

Abstract
Depending on logistics, whole blood has to be stored for several hours after collection. If storage time exceeds 8 h, storage has to be at 1–6°C to comply with FDA regulations. In the Netherlands, however, whole blood is also stored for 12–15 h at 20–24°C using butane-1,4-diol cooling devices. We compared these two storage methods for factor VIII recovery in plasma and cryoprecipitate. At laboratory scale a significantly higher factor VIII recovery was found in plasma prepared from whole blood stored at ambient temperature; in cryoprecipitate this was confirmed but differences were not significant. However, as a result of inefficient cooling in routine procedure, no significantly different factor VIII recoveries were found in plasma prepared from whole blood stored at either of the two temperatures. From our study we conclude that overnight storage of whole blood at ambient temperature using butane-1,4-diol cooling devices has to be favoured.

Introduction
Whole blood is collected from donors for the preparation of fresh frozen plasma and cell concentrates. After collection, whole blood can be stored under different conditions: at room temperature for up to 24 h, as well as at 1–6°C for a maximum of 15 h [1]. These storage conditions are related to the blood component which is to be prepared from the whole blood donation; they also depend on collection and processing logistics and have to comply with (national) regulations [1].

Storage of whole blood at 1–6°C is preferable to room temperature for red cell preparation; for platelets, whole blood storage below 20°C is advocated [1]. For factor VIII it has been demonstrated that storage of whole blood for 24 h at room temperature or at 4°C results in a factor VIII recovery in plasma of about 75 and 50%, respectively [2, 3]. From a microbiological point of view whole blood storage at 4°C has been advocated because of bacterial growth inhibition at that temperature [1]. The self-sterilising properties of freshly drawn blood, however, have to be taken into account as well and so far spiking experiments showed no differences for storage at 4 or 20°C [1]. For Yersinia enterocolitica, storage of whole blood at 22°C for 24 h even has a positive effect on the removal of this psychrophilic micro-organism during processing, probably because of ingestion of the bacteria by granulocytes [4].

Whole blood can be stored at room temperature for maximally 8 h, after which it has to be stored at 1–6°C to comply with the regulations of the United States Food and Drug Administration (FDA). When cooled to 1–6°C it can be kept for up to 72 h for the purpose of red cell preparation and for 15 h if fresh frozen plasma is to be prepared. In our situation whole blood is collected by mobile teams in the evening. So if we are to comply with FDA regulations, red cell concentrates have to be processed at night if blood is stored at room
Table 1. Whole blood cooling and storage conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>Scale</th>
<th>Coolant</th>
<th>Environmental temperature, °C</th>
<th>Process temperature, °C</th>
<th>Storage time, h</th>
<th>Time to reach specification, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>laboratory (60)</td>
<td>butane-1,4-diol (20)</td>
<td>ambient</td>
<td>20–24</td>
<td>3 maximal</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>laboratory (60)</td>
<td>butane-1,4-diol (20)</td>
<td>ambient</td>
<td>20–24</td>
<td>12–15</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>laboratory (60)</td>
<td>melting ice (0)</td>
<td>1–6</td>
<td>1–6</td>
<td>12–15</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>routine (8 lots of 500)</td>
<td>butane-1,4-diol (20)</td>
<td>1–6</td>
<td>1–6</td>
<td>12–15</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>routine (9 lots of 500)</td>
<td>butane-1,4-diol (20)</td>
<td>ambient</td>
<td>20–24</td>
<td>12–15</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Number of units of whole blood in parentheses.

2 Temperature (°C) in parentheses.

temperature, whereas processing the next day is possible if the whole blood donations are cooled and stored at 1–6°C. To study the effect of these two alternatives for whole blood storage conditions on factor VIII recovery, we analyzed factor VIII in fresh frozen plasma and cryoprecipitate in a comparative laboratory scale study. Three storage conditions were compared, two of which complied with FDA regulations (A and C): (A) 20–24°C for maximally 3 h (B) 20–24°C for 12–15 h and (C) 1–6°C for 12–15 h. Storage at room temperature and at 1–6°C for 12–15 h was evaluated by assaying factor VIII in plasma obtained from routine production as well.

Materials and Methods

Comparative Laboratory Scale Study

Whole Blood Collection. Three groups of 60 donors each were selected at random and stratified for blood group and sex. 500–550 ml of whole blood was collected within 12.5 min in a quadruple-bag system, using citrate-phosphate-dextrose solution as anticoagulant (Baxter, USA). To obtain a reference sample (t = 0 h), a sample from each donation was drawn immediately into a polypropylene tube after collection and before cooling. These samples were centrifuged at room temperature (10 min at 3,000 rpm) and the supernatant was transferred into polypropylene tubes. The samples were frozen on dry ice.

Whole Blood Cooling and Storage. Within 15 min after collection the donations in groups A and B were placed on cooling elements filled with butane-1,4-diol, to obtain a temperature of 20–24°C within about 2 (table 1) [3]. The donations in group C were placed on packs with melting ice within 15 min after collection; a 2-mm polystyrene layer was used to separate the ice packs from the whole blood units in order to prevent whole blood temperatures under 1°C. In this way a temperature of 1–6°C was obtained within about 5 h for donations in group C (fig. 1).

Fig. 1. Temperature profiles of whole blood units cooled on melting ice and stored at 1–6°C (method C; ■) or cooled and stored with butane-1,4-diol cooling devices in the routine procedure (method D; □).

The donations in groups A and B were stored on the butane-1,4-diol cooling plates at ambient temperature, which guarantees a temperature of 20–24°C for the whole storage time [3]. The donations in group C were stored in a 1- to 6°C cold storage room together with the ice packs. Storage time before plasma-cell separation was maximally 3 h (group A) or 12–15 h (groups B and C), respectively.

Components Preparation. After storage the donations were centrifuged (10 min at 3,000 rpm, at room temperature for groups A and B and at 4°C for group C). The plasma was separated from the red cells and buffycoat automatically by using a Compomat system (NPBI, Emmercompascuum, The Netherlands). The plasma was sampled im-

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mediate after separation into polypropylene tubes (2×1 ml of plasma) for factor VIII assay, and in a Falcon tube (40 ml of plasma) for cryoprecipitate preparation; samples were frozen immediately in liquid nitrogen and stored at ≤−30°C.

Cryoprecipitate Preparation. The fresh frozen plasma was thawed at 4°C in a glycol bath and centrifuged for 10 min at 3,000 rpm at 4°C. The cryosupernatant plasma was decanted and the approximately 0.4 ml of cryoprecipitate was reconstituted in 2 ml of buffer (200 mmol/l sodium citrate, 20 mmol/l calcium chloride, 800 mmol/l sodium chloride, pH 6.6) at 30°C and sampled. Samples were frozen immediately in liquid nitrogen.

Routine Production
Whole Blood Collection. In routine production lots of about 500 whole-blood donations were collected by mobile teams. Collection took place as described above.

Whole Blood Cooling and Storage. All donations were cooled immediately after collection using butane-1,4-di dol cooling plates to obtain a temperature of 20–24°C in about 2 h (table I) [3]. Within 3 h after collection, a part of each lot was stored at 1–6°C in a cold storage room (group D). In this way a temperature of 1–6°C was obtained within about 10 h (fig. 1). The other part of each lot was stored at the butane-1,4-diol cooling plates at ambient temperature until processing (group E).

Components Preparation. Components were prepared as described above within 12–15 h after whole blood collection. The plasma units were frozen in circulating nitrogen gas of −60°C: within 30–45 min the plasma had reached a temperature of −30°C.

Lot Sampling. According to standard procedures for the in-process quality control of production, from every lot of about 500 units 20 units of frozen plasma bags were selected at random. With a hollow punch these frozen plasma bags were sampled in the centre. The 20 samples were thawed and mixed, giving the lot sample. This lot sample was analyzed for factor VIII activity and total protein.

Data Analysis. The regular in-process results were used to compare the two storage conditions. Therefore 8 lot samples of group D and 9 lot samples of group E, at random selected and stratified for blood group, were compared.

Assay Methods
Factor VIII Assay. Factor VIII was assayed by a chromogenic method (Chromogenix AB, Mölndal, Sweden). Reagents were used in combination with a Biomek-1000 Automated Laboratory Workstation (Beckman Instruments, Inc., Fullerton, Calif., USA). A plasma factor VIII standard, calibrated against the 2nd International Standard for Factor VIII in Plasma (87/718) of the World Health Organisation was used as a reference.

### Table 2. Factor VIII activity in the reference samples, plasma units and cryoprecipitates and process efficiency (mean values ± standard error of mean)

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cond. n</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Reference sample, IU/ml</td>
<td>0.91±0.03</td>
<td>0.90±0.02</td>
<td>0.90±0.03</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Plasma, IU/ml</td>
<td>0.82±0.03</td>
<td>0.73±0.03</td>
<td>0.66±0.02</td>
<td>0.78±0.09</td>
<td>0.84±0.06</td>
</tr>
<tr>
<td>Cryoprecipitate, IU/ml</td>
<td>6.35±0.31</td>
<td>6.18±0.27</td>
<td>5.49±0.23</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Process efficiency</td>
<td>7.65±0.17</td>
<td>8.38±0.17</td>
<td>8.37±0.17</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Temperature. The cooling times and temperatures were validated by temperature measurements inside the blood or plasma bag. Simulated whole blood temperature profiles were obtained using bags filled with 500 ml of a 50/50 glycerol-water mixture and a starting temperature of 37°C. Plasma temperatures were obtained using bags filled with plasma.

Statistical Analyses. In the comparative laboratory scale study, process efficiency was defined as the factor VIII content in cryoprecipitate (in IU/ml) divided by the factor VIII content in the plasma (in IU/ml) prior to cryoprecipitation.

Whether the three storage methods influenced the factor VIII recovery and process efficiency in the groups was determined by analysis of variance. Groups were also compared mutually by unpaired two-tailed t-test; p values ≤0.05 were considered to be statistically significant.

Results
Comparative Laboratory Scale Study
Statistical analysis of the distribution of blood groups and sex of the donors over the three groups showed no differences. Factor VIII activities in the reference samples (t = 0 h) of the three groups also showed no statistical differences (table 2).

For the plasma factor VIII activities (table 2), analysis of variance showed significant effect of the storage methods (p < 0.001). By paired comparison a significant difference was found between all groups respectively. The highest plasma factor VIII activity was found for storage condition A and the lowest activity for C.

Analysis of variance showed no significant effect (p = 0.086) of the storage conditions on factor VIII activity in cryoprecipitates (table 2). A paired comparison, however, showed a significant difference in factor VIII activity when method A and C were compared. In the cryoprecipitates, method A resulted in the highest activity and C gave the lowest activity.

Analysis of variance showed a significant effect (p = 0.004) of the storage conditions on process efficiency (table 2). When the methods were compared in pairs, both method B and C differed significantly from A. The highest
process efficiency was found for storage condition B and the lowest efficiency for A.

**Routine Production**

In routine production no significant difference was found in factor VIII activity in plasma (table 2), when storage at 1–6°C was compared with 20–24°C.

**Discussion**

Our results showed that, on laboratory scale, the 1–6°C storage of whole blood for 12–15 h was inferior compared to 3 h as well as 12–15 h of storage at 20–24°C for factor VIII recovery in both plasma and cryoprecipitate. These results are in agreement with the findings of others [2, 3]. Vermeer et al. [5] demonstrated that the temperature of stored whole blood should not decrease to 8°C; they suggest that low recovery for 4°C storage probably is due to spontaneous cryoprecipitation. Hughes et al. [2], who studied factor VIII recovery in whole blood stored at 22 and 4°C, suggest that inactivation of labile factor VIII is the cause of the lower recoveries at 4°C.

If storage at 20–24°C for 3 and 12–15 h was compared, the differences in factor VIII activity in cryoprecipitate were not significant. This might be due to the two-phase decay of factor VIII [6, 7], which will result in the main factor VIII loss within the first hours of storage, and the fact that in both conditions most of the recovered factor VIII is of the stable form [2, 6, 7]. Storage for 12–15 h might be favoured as far as process efficiency is concerned.

The difference between storage for 12–15 h at 20–24°C and 1–6°C respectively that we observed in the laboratory scale test was not confirmed in routine production. We believe that this is due to less efficient cooling to 1–6°C in routine production, which is in agreement with the findings of Rock and Tittley [8]. Griffin et al. [9] also found no statistical difference for FVIII recovery in plasma when whole blood was stored at 4°C or at room temperature, which was probably also due to the inefficient cooling at 1–6°C.

Our results show that storage of whole blood at 1–6°C is inferior to storage at 20–24°C when these temperatures are rapidly imposed on the blood by forced cooling. However, when the cooling was insufficient to reach the desired temperature within 2–3 h the phenomenon of factor VIII loss by cryoprecipitation at temperatures below 8°C was not noticed. Apparently the formation of cryoprecipitate at 1–6°C required more time than obtained during our routine procedure. So if for whole blood, collected by mobile teams, the FDA regulations are applied and donations are sufficiently cooled, substantial amounts of factor VIII will be lost. Moreover, efficient and reproducible cooling to 1–6°C is difficult to achieve in whole blood collection by mobile teams. As low temperatures are critical in relation to factor VIII recovery [2, 5], we believe that storage procedure and process have to be validated and temperature controlled equipment is needed for this method.

In a system where whole blood is collected by mobile teams, blood often has to be stored for more than 8 h to avoid the team being forced to process blood by night. The FDA regulations dictate storage at 1–6°C if a time period of 8 h is exceeded. However, this method is in discussion, while storage of whole blood at room temperature for long periods is also successfully applied [1]. We believe that storage at 20–24°C, using butane-1,4-diol cooling devices for up to 15 h, is the method of choice for mobile whole blood collection systems. For factor VIII recovery in cryoprecipitate we showed that these conditions yield better, although not significantly different, results compared to 1–6°C storage. Moreover, validated reproducible whole blood storage conditions at 20–24°C are guaranteed and for the preparation of cell components these circumstances are also satisfactory [3]. Storage at 20–24°C will not result in a higher risk of microbiological contamination, as was shown by spiking experiments [1, 3]. These results are confirmed by data we obtained in our quality control programme on sterility testing of red blood cell concentrates prepared from whole blood donations stored at butane-1,4-diol devices for 12–15 h. In these tests 0.2% nonsterile units were found, which is in agreement with what was found by others [10, 11].

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References


