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The Effect of Holding Times of Whole Blood and Its Components During Processing on In Vitro and In Vivo Quality

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ABSTRACT

Whole blood is not usually collected close to the processing site, which results in a holding time between collection and processing. In some countries, the holding time is limited to 8 hours, after which the units are cooled, rendering them useless for platelet preparation. Other countries allow a 24-hour ("overnight") ambient hold to allow platelet preparation. The impact of this holding time on subsequent blood components will be reviewed in this article. In addition, there are various "in-process" holding times that further prolong the time before the final blood component is ready. Particularly, these in-process holding times are not well defined and poorly controlled, but can nevertheless affect the biochemical and functional characteristics of blood components. Furthermore, current, non–evidence-based, guidelines have restricted the length of some of these holding times. This article summarizes the evidence and fills gaps where evidence is lacking.

Contents

Blood is a mixture of living cells in a plasma environment, and outside the body—in plastic bags in the blood bank—these cells continue to metabolize nutrients and consume oxygen. Under these artificial circumstances, cells and plasma can lose their functionality, and even to the current day, studies are ongoing that aim to maintain and improve clinical effectiveness of these cells and plasma.

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The changes to the blood constituents commence immediately after collection and continue up to the moment a blood component is administered to the patient. Whole blood and its components are stored for various periods at various temperatures, all which affect the quality of the transfused product.

Although much is known about the changes during storage of the various blood products such as red cells and platelets [1,2], little is known about the changes in the characteristics of the products during the hold time during the various processing steps that precede storage. Many of these holding steps are often not specified and can vary considerably on a day-to-day basis in normal routine blood banking. As an example, at our blood center, if few collections are performed, the holding time of buffy coats will be much shorter before platelet concentrates are prepared than on a busy day, where first the large bulk of whole blood must be processed before platelets can be prepared. To prevent the fluctuations becoming too large, some of the holding steps have found their way into the guidelines, as it seemed good to set limits to some of them, but often without evidence *why* certain limits were set. Sometimes these limits are unnecessarily restrictive and may potentially lead to loss of products.

This review aims to summarize the available literature on the effect of holding times during processing of whole blood into its components, namely, red cells, platelets, and plasma. Also, additional experiments are discussed where we feel that evidence is lacking, but suspect that the holding time may affect the quality of blood components. The effect of holding temperature has been studied extensively with the introduction of overnight hold of whole blood at ambient temperature and will only be discussed briefly.

Effect of Holding Time of Whole Blood Prior to Processing

Historic Perspective

In the early days of transfusion therapy when hospitals collected whole blood for their own patients, there was generally little time between blood collection and subsequent transfusion. Improvements in anticoagulants, by addition of glucose and adenine, and the ensuing development of blood component therapy, allowed longer storage and thus "banking" of blood components. Blood transfusion services began to specialize and conglomerate, resulting in the formation of (regional) blood banks located at a distance from the hospital. Donations were performed more and more outside the hospital and transported to the blood bank for further processing into its various components, introducing a holding time of several hours before whole blood was separated into components. To allow for transportation, it became common practice that there was a lag time of several hours before whole blood was separated into components.

Currently, the allowed holding time of whole blood at ambient temperature before processing varies between 8 and 24 hours, depending on the requirements of regulating agencies and/or guidelines in force. During this hold at ambient temperature, some changes in blood already take place. One of the most notable changes is the decrease in 2,3-diphosphoglycerate (DPG) concentration in the red cells. In a study comparing immediate vs 6-hour delayed processing, where platelet-rich plasma (PRP) was removed from centrifuged fresh whole blood and split in 2 identical portions, the portions that were held for 6 hours at room temperature had a 10% lower 2,3-DPG concentration at onset of storage than the portions that were refrigerated immediately [3]. Also, the portions held for 6 hours before cold storage subsequently showed a more rapid decrease in concentration, although the differences dissipated after day 12 as 2,3-DPG became depleted, irrespective of the initial holding time. However, ATP content was not affected by the 6-hour hold. ^{51}Cr recovery 24 hours after retransfusion of 21-day-old red cells was slightly better for units cooled immediately ($P = .05$), but with the average value above 85%, both values were deemed to be excellent. No effect on the in vitro quality of platelets was found, and all 10 platelet concentrates were negative when cultured at the end of the 72-hour storage period. Factor VIII concentration (factor VIII:C) in plasma was also similar in both units. In the following years, the maximum holding time was extended from 6 to 8 hours at room temperature, based on a study where after removal of PRP and subsequent 35-day storage, red cells in CPDA-1 showed a satisfactory 78.0% ± 8.1% recovery 24 hours after retransfusion [4].

In the early 1980s, blood centers began experiments to extend the hold time beyond 8 hours. Using a modified anticoagulant, CPD-AD, containing 0.4 mM adenine and a 1.5 times higher glucose than in regular CPD, an overnight holding time of 15 hours could be achieved [5]. ATP was similar during a 5-week storage compared with units held for less than 4 hours, but initial 2,3-DPG was less than half of the controls. There was no effect on platelet quality in vitro. Pietersz et al [6] finally extended the holding time to a full 24 hours after collection. They used butane-1,4-diol cooling plates to ensure that all units reached a temperature of approximately 20°C within 2 hours after collection. ATP was maintained during the first 24 hours and dropped to 81% ± 5% of the initial value after 5 weeks of storage. 2,3-DPG was reduced by two-thirds after 24-hour hold at room temperature and declined further during storage, until it was completely depleted by week 2. Platelet yield was 84% ± 6% for overnight-held units and was slightly higher than the 76% ± 18% found in units processed within 3 hours. Factor VIII concentration declined to 80% ± 3% of the initial value after 24-hour hold, but routinely processed units held for 16 to 20 hours showed sufficient factor VIII:C levels.

Routine overnight storage of whole blood at room temperature for up to 24 hours became standard procedure at the Amsterdam blood center in 1987 [7] and is now common practice in many countries. This practice was initially introduced mainly in Europe and has been subsequently incorporated into the internationally recognized Council of Europe Guide for the Preparation, Use and Quality Assurance of Blood Components [8], and later by the Canadian Blood Services [9]. More recently, the Food and Drug Administration allowed 24-hour room temperature hold for apheresis plasma [10], although overnight hold of whole blood with use of all components (red cells, platelets) has not yet been approved.

Overnight hold of whole blood therefore allows ample time to transport units from the donation site to the processing center. Fewer transport runs between the donation and processing sites are needed when the necessity to comply with a short holding time is no longer required. When all units are available for processing in the morning, efficiency increases (rather than waiting for units to come in) as the workload can be distributed more evenly. Working during business hours is not only more economical but less prone to error than night shifts, as night shifts are associated with increased error rates when performing tasks [11,12]. With the buffy coat method, where multiple units are pooled prestorage to make one platelet concentrate, the different blood groups are available around the same time, to facilitate pool formation.

Effect of Holding Time of Whole Blood on the Quality of Red Cells

As indicated above, 2,3-DPG rapidly declines at room temperature hold, but ATP remains almost unaffected. This has consistently been reported in numerous studies [13–18]. Other parameters also remain more or less unaffected by the preprocessing holding time. One study comparing 7- to 8- vs 23- to 24-hour hold showed no effect on hemolysis and supernatant K^+ [19] after the initial holding time, or throughout 42 days of storage. The same group also conducted a radiolabeling study in 2 collaborating laboratories, that each used slightly different radiolabeling techniques to determine the recovery 24 hours after retransfusion. At site A, using a single ^{51}Cr radiolabel, red cells stored for 35 days in AS-1 showed a 79.2% ± 4.3% vs 79.4% ± 3.9% recovery when produced from 8- or 24-hour-held whole blood. Site B reported 79.7% ± 6.5% vs 83.4% ± 7.2% with a dual label ^{51}Cr/^{99m}Tc method, respectively; all differences were not statistically significant. For units stored for 42 days, site A

showed no difference in recovery, whereas site B demonstrated a significantly lower recovery for whole blood held for 24 hours. Also, the combined single-label data of sites A and B were statistically different, 76.0% ± 5.4% for the 8-hour holding time and 72.9% ± 6.5% for 24-hour holding time ($P = .036$). For the dual-label procedure, however, neither the data from the individual sites nor the combined data showed statistically significant differences for 42-day stored red cells. The authors commented that the single statistically significant difference could be considered to be due to chance and of no clinical relevance. Moreover, the half-life (T-50) was not different at 26.2 ± 2.0 days for red cells from whole blood held for 8 hours vs 26.3 ± 2.6 days for those from whole blood held for 24 hours. The red cells were processed according to the PRP method, and white cells were not removed.

In a similar study (with holding times 6-8 vs 22-24 hours) with PRP-removed leukodepleted red cells in AS-3, almost identical in vitro measures were found, with a small but significant lower pH at onset of storage for the latter group, caused by a higher lactate concentration after the longer room temperature hold; however, this difference disappeared rapidly [13]. Hemolysis was marginally lower on day 1 and throughout storage in red cell concentrates from units held for 8 hours. Others mainly confirmed these in vitro measures [15], a comparison of less than 8- vs 24-hour hold at room temperature resulted in significantly lower hemolysis for the short-held group, throughout subsequent storage. Despite no difference at onset, free K^+ was higher from day 14 onward in the short-held units, possibly due to a higher temperature during processing. A comparison of a number of collection systems under various holding conditions showed that overnight hold of whole blood at room temperature was associated with lower potassium levels both at onset and during subsequent storage of the red cell concentrates produced from the whole blood collections [16]. These effects were consistent irrespective of the blood bag system. A study comparing holding times of 8, 12, 16, or 19 hours also found higher lactate and a lower pH with a longer holding time [17]. Hemolysis was not different initially, but was 0.30% ± 0.05% for 8-hour hold by day 42, and 0.52% ± 0.17%, 0.53% ± 0.16%, and 0.54% ± 0.22% for 12-, 16-, and 19-hour hold, respectively. A multisite study including 9 sites, 7 using the buffy coat method and 2 the PRP method [18], compared the quality of components made from whole blood either 3:09 ± 1:20 or 24:21 ± 1:14 hours after collection. The same patterns were seen for lactate and pH as previously found, confirming the trend that overnight-held whole blood shows a somewhat higher hemolysis at the end of storage, though still well below current guidelines for hemolysis during a 35- to 42-day storage period. Another exception was that potassium concentrations were not different among the different holding periods.

Finally, a recent radiolabeling recovery study was published where red cells had been processed with the Reveos automated separator [20]. Using whole blood held for 2 to 8 vs 10 to 24 hours and stored for 42 days, ^{51}Cr 24-hour recovery was 88.9% ± 4.6% and 89.1% ± 5.9%, respectively, which is statistically not significant and well above current-day acceptance criteria. In this study, hemolysis at day 42 was 0.30% ± 0.16% and 0.26% ± 0.04% for the fresh and overnight-stored groups, respectively.

Summarizing, ambient hold of whole blood results in a build-up of lactic acid and lowering of pH in red cell concentrates. Probably depending on specific circumstances (collection system, holding conditions, method of processing, type of leukoreduction, additive solution) as a number of reports describe a somewhat higher hemolysis rate and lower free potassium, whereas others report no difference. It can be concluded that overall red cell recovery is not affected by the holding time of whole blood.

The Decline of 2,3-DPG in Red Cells from Whole Blood Held at Room Temperature

As summarized above, the holding time of whole blood at room temperature is associated with a decreased concentration of 2,3-DPG in red cells. As a consequence, the oxygen dissociation curve of hemoglobin (Hb) shifts, resulting in reduced oxygen delivery to the tissues, although other factors like pH and ATP content also play a role in the actual delivery process. Some argue that this is of no consequence as 2,3-DPG is rapidly restored; others believe it to be clinically relevant, particularly in patients where immediate tissue oxygen delivery is required. A study in rats which were hemodiluted to a hematocrit of about 14% prior to receiving an exchange transfusion with either fresh, 2- to 3-week-old or 5- to 6-week-old red cells indicated that intestinal oxygen pressure was maintained with fresh or 2- to 3-week-old units, despite the complete depletion of 2,3-DPG after 2- to 3-week storage. Oxygen pressure declined by 26%, after administration of 5- to 6-week-old red cells [21]. On the other hand, a randomized study with volunteers that received fresh (3.5 hours after collection) or stored (23 days) autologous red cells showed that transfusion in itself caused a slight increase in alveolar to arterial difference in oxygen partial pressure ($AaDO_2$) before and 60 minutes after transfusion, but the effect was equal for fresh and stored red cells [22].

The importance of the decline of 2,3-DPG was acknowledged early; in the late 1960s, 2 studies were published almost simultaneously. Beutler and Wood [23] transfused type O red cells (stored for 17-20 days as whole blood in ACD-B) to anemic type A patients. In the hours after transfusion, samples were obtained from these patients and the transfused cells were separated from the patient's cells by differential agglutination. Thus, it was elegantly demonstrated that 2,3-DPG levels rapidly increased to about half of the original value within 4 hours of transfusion; after about 24 hours, the levels were within the physiologic range [23]. Valeri and Hirsch [24] used the same method to discriminate between donor and patient red cells, and found that at least 25% of the 2,3-DPG is restored within 3 hours after transfusion and that greater than 50% was restored after 24 hours. In their discussion, the authors stated that at this level of 2,3-DPG recovery, oxygen affinity of the red cells is normalized within 24 hours.

These data were confirmed for red cells stored in saline-adenine-glucose solution, with 50% of the 2,3-DPG restored after 1 hour, and in anemic patients, full recovery was observed after 2 hours [25]. Others found that in volunteers, at least 50% of the 2,3-DPG was regenerated within 7 hours posttransfusion, and 95% of the recipient's mean was restored after 72 hours posttransfusion for red cells stored in AS-1, AS-3, or CPDA1-plasma [26].

These publications all demonstrate that 2,3-DPG rapidly restores after transfusion, although somewhat different rates are reported. It may be that the 2,3-DPG restoration rate is associated with the clinical status of the patient/volunteer. However, as Heaton [26] showed the regeneration rate to be correlated with the ATP content, it is more likely that it is associated with the quality of the red cells. Also, older studies with whole blood [23,24] showed slower regeneration rates than more recent studies with red cells stored as components in additive solutions [25,26] suggests that the regeneration rate depends on overall product quality. It is not known whether the number of transfusions affects 2,3-DPG recovery. Solheim and Hess [27] warned that looking at the 2,3-DPG concentration alone is too simple, because some data about the need for 2,3-DPG were generated in animal models at critically low Hb levels, far below standard transfusion triggers in humans. Also, 2,3-DPG affects the lower end of the oxygen dissociation curve, whereas oxygen transport takes place at the top of the binding curve, which is relatively unaffected. Therefore, it can be assumed that for most clinical applications, stored red cells that are completely depleted of 2,3-DPG can be transfused, as the level of 2,3-DPG is quickly restored to levels where oxygen delivery to tissues is adequate. In summary, the oxygen delivery capacity of stored red cells is much higher than normally required; only in critical situations is the immediate assistance of 2,3-DPG essential. In general, the 2,3-DPG recovery time is a nonissue after red cell transfusion.

Effect of Holding Time of Whole Blood on the Quality of PRP-Derived Platelet Concentrates

Platelets after red cells are the second major cellular component derived from whole blood donations. Platelets need to be stored at room temperature to maintain their ability to circulate after transfusion [28]. The requirement to transport and process whole blood collections within 6 or 8 hours was challenging, as units held beyond that cut off time had to be cooled to 4°C. When platelet demand was low, that challenge could be managed by using whole blood from nearby collection sites specifically for platelet preparations, whereas the remaining collections could immediately be stored at refrigerated temperatures, facilitating longer hold times. However, with increasing demand for platelets, almost all units had to undergo the removal of PRP before storing the red cells at 4°C, with the concomitant limitation of the holding time. Nevertheless, this has been the standard in many countries for many years.

Differences in platelet counts can already be observed at the end of the whole blood holding time, even prior to the production of platelet concentrates. It was demonstrated that in whole blood sampled within 4 to 8 hours after collection, the platelet counts in whole blood appeared to be 10% to 20% lower than in units sampled after 8 hours and up to 24 hours after collection [29]. This suggests that initially after collection, platelets are activated and form small aggregates, which leads to an underestimation of the number of platelets in the unit when counted with a hematology analyzer. As will be outlined below, this also has consequences for the number of platelets in the final platelet concentrates.

In the late 1970s, at the time when studies were performed to extend the holding time from 6 to 8 hours, the in vivo quality of platelet concentrates from 8-hour-held whole blood, stored for 64 hours (making it 72 hours after blood collection), was determined. The recovery was 56% ± 15% with a survival of 7.3 ± 1.8 days [30], which was in line with other platelet recovery and survival studies (from whole blood that was stored for up to 4 hours) published at that time. A subsequent comparison of platelets from whole blood that was processed immediately or held for 8 hours and stored for 5 days revealed 44.4% ± 9.4% platelet recovery from the first group vs 44.5% ± 8.4% from the second [4]. The half time platelet survival was 4.0 ± 0.5 days for immediately processed vs 4.1 ± 0.5 days for 8-hour hold, respectively. These differences were not statistically significant. Moroff et al [19] in the mid-1990s experimented with 24-hour-held whole blood and found that platelet recovery after autologous retransfusion of (nonleukoreduced) PRP-derived platelet units stored for 5 days was 51.1% ± 14.9% when from 8-hour-held whole blood vs 50.6% ± 17.7% when held for 24 hours (not significant). Survival was 167.9 ± 30.7 vs 152.9 ± 51.5 hours (not significant). Platelet in vitro variables also indicated no difference between the 2 holding times, including no difference in platelet content. For leukoreduced PRP-derived platelet concentrates, in a paired comparison of 8- vs 24-hour room temperature hold, there were some differences in in vitro quality: pH was lower after a 24-hour hold, with initially a lower hypotonic shock response (HSR), but these differences resolved by day 5 of storage [13]. Neither platelet counts nor in vitro quality (ATP content, extent of shape change, thrombin response; CD62P expression was comparable initially but marginally lower in favor of the 24-hour hold group on day 8) differed. In a BEST Collaborative study comparing 3- vs 24-hour½ hold, with blood processed according to the PRP method, the platelet concentrates contained 30% more platelets when whole blood was held overnight [31]. No difference in CD62P expression was seen; initially, a lower HSR was observed for 3-hour-held whole blood, but during storage, this difference disappeared. Even more recently, a study was conducted in which whole blood was held for 22 ± 2 hours using either butane-1,4-diol plates or a room temperature incubator [32]. The in vivo quality of these platelets was compared with fresh platelets collected from the volunteer on the day of retransfusion of the stored platelet concentrates. After a 7-day storage period of the nonleukoreduced PRP-derived platelet concentrate, the recovery was 47% ± 13% for the group cooled with butane-1,4-diol plates and 53% ± 11% for those kept in the incubator; recovery of the "fresh" platelets was 63% ± 14%. In summary, the values of the stored platelets were 74% ± 11% of the fresh values and conformed to the preset requirements of greater than 66% [33]. Survival of stored platelets was 4.6 ± 1.7 days for butane-1,4-diol plates and 4.7 ± 0.9 days for the incubator vs 8.0 ± 1.5 days for fresh. Thus, the results of the stored platelets were 57% ± 14% of fresh and marginally did not conform to requirements of greater than 58%. It should be kept in mind that at present, PRP-derived platelet concentrates have a 5-day outdating, not 7, and it can thus be assumed that with the current outdating period, PRP-derived platelet concentrates can be prepared from overnight-held whole blood with good recovery and survival, with the potential to extend to 7 days under optimal storage conditions.

Effect of Whole Blood Holding Time on the Quality of Buffy Coats and Subsequently Prepared Platelet Concentrates

For platelet concentrates prepared from buffy coats, a more complicated 2-step procedure is involved; whole blood is first held for variable periods and then centrifuged, after which the buffy coat is isolated. These buffy coats need to rest for at least an hour, allowing postcentrifugation aggregates to dissolve (personal observation). Nowadays, most blood centers pool multiple buffy coats to have sufficient platelets for an adult, which again introduces a holding time, as not all buffy coats with the same blood group are simultaneously ready for further processing. Thus, the buffy coat pooling process also has a holding step. Once pooled, they are again centrifuged, after which the platelet-rich supernatant is expressed to a storage container resulting in the final platelet concentrate. Currently, during this final processing, the product is in most cases leukoreduced over an integrated white cell removal filter.

As indicated above, units processed close to the time of donation appear to have lower platelet counts, most likely due to small aggregates. The apparently lower platelet content immediately after donation translates into lower platelet numbers per platelet concentrate, as shown by a study [34] that compared the composition and storage of buffy coat–derived platelet concentrates from whole blood processed into platelet concentrates 4 to 6 hours after collection, with those from whole blood stored overnight. Platelet content was about a third lower when made from buffy coats at the day of collection rather than after overnight hold, irrespective of the 4 buffy coat pooling systems used in this study. On day 7, 1 of 5 platelet concentrates had a $pH_{22°C} > 7.4$ in platelet concentrates prepared on the day of collection in all 4 storage containers tested. For platelets from overnight-held whole blood, 0, 0, 2, and 1 of 5 experiments, respectively, had a $pH_{22°C} < 6.8$ by day 7 storage and none had a $pH_{22°C} > 7.4$ in all 4 platelet storage containers tested. It is quite probable that the different platelet concentrations at the various holding conditions caused this difference in pH; indeed, a subsequent analysis based on platelet concentration, for whole blood held for 16 to 20 hours, showed a strong relationship between platelet concentration and pH [35]. In a comparison of 8- vs 24-hour hold [34], glucose by day 7 of storage was lower in platelet concentrates from overnight-held whole blood, reflecting elevated glucose consumption, but the remaining glucose was still more than sufficient to allow adequate platelet metabolism. Consequently, lactate concentrations were higher in units from overnight-held whole blood. Platelet activation determined by CD62P expression was not different either at the beginning of storage or on day 7, but annexin A5 binding, a marker of apoptosis, was significantly higher at onset and remained higher throughout storage in units from fresh whole blood. Swirling, which is the ability of functional discoid platelets to align to a current in the storage medium, was not different. Others found a higher CD62P expression and annexin A5 binding at onset for platelet concentrates from 8- vs 24-hour-held whole blood, but this difference disappeared

on day 3 [36]. In this study, platelet yield was 14% lower in the 8-hour hold group.

A BEST study comparing 3- vs 24½-hour hold showed slightly more platelets in the whole blood when held overnight, which were somewhat more activated, 14 ± 8% vs 22 ± 11% CD62P expression ($P < .01$) [18]. Sampling of subsequently produced buffy coats showed that the platelet concentration was about 25% higher when held for more than 24 hours, but also that these platelets were less activated, 25% ± 12% CD62P-positive when processed from less than 8-hour-held whole blood vs 16% ± 10% CD62P-positive platelets when held for more than 24 hours ($P < .01$).

In summary, up to 6 hours after collection, small aggregates are present in the whole blood that lead to lower platelet numbers in the platelet concentrates (both PRP and buffy coat method), whereas an 8- to 26-hour hold results in higher platelet yields in the concentrates. Platelet activation is higher on the day of collection, both when measured in whole blood and in the platelet concentrate. This activation lowers during overnight hold as whole blood. In contrast, some authors found no effect of the holding time on platelet activation.

Holding of Red Cells Before Refrigerated Storage

Before final storage at 2°C to 6°C, red cell concentrates in additive solution may be held for a couple of hours to allow for filtration, segmenting of the tubing, and/or labeling of the units. This period can vary, and we previously performed a study comparing 0-, 6-, 12-, 18-, and 24-hour hold of red cell concentrates at room temperature from overnight-held whole blood, followed by (slow, 10-24 hours) cooling to 2°C to 6°C [37]. During this holding time, pH declined, and on day 3 (which was the first day all units had reached a temperature of 2°C-6°C), pH was 6.85 ± 0.02 for units with no holding time vs 6.68 ± 0.01 for units with a 24-hour holding time ($P < .001$). ATP was 5.2 ± 0.3 and 6.1 ± 0.3 μmol/g Hb ($P < .001$), and 2,3-DPG was 7.4 ± 1.3 and 0.9 ± 0.3 μmol/g Hb ($P < .001$), for 0- and 24-hour hold, respectively. These data indicate that during the holding time, adenine is taken up from the additive solution and converted into ATP at the expense of 2,3-DPG. Toward the end of storage on day 42, no lasting beneficial effect of room temperature holding time could be observed, as ATP was 2.5 ± 0.3 vs 2.6 ± 0.3 μmol/g Hb for 0- and 24-hour hold, respectively. Hemolysis was also not different. Thus, postprocessing room temperature hold of red cells gives initially higher ATP values, but toward the end of storage, the difference has disappeared.

Effect of the Holding Time of Buffy Coats on the Quality of Platelet Concentrates

The results described in the previous paragraph are from studies where the buffy coats were processed into platelet concentrates close to the time when the whole blood was centrifuged and the buffy coat isolated. However, buffy coats can also be held for a couple of hours or, in case of whole blood processing within 2 to 8 hours after collection, even overnight at room temperature for pooling and processing in platelet concentrates the next day. One study evaluated a number of holding times of the buffy coat, prepared after 3- to 6-hour hold of the whole blood, and saw an increase in platelet yields in those buffy coats: 52% ± 7% when buffy coats were immediately processed into platelet concentrates, 53% ± 10% when held for 90 minutes, 73% ± 4% when held for 3 hours, and lastly 74% ± 9% when the buffy coat was held for 12 hours before processing [38]. A more in-depth evaluation of 5-day stored platelet concentrates from the 3- vs 12-hour-held buffy coats showed no difference for pH, HSR, morphology scores, dense granula content, collagen-induced aggregation, and thromboxane production, but, ß-thromboglobulin content and thrombin-induced aggregation were somewhat better for platelet concentrates from buffy coats held for 12 hours than for 3 hours. Others, comparing a 4-hour hold of the buffy coat vs 24-hour hold (with buffy coats prepared from 2-hour-held whole blood) found some differences in platelet-derived growth factor concentrations initially, but by day 5 of storage, these effects had disappeared [39]. They also found no effect on fibroblast growth-promoting activity and ß-thromboglobulin, suggesting that the hold period of buffy coat did not affect granule content release. A study where whole blood was processed into buffy coats within 4 hours after collection followed by sampling of the buffy coat after 1, 3, 6, 12, and 24 hours revealed that the expression of activation antigens CD41, CD62P, and CD63 on platelets was fairly stable up to $t =$ 12 hours, but that significantly elevated values were found at $t = 24$ hours [40]. Interleukin (IL)-1ß, IL-6, and tumor necrosis factor α did not change, IL-8 rose somewhat, and in particular platelet factor-4 increased from 4.4 ± 3.6 to 14.1 ± 21.0 μg/mL between $t =$ 1and $t =$ 24 hours, indicating some α granula release. The authors concluded that storage of buffy coats should not greatly exceed 12 hours under these conditions.

An evaluation of platelets processed from buffy coats on the day of collection (holding times of whole blood not specified), from buffy coats held overnight for processing the following day, or from whole blood held overnight, showed no difference in platelet content [41]. Also, no significant difference in pH was found after 5-day storage, and neither for ADP aggregation at 37°C and lactate dehydrogenase (showing platelet lysis) release under any of the conditions tested.

A study comparing 4-hour-held buffy coat prepared from overnight-held whole blood vs a less than 4-hour hold of whole blood followed by 18-hour hold of the buffy coats prior to buffy coat pooling showed no differences in cell counts and minor differences in storage characteristics of subsequently prepared platelet concentrates [42]. They saw a slight but significant increase in platelet activation markers (such as CD62P expression, lysosomal integral membrane protein, annexin A5, factor V-Va, bound von Willebrand factor [vWF], and bound fibrinogen) during the holding period, but similar to the findings of Van der Meer et al [31], this dropped to baseline levels once the platelet concentrates were prepared. At onset of storage, no relevant differences were demonstrated. By day 7, CD62P, lysosomal integral membrane protein, and annexin V were again higher in the group where the buffy coat was stored for 18 hours rather than 4 hours. We gathered additional data with pooled buffy coat–derived platelet concentrates, as summarized in Table 1. The study design was close to our current routine within regular working hours: whole blood was collected in the morning or afternoon, processed later in the afternoon (if the buffy coat was held overnight), and processing was continued the next morning (if whole blood was held overnight). Platelet concentrates were then prepared by pooling 5 buffy coats and a unit of plasma, followed by soft spin centrifugation and transfer of the PRP to a storage container. Platelet numbers in the platelet concentrates were not different, but pH was lower at onset due to higher lactate levels when buffy coats were held overnight (16-20 hours), presumed to be originating from maintaining a high concentration of cells (platelets, white cells) in a small container. CD62P expression and annexin A5

Table 1
In vitro quality of platelet concentrates in plasma from pooled buffy coats, either from buffy coats held overnight or from whole blood held overnight (n = 12, mean ± SD)

	Buffy coat held overnight	Whole blood held overnight
Platelets ($\times 10^9$/U)		
Day 1	341 ± 48	342 ± 22
pH		
Day 1	6.92 ± 0.03	7.04 ± 0.01*
Day 8	7.04 ± 0.06	7.07 ± 0.05
Lactate (mmol/L)		
Day 1	7.9 ± 0.7	4.9 ± 0.5*
CD62P expression (%)		
Day 1	29 ± 10	6 ± 2*
Day 8	26 ± 8	15 ± 3*
Annexin A5 binding (%)		
Day 1	24 ± 13	4 ± 2*
Day 8	32 ± 9	20 ± 4*

* $P < .001$, unpaired t test.

were also higher, and this difference remained present until day 8 of storage. Experiments with SSP+ platelet additive solution showed the same patterns (data not shown), although the use of SSP+ resulted in somewhat lower CD62P expression of 26% ± 6% in the units where the buffy coat was held overnight vs 12% ± 1% for whole blood held overnight by day 8 ($P < .001$), as well as lower annexin A5 binding, 24% ± 7% when from overnight-held buffy coats and 10% ± 3% for overnight-held whole blood ($P < .001$).

Dijkstra-Tiekstra for the BEST Collaborative coordinated a multisite study and found that processing into platelet concentrates after 2 to 8 hours resulted in low platelet yields with platelet concentrates containing $201 \pm 75 \times 10^9$ platelets [43]. Those from overnight-held buffy coats contained $285 \pm 55 \times 10^9$ platelets and those from overnight-held whole blood, $338 \pm 55 \times 10^9$ platelets (all $P < .05$). These different platelet counts remained present throughout the remainder of the storage period. Those processed into platelet concentrates on the day of collection showed a higher initial CD62P expression that remained present throughout storage when compared with overnight-held whole blood. For annexin A5, ADP and collagen aggregation, and HSR, the differences were minimal, although the best values were always for overnight-held whole blood.

Using the fully automated separators Atreus (to separate whole blood into red blood cell, plasma, and buffy coat) and OrbiSac (to produce a platelet concentrate from pooled buffy coats) [44], platelet count was about 10% lower when blood was processed within 8 hours after collection with overnight hold of the buffy coat, compared with overnight-held whole blood and buffy coats processed within 4 hours. During storage, glucose, lactate, intracellular ATP content, extent of shape change, and the release of RANTES were not different. Hypotonic shock response was somewhat better preserved in units from overnight-held whole blood. A complete panel of platelet markers (CD62P, CD61, CD63, CD42b, CD41) showed minor differences between the holding conditions and were deemed to be of no clinical relevance.

The above data show that not only the holding time of whole blood (a longer holding time seems better) but also the holding time of the buffy coat (a shorter holding time seems better) influences the platelet concentrates produced. We wanted to investigate if there was an optimum between these variables; we aimed to facilitate platelet production of from morning whole blood collections as these are usually centrifuged on the day of collection and after limited hold processed into platelet concentrates. To investigate whether buffy coat processing could be performed on the day of collection, but platelet concentrate preparation could take place the next day, we carried out unpaired experiments; 4 conditions were compared where whole blood from morning collections was processed either after 4 hours or after 8 hours, and held overnight as individual buffy coats or as pooled buffy coats. In order to have the platelet concentrates ready within 24 hours after blood collection (a regulatory requirement in the Netherlands), the units processed after 4 hours had a holding time of the buffy coat of 18 hours, and those processed after 8 hours, a holding time of 14 hours. The various holding times are shown in Table 2. In these experiments, a 4-hour holding time for whole blood was chosen, as this is the minimum resting time according to our guidelines; 8 hours was chosen to have reasonable working hours if whole blood was to be processed on the day of collection. The 14- and 18-hour holding times for the buffy coats reflect the remaining allowed holding time before the 24-hour room temperature holding time of whole blood has elapsed. Volumes and platelet counts were not different, but the lactate concentration was higher and thus pH lower if held overnight as a buffy coat pool than as single buffy coats. Platelet activation (CD62P positive) was initially higher in units stored as pooled buffy coats (C and D) and might be related to the lower pH, but the annexin A5 was higher in units that were processed into buffy coats after 4 hours (B and D), suggesting a link with the holding time of whole blood. In any case, the differences disappeared during storage.

Our study shows that there are differences in CD62P expression and annexin A5 binding of the platelets immediately after the holding period, but these differences disappeared at the completion of their maximal storage time.

Summarizing, a certain holding time (1-4 hours) of buffy coats before processing into a platelet concentrate might be beneficial and result in higher numbers of platelets per unit. A long hold (12-24 hours) of the buffy coat might, however, lead to platelet activation because a high concentration of cells is stored in a small volume.

In-Process Holding Times

Besides whole blood and buffy coat holding times, which have been studied in-depth, there are also some in-process holding times for which data are limited. An example is the holding time after pooling of buffy coats but before centrifugation. Dutch guidelines limit this time to 4 hours. A comparison of buffy coat pools from overnight-held whole blood that were either held for 4 hours after pooling (A, n = 6) or held for 1 hour (B, n = 3) was performed. At $t = 0$ hours, there were no differences between the 2 groups for any of the in vitro measures (data not shown). By $t = 4$ hours, pH had dropped from 6.95 ± 0.03 at $t = 0$ to 6.89 ± 0.04 ($P < .01$, analysis of variance, Dunnett post-test) in group A, caused by an increase in carbon dioxide concentration from 82 ± 12 mm Hg at $t = 0$ to 92 ± 16 mm Hg at $t = 4$ hours ($P < .01$). Platelet activation was unchanged, and a minor increase in annexin A5

Table 2
In vitro quality of platelet concentrates in plasma from whole blood held for 4 or 8 hours, with the buffy coats or buffy coat pools held for 18 or 14 hours (n = 12, mean ± SD) Statistics

	A	B	C	D	
Resting periods (h)					
Whole blood	8	4	8	4	
Buffy coat	14	18	1	1	
Buffy coat pool	1	1	14	18	
	A	B	C	D	
Day 1					
Volume (mL)	363 ± 20	355 ± 26	349 ± 17	353 ± 18	Not significant (ns)
Platelets ($\times 10^9$/U)	367 ± 36	360 ± 46	379 ± 51	377 ± 79	ns
pH	6.92 ± 0.02	6.90 ± 0.03	6.74 ± 0.05	6.72 ± 0.05	All $P < .001$, except A vs B; C vs D: ns
Lactate (mmol/L)	8.8 ± 0.7	9.2 ± 0.8	12.4 ± 1.6	12.5 ± 1.5	All $P < .001$, except A vs B; C vs D: ns
CD62P expression (%)	19 ± 7	20 ± 6	27 ± 8	30 ± 12	ns except A vs D: $P < .05$
Annexine A5 binding (%)	12 ± 6	18 ± 7	12 ± 4	16 ± 7	ns
Day 8					
pH	7.00 ± 0.06	6.99 ± 0.07	6.92 ± 0.11	6.96 ± 0.09	ns
Lactate (mmol/L)	19.0 ± 1.5	17.7 ± 2.0	19.5 ± 2.1	18.5 ± 2.1	ns
CD62P expression (%)	26 ± 6	23 ± 7	23 ± 7	23 ± 5	ns
Annexine A5 binding (%)	35 ± 7	36 ± 8	32 ± 7	36 ± 7	ns

was seen from 4% ± 1% to 6% ± 2% (not significant). Additional sampling during this study at $t = 1$ hour (which is approximately the current hold time in our routine) and $t = 5$ hours (beyond the allowed hold time) showed that without adverse consequences, buffy coat pools can be held for at least 4 hours before centrifugation.

Longer holding times of buffy coats that contain high numbers of white cells may mean that there are differences in cytokine concentrations excreted by these white cells. This was illustrated by a study that compared holding buffy coats for 6 or 16 hours (produced within 4 hours after collection) [45]. This study showed minimal changes in IL-6, tumor necrosis factor α, and IL-1β, but an increase in high mobility group box 1 at $t = 20$ as well as IL-8. In a follow-up experiment, it was demonstrated that the sharp increase in IL-8 was indeed caused by release from white cells and not platelets. The authors comment that the concentrations are probably not high enough to cause febrile reactions in patients.

Also for PRP-derived platelet concentrates, there is one in-process holding time, namely, the postcentrifugation resting of the platelet pellet. A comparison of 5-minute, 1-hour, or 4-hour resting of the platelet pellet after removal of platelet-poor plasma showed minor differences during 5 days of storage [46]. More units showed a "substantial amount" of aggregates if held for only 5 minutes vs those held for 1 or 4 hours. A radiolabeled recovery study confirmed these results with a recovery of 49.9% ± 15.3% for 0- to 5-minute resting vs 50.9% ± 20.2% for 1-hour resting; survival times were 111.2 ± 50.7 and 114.9 ± 43.8 hours, respectively.

Effect of Holding Time on the Quality of Plasma

Plasma is the remaining component that is affected by the holding time of whole blood. As indicated previously, a 6-hour hold had no effect on factor VIII:C [3]. Eight-hour hold led to a decrease by about 10% in the levels of fibrinogen, plasminogen, fibronectin, and factor V, but factor VIII activity was not affected [47]. A full 24-hour holding time led to a decline in factor VIII:C of about 20% [6], but the implication was thought to be small for routine use. In a follow-up study, whole blood held less than 3 hours had a factor VIII activity of 0.82 ± 0.03 IU/mL, whereas those held for 12 to 15 hours contained 0.73 ± 0.03 IU/mL factor VIII:C ($P < .001$) [48], which was higher than after the same hold period at 4°C. However, in the small-scale cryoprecipitate made from these units, the difference in factor VIII content was not statistically significant. In another study, plasma factors were determined in blood processed immediately, or held for 8 or 24 hours at room temperature [49]. This study showed that factor V, factor II, factor X, fibrinogen, AT-III, protein C, and protein S were stable under all conditions tested. Factor VIII dropped by 14% during the 8-hour ambient hold and dropped further to values that were about 26% lower than the initial values. Others confirmed these results for 18-hour-held whole blood [50] but also found a slight increase of C3a-desArg, indicative of complement activation with no clear cause; however, the authors suspected that the clinical relevance was low because it is quickly detoxified after release. Serrano et al [51] compared freshly processed PRP-derived plasma clotting factors with overnight-held buffy coat–derived plasma and demonstrated that for an extended panel of clotting factors (14 in total), values for various plasma clotting factors from overnight-held whole blood fell between 84% and 130% of fresh. Only factor VIII in the plasma was about 28% lower, whereas cryoprecipitate from overnight-held whole blood had about 20% lower factor VIII than fresh blood. Factor XIII was 3 times higher and vWF multimers (band count) was 20% lower, but fibrinogen, vWF (antigen level, activity, and band density), and A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS)-13 were not affected. A comparison of 8 vs 24 hours found no difference for factor V, vWF, and fibrinogen in fresh-frozen plasma, cryoprecipitate, or cryo-poor plasma, and the differences in factor VIII:C reached only statistical significance in cryoprecipitate, 3.9 ± 0.8 U/mL for 8-hour-held whole blood vs 3.5 ± 0.7 U/mL for 24-hour-held whole blood [13]. Our group found a decline in factor III:C from 1.22 ± 0.31 IU/mL when processed within 2 hours of collection vs 1.05 ± 0.25 IU/mL when processed after 16 to 20 hours (14% lower; $P < .001$) [14]; others found no difference in factor V and a 33% or 12% lower value for factor VIII, in a study that reported the results of 2 processing sites [19]. An evaluation of 9 clotting factors showed the same pattern as others [36]. Finally, a multisite study of the BEST Collaborative [52] demonstrated a 6% lower protein C and a 14% lower protein S, but no change in antithrombin for whole blood held for 24 to 26 vs less than 8 hours in a paired study; factor VIII was 23% lower.

In summary, mainly factor VIII:C is affected by the room temperature holding period and decreases by about 1% per hours of hold. Despite the changes in coagulation factors, the overall ability to generate thrombin or form a clot as demonstrated by various assays is unaffected.

Leukoreduction

As shown above, platelets are more or less activated, depending on the timing of the various holding times of whole blood and/or platelet concentrates. Platelets play a role in the leukoreduction process particularly in red cell concentrates [53], and their activation status might affect the efficacy of leukoreduction. It can be further speculated that the white cells themselves could have various levels of activation, which might influence their ability to adhere to filter fibers.

Most of the data to date show that leukoreduction by filtration closer to the time of blood collection results in better white cell removal. For whole blood filtration, leukoreduction of units held for 8 or 18 hours showed twice the amount of residual white cells in the latter group, although leukoreduction conformed to the requirement of $<1 \times 10^6$ per unit and was in fact below 0.5×10^6 per unit [54]. Whole blood held for 8 or 24 hours after removal of PRP and subsequent filtration of the red cell concentrates resulted in $0.48 \pm 0.24 \times 10^6$ residual white cells for 8-hour hold vs $2.27 \pm 2.05 \times 10^6$ for 24-hour hold ($P < .05$) [13]. In the 8-hour group, all complied with the required less than 5×10^6 per unit, but 12% failed for 24-hour hold. Evaluating different leukoreduction filters for red cell concentrates, we found that there was an increase in the number of cells with longer holding times of the whole blood prior to processing [29]. As an example, use of the Baxter filter resulted in a median of 0.03×10^6 white cells per unit when processed within 4 to 8 hours vs 0.17×10^6 per unit when held for 20 to 24 hours. Fresenius and MacoPharma filters showed the same trend. Also, others found the best leukoreduction in red cells processed on the day of collection within 8 hours of collection, whereas those held for 24 hours sometimes had values that were "higher than expected," but nevertheless conformed to the requirement that 95% of the units contained less than 5×10^6 white cells [15]. Others found no effect of the holding time on white cell reduction, using a filter for red cell concentrates, comparing 4- to 6-hour hold of whole blood vs 22- to 24-hour hold with subsequent component separation and immediate filtration of the red cell concentrates [55].

In the recent years, whole blood and red cell filters have been optimized for leukoreduction of either fresh or overnight-held whole blood. Nowadays, these are minimal differences in residual numbers of white cells among the groups [20] (unpublished observations).

For platelet concentrates, leukoreduction by filtration was about 3 times more efficient when performed on buffy coats from overnight-held whole blood than on those processed on the day of collection and was independent of the combination of buffy coat pooling system and filter used [34]. Our study comparing overnight hold of buffy coats vs whole blood (Table 1) showed a median residual number of white cells of 0.13×10^6/unit for buffy coats held overnight vs 0.06×10^6/unit if whole blood was held ($P = .005$).

In summary, leukoreduction by filtration is more efficient when performed closer to the time of collection, although even after 24 hours, the number of white cells conforms to current requirements. Newly developed filters seem to be less affected by the holding time of whole blood.

Bacteriologic Aspects to the Holding Time of Whole Blood

Storage of blood at room temperature has always been looked upon with suspicion, as this is a temperature that propagates bacterial growth. However, the contrary was found true as the risk of bacterial growth is related both to the timing and efficiency of white cell removal.

Most studies have been conducted with *Yersinia enterocolitica*, as it can be present in donated blood and is able to survive and proliferate in red cell concentrates at 4°C. Paired studies were performed where various inocula were spiked into whole blood, which was processed into red cell concentrates after 6-hour hold of whole blood at 4°C as control group, or had the buffy coat (with about 70% of the white cells) removed after 20-hour hold of whole blood at ambient temperature, or had the buffy coat removed after 20-hour hold with subsequent filtration to remove more than 99% of the white cells [7]. The results indicated that at the highest inoculum of 3×10^4/mL, all red cell concentrates showed growth, but those from whole blood held for 6 hours at 4°C tested positive almost instantly, whereas those held overnight at room temperature with buffy coat removal or filtration showed growth at a later time point, ranging from 1 to 5 weeks. At an inoculum of 1×10^2 or below, the overnight-held units remained sterile, whereas 2 of 5 units processed after 6-hour hold at refrigerated temperatures still showed growth. This shows that a certain holding time is needed to ingest bacteria by white cells, which needs to be followed by adequate removal of white cells by filtration. Another spiking study with *Y enterocolitica* showed that in units that were filtered 5 hours after spiking during room temperature hold, all tested negative for bacteria for the remainder of the 42-day storage period, whereas unfiltered units showed high bacteria titers [56]. Other studies confirmed that reduction of the number of white cells, either by buffy coat removal or filtration prevents bacterial outgrowth in blood components, provided that there is sufficient time for the white cells to ingest bacteria [57,58]. One study is of particular interest where a panel of 5 different bacteria was used to spike white cell–containing or white cell–reduced preparations of either buffy coat or whole blood, at "moderately large" and "large" numbers of bacteria [59]. Units were sampled immediately after spiking and after 2, 5, 18, 24, and 48-hour room temperature hold. Inhibition of bacterial growth was shown only when white cells were present. Antibiotic-sensitive *Staphylococcus epidermidis* could not be demonstrated after 24 hours and thereafter, and antibiotic-resistant *S epidermidis*, after 5 hours or thereafter; *Staphylococcus aureus* showed no signal after 5 hours at the lower inoculum but showed growth at $t = 18$ hours and thereafter, and the high inoculum showed growth at all time points; *Escherichia coli* was still demonstrable at 18 hours but not at $t = 24$ hours and thereafter; and *Propionibacterium* sp at the lower inoculum was cleared immediately, whereas at the higher inoculum, bacteria could be shown at $t = 24$ hours, but not at $t = 48$ hours. Lastly, *Pseudomonas aeruginosa* was absent in all preparations at all time points, also in the leukoreduced ones. These data show that most bacteria species are ingested by white cells and that, depending on the bacterium and concentration tested, it takes somewhere between 5 and 24 hours at room temperature for the white cells to clear the bacteria, resulting in culture-negative units. In the absence of a leukoreduction step, a comparison of 8- vs 24-hour room temperature hold of spiked whole blood, followed by component separation according to the PRP method, showed significantly (1-1.5 $\log_{10}$) more bacteria in the 24-hour group in the red cell concentrates, but no difference for platelet concentrates or plasma [60]. Additional experiments where the 24-hour group was leukoreduced by filtration showed a reduction of the bacterial titers in the red cell concentrates to those that were held at room temperature for only 8 hours without leukoreduction; subsequent 42-day storage of the red cell concentrates showed almost no persistent effect for 8 of the 10 tested bacteria, except *Staphylococcus pyogenes* with a titer of 0.8 $\log_{10}$ in the 8-hour-held nonleukoreduced group vs 2.3 $\log_{10}$ in the 24-hour-held leukoreduced group, and *Y enterocolitica* with a titer of 8.0 $\log_{10}$ vs 0.4 $\log_{10}$ in these groups, respectively. Others compared growth of *S epidermidis* and *E coli* after 6- or 16-hour hold of whole blood at room temperature and showed that for subsequently prepared platelet concentrates, there was no benefit of an extended holding time for the first bacterium, but for *E coli*, already by day 2 of storage, all contained spurious amounts of bacteria, whereas 4 of 5 in the 16-hour group showed no growth, and even by day 5, 2 showed no growth, 2 showed less than 10 colony-forming units/mL, and only 1 contained high numbers of bacteria [61].

Lastly, a meta-analysis compared the risk of occurrence of bacterial contamination of PRP-derived and buffy coat–derived platelet pools vs apheresis platelet concentrates [62]. The risk was modeled assuming that both PRP- and buffy coat–derived pools were formed from 5 donations and indicated that PRP-derived platelet pools had an approximately 5 times higher risk of bacterial contamination than single-donor apheresis platelets, but that buffy coat–derived platelet concentrates showed no increased risk. Because 2 of the 3 publications used as source for the meta-analysis described pools from leukoreduced PRP-derived platelet concentrates, leukoreduction is an unlikely candidate to explain the observed difference. An alternative explanation might be that PRP-derived platelet pools are from whole blood held for at most 8 hours (as required in the United States), whereas the publications cited for buffy coat–derived platelet pools all had an overnight holding time for the buffy coat (1 publication) or of the whole blood (3 publications).

Summarizing, holding of whole blood for at least 8 hours after collection promotes ingestion of bacteria by white cells; subsequent removal of leukocytes by filtration, before they disintegrate, reduces the risk of bacterial contamination of blood components made from this whole blood.

Is Overnight Hold the Same as 24-Hour Hold?

An issue that arises every so often is the definition of the term "overnight." The general perception is that overnight hold means that whole blood is held as long as possible from the day of collection to the day of processing. In blood bank practice, however, this is often not the case. In many countries, the holding time is limited to 24 hours, also because the European Pharmacopeia requires freezing of plasma within 24 hours after donation [63]. This means that for units collected during morning sessions, processing on the following day is too late, certainly if secondary processing like leukoreduction and/or platelet processing from buffy coats needs to be performed. Consequently, units from morning collections are often processed the same day in order to be able to comply with the 24-hour rule. Whole blood collected in the afternoon and the evening is held overnight and processed the next day and is routine practice at our blood center in Amsterdam. To illustrate this, the collection and processing time of all units processed between January 2, 2013, and August 12, 2013, at the Amsterdam blood center were acquired from our computer information system. At our center, morning

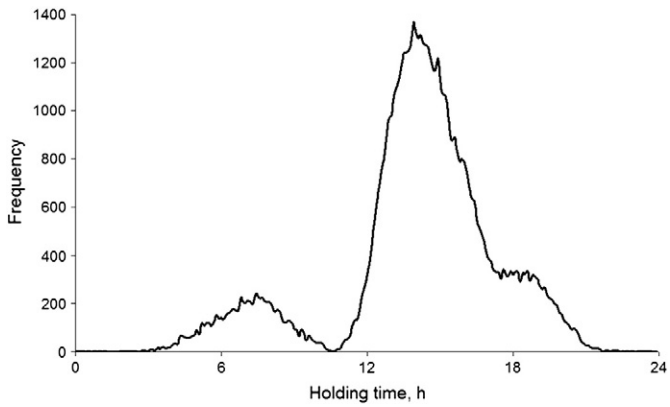

Figure. Holding times of routinely processed units of whole blood from the Amsterdam blood center (January-August 2013, n = 65535).

donations are separated into components between 4 PM and 7 PM on the same day; this amounts to about 12% of the total number of units processed daily. The remaining 88% are from afternoon and evening collections and are separated between 7.30 AM and noon of the following day. The holding times are shown in Figure, indicating peaks between 5 and 9 hours after collection (these are the morning donations processed on the same day) and between 11 and 20 hours after collection (units held overnight). Thus, with the practice of overnight hold of whole blood, the median processing peak is 14 hours after donation, and all units are separated well within 22 hours of donation.

Although overnight hold might allow holding whole blood for 24 hours, at our blood bank routine, the holding time is on average 14 hours with a maximum of 22 hours after donation. This can vary from center to center.

Holding of Whole Blood Beyond 24 Hours

Is storage beyond 24 hours possible? Such a question has only been addressed for whole blood that underwent no further component processing [64]. Apart from a depletion of 2,3-DPG at $t = 48$ hours when either stored at 19°C or 25°C, small changes in red cell quality with respect to hemolysis, ATP, and free potassium were found. For clotting ability of the plasma, the PT was prolonged from 13.3 ± 0.6 to 14.7 ± 0.7 seconds ($P < .05$), but overall clotting factors, including factor VIII, were not affected. Changes in platelet quality were modest.

Practically, there is no need to hold whole blood for long periods after blood collection, but a slight extension of the holding time might be attractive. This mainly concerns units collected in the morning that, due to the 24-hours limit, must be processed on the day of collection. This may not be a problem, as staff can perform this in the afternoon during business hours, but it might add flexibility if those units could be processed with the bulk of the units the following day. Thus, a holding time up to 30 hours might be appealing. Both the publication by Hughes et al [64] with a 72-hour storage and by Van der Meer et al [18] with a 24- to 26-hour storage time show that after 24 hours, there is no sudden decline in quality of whole blood or its components, and it should be emphasized that the 24-hour time limit was chosen arbitrarily based on data of the longest storage time that was investigated [6], not by proof that after 24 hours, the units fall off a cliff.

Discussion

Overnight hold of whole blood at ambient temperature has brought many logistic benefits, where units can be separated into components uniformly during business hours. The effect of overnight hold is surprisingly small: both red cell recovery [4,20] and platelet recovery and survival [4,19,32] are virtually unaffected. In vitro, though, there are changes. In red cell concentrates, the 2,3-DPG concentration rapidly decreases, which seems to indicate that the quality diminishes rapidly. However, at a somewhat slower rate, this also occurs in units held up to 8 hours; 2,3-DPG is 0 after 2- to 3-week storage regardless of the initial holding time. Moreover, the clinical relevance of this observation is probably small. A longer holding time results in the accumulation of lactate and consequently a slightly lower pH, but this does not adversely affect red cell quality. Although there is some variation between studies, for other red cell quality measures like free potassium and free Hb are more or less unaffected by overnight hold of whole blood.

The lower pH of whole blood after overnight hold causes a lower pH of PRP-derived platelet concentrates, which is reflected in a lower HSR at the onset of storage. Other in vitro measures show no difference and, if present, disappear during storage. There is some discrepancy between studies that show no difference in platelet content in the platelet concentrate after overnight hold [13,19] or 30% higher platelet concentration [18]. This is also seen with the buffy coat method where some find a difference [34], whereas others do not. It seems that the precise holding time is critical, as units processed within 4 to 6 hours show lower platelet contents than those processed 8 to 26 hours after collection, but further studies are needed. In general, it can be stated that most studies indicate a higher recovery from whole blood after a longer holding time; the minimal holding time is to attain a higher recovery remains unknown. Because the in vitro tests indicate that the platelets are initially more activated, it could be that specific centrifugation conditions result in platelet aggregates, which are lost during transfers to satellite bags. Alternatively, certain foils used for blood bag containers could stimulate adhesion of platelets to the bag, which might also depend on centrifugation conditions [65]. Although less likely, the method of cooling whole blood to room temperature might affect the activation status of the platelets. With the differences observed, it might be interesting to investigate this finding in more detail.

Holding the buffy coat instead of whole blood seems to be less optimal, based on in vitro measures. Platelets become more activated and show markers of apoptosis, and the maximum hold time of buffy coats of 12 hours before processing in platelet concentrates seems, though executed with individual buffy coats [40], still valid. Our data with buffy coat holding times of 14 and 18 hours seem to confirm this finding, although the platelets still show acceptable quality. Any in-process holding time, that is, after buffy coat pooling before centrifugation, during the resting time of PRP-derived platelets after removal of the platelet-poor plasma, appears to have no effect.

In plasma, the 10% to 30% reduction in factor VIII is most noticeable. However, the overall functionality of the plasma seems unaffected [52]. Other factors show some fluctuations between publications, but fall within the biological variation among blood donors and the analytical variation of the tests used.

Possibly because of initial platelet activation, leukoreduction (by adherence of platelets to filter fibers, which causes adherence of white cells) is more efficient close to the moment of collection; in almost all studies, the number of residual white cells is higher both in red cells and in platelet concentrates. Nevertheless, the difference is not large enough for these products to fall outside the upper limit for white cell content, especially after introduction of the newest filters.

Lastly, the elegant study by Högman et al [59] demonstrates that white cells ingest bacteria, which either are killed or can be removed by buffy coat removal or filtration before the white cells starts to disintegrate and release the remaining viable bacteria. The optimum room temperature holding time appears to be somewhere between 5 and 24 hours. A meta-analysis [62] showed that buffy coat–derived and single donor apheresis platelet concentrates have equal risk of bacterial contamination. The publications used to calculate the risk for PRP-derived platelet concentrates did not specify the holding time of the whole blood used for their platelet concentrates, but was shorter than 8 hours. We can conclude that within the time frame of 5 to 8 hours after collection, there is little protective effect against bacterial contamination and that indeed overnight hold (>8 and <24 hours) is beneficial for bacterial safety. A summary is given in Table 3.

Overall, holding whole blood at room temperature for at least 24 hours has no deleterious effect on red cell recovery, platelet recovery and survival, and plasma functionality, despite some differences in in vitro measures. The 24-hour limit has been chosen because that is the longest investigated time reported in the literature, but there is no evidence that support a sudden decline in quality after this 24-hour hold. Logistically, to allow for overnight hold of units collected in the morning time, an extension to 30 or 36 hours might be attractive and should be an area of research. A hold time of 72 hours for whole blood has shown to give no large differences compared with shorter holding times, but no components were prepared. Changes in 2,3-DPG seem dramatic but have little clinical impact.

Overnight hold of whole blood results in an increased number of platelets per concentrate, but other factors than the holding time itself are likely to influence this finding. Holding the buffy coat rather than whole blood for longer than 12 hours leads to platelet activation and degranulation, and there is no improvement if buffy coats are held as a

Table 3
Summary of the effects of overnight hold of whole blood on the quality of various blood components

Measure	Effect of overnight hold	References
Red cell concentrates		
^{51}Cr survival	No effect	[19,20]
ATP concentration	No effect	[5,6,13–20]
2,3-DPG concentration	Decreases by two-thirds. Also decreases by ~10% in units processed on the day of collection. After day 14 of storage, no difference between groups. Clinical consequence probably low	[6,13–20]
pH, lactate	pH lower, lactate higher, but disappears during storage	[13–20]
Hemolysis	Somewhat higher, but within specifications	[13–20]
Other red blood cell quality measures	Minor effects that disappear during storage	[13–20]
Leukoreduction	Less efficient with older types of filters, but residual white cells within specifications. For currently marketed filters no difference	[13,15,20,29,55]
Platelet concentrates		
Recovery/ survival	No effect (shown for PRP-derived platelets only)	
Platelet concentration	0-30% higher	[4,19,30,32]
pH, lactate	pH lower, lactate higher, but disappears during storage	[6,13,29,31,34,36]
HSR	HSR initially lower, but disappears during storage	[13,31,34,36]
Other PLT quality measures	Minor effects that disappear during storage	[13,31,34,36]
Leukoreduction	Less efficient with older types of filters, but residual white cells within specifications. For currently marketed filters no difference	[34],Table 1
Risk of bacterial outgrowth	Optimal when leukoreduction performed after 8- to 24-h room temperature hold (shown for buffy coat–derived platelets only)	[7,56–61]
Plasma		
Factor VIII	10%-30% lower, but clinical consequence probably low	[3,6,13,14,19,47–52]
Other clotting factors	no effect	[13,19,47,49–52]

Details are given in the text.

pool. In-process holding of the buffy coat pool up to 4 hours has no effect. For PRP-derived platelets, a resting time of the platelet pellet before resuspension up to 4 hours has no effect. Leukoreduction for all cellular components is more efficient when close to the time of collection, but up to 24 hours, the units still conform to current requirements. More recently introduced white cell removal filters seem less sensitive to this difference. Besides the logistic advantages, the prevention of bacterial contamination is probably the other large benefit of overnight hold. However, evidence needs to be gathered in a phase IV trial to determine whether this is also true for PRP-derived platelet concentrates.

Conflict of Interest

None of the authors report a conflict of interest.

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