

**UKBTS General Information 03**

**Deviations from 4 °C temperature storage for red cells: Effect on viability and bacterial growth**

**December 2009**

**Prepared by:** Standing Advisory Committee on Blood Components

***This document will be reviewed whenever further information becomes available. Please continue to refer to the website for in-date versions.***

**1. Introduction**

**a) Regulatory requirements for storage temperature**

The current requirement in the UK [1] is that red cell components must be stored within the temperature range 2 – 6 °C. Exceptionally, it is allowed that the storage temperature may extend from 1 °C up to 10 °C, providing that this deviation has happened on one occasion only, and that the duration is no longer than 5 hours.

In addition, the UK Guidelines allow temperatures up to 10 °C for up to 12 hours during transport. The Council of Europe Guidelines [2] allow up to 10 °C for 24 hours during transit. The aaBB Standards [3] and aaBB Technical Manual [4] state that blood storage and transit temperature should not exceed 10 °C but no time limit is stated.

No amendment to these rules is proposed.

**b) The '30 minute' rule**

The other occasion when red cells are removed from their normal refrigerated storage is prior to transfusion. Although the UK Guidelines (the Red Book) do not give any guidance on how long blood can be out of controlled temperature before transfusion is commenced, the British Council for Standardisation in Haematology (BCSH) guidelines [5] refer to the Handbook of Transfusion Medicine [6] and state that: "If a unit of blood has been out of the refrigerator for more than 30 min and there is no prospect of its imminent transfusion, the hospital blood bank should be informed that it has been unrefrigerated for more than 30 min, and the blood returned to the hospital blood bank for disposal because of the risk of bacterial growth".

The 30 minute rule can be problematical, as it may not always be possible to commence the transfusion within this timeframe, and subsequent return of the unit to blood bank for discard is wasteful and can delay transfusion further.

The Handbook of Transfusion Medicine also has the instruction "complete the infusion within four hours of removal from controlled temperature storage".

This paper reviews the literature on red cell temperature deviations and their effects on red cell viability and potential for increasing the risk of bacterial contamination of the unit, and recommends amendment of these rules based on the evidence available.

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**2. What is the origin of the '30 minute rule'?**

It is likely that this rule originated as a result of the work of Pick and Fabijanic [7] who investigated the time taken for a unit of cooled blood to reach 10 °C when removed from the refrigerator. They found that, whether the unit was handled or not, the surface temperature reached 10 °C between 15 and 30 minutes after removal into ambient conditions, whereas core temperature took 45 – 60 minutes. 30 minutes thus would appear to be a reasonable cut-off to ensure that the core temperature did not rise above 10 °C .

**3. Viability of red cells**

**In-vitro studies**

Storage of red cells at 4 °C decreases the metabolic rate of the cell and enables blood to be stored for longer periods. At higher temperatures, the rate at which glucose is consumed and lactate produced is increased, leading to a lowering of pH. This in turn stimulates 2,3 DPG phosphatase, resulting in a rapid reduction of 2,3 DPG, a molecule that competes with oxygen for the same site on the haemoglobin molecule, reducing the oxygen affinity of haemoglobin and increasing oxygen delivery to the tissues. At 30 °C it has been estimated that within 4 hours 2,3 DPG will have fallen to 35% of the initial concentration, and it will be totally depleted within 18 hours [8].

Several workers have looked at the effect of intermittent and repeated warming on red cell metabolism. Strauss et al [9] stored ACD blood at different temperatures, and on the basis of changes to 2,3 DPG, ATP, pH, extracellular K<sup>+</sup> and Hb concentration, concluded that acceptable shelf-life was 9 days at 10 °C, 6 days at 15 °C and 3 days at 20 – 25 °C. If adenine and guanosine were added, the storage times were increased to 20, 10 and 5/4 days respectively.

Shields [10] stored units of whole blood in ACD-A at 4 °C or at 10 °C for 28 days, and saw no difference between the two groups for plasma Hb, K<sup>+</sup>, haematocrit or osmotic fragility. This provides some evidence to support the upper limit of 10 °C being acceptable with respect to red cell metabolism.

Shields [10] also exposed WB units (otherwise stored at 4 °C) repeatedly to either 10 °C or 22 °C for varying periods of time (between 1 – 24 hours). Those exposed to 10 °C at weekly intervals showed no difference from the controls for parameters tested (plasma Hb, K<sup>+</sup>, Hct). However, repeated exposure to 22 °C showed elevation of plasma Hb at day 21, though not earlier in the storage period. Unfortunately, data is only provided for 16 and 24-hour periods of exposure at this temperature and not shorter periods.

Ruddell et al [11] compared CPDA-1 packed cell units warmed to 25 °C for 24 hours at either day 6 or day 20 with control units. The warmed units had lower concentrations of glucose, higher lactate, and lower pH than controls. The rates of decrease in ATP were greater in the

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warmed units during the week after warming compared with controls. There was no statistically significant increase in plasma Hb, and in all units haemolysis did not exceed 1%. The authors concluded that one day of storage at 25 °C accelerates essential metabolic breakdown equivalent to 10 days of storage at 1 – 6 °C, and extrapolated this observation to predict that a single 2-hour exposure to ambient temperature might be expected to reduce the storage life of a unit by one day.

Reid et al [12] studied red cells in additive solution (AS-5). Units were warmed to 25 °C for 24 hours on day 14 or day 28. Glucose, ATP and pH declined more in the warmed units, and haemolysis was less than 1% in all units. Mean cell ATP concentrations in the warmed cells at day 30 of storage were approximately equivalent to those in cold-stored cells at 42 days, suggesting an enhanced aging of the cells.

Ecker & Hitzler [13] performed a similar study, but exposed units to ambient temperature for a shorter length of time. CPDA-1 red cells were exposed to 20 °C for 6 hours on day 5, day 15 or day 30 of shelf life and compared to continuously refrigerated controls. The warmed units had a lower ATP content than controls, but this was greater than 50% of the initial concentration and all values were above the level sometimes considered adequate of >2 µmol/g Hb. There was no significant difference between the groups for lactate, glucose, Na<sup>+</sup> or K<sup>+</sup>, and haemolysis was < 0.5%.

**In-vivo studies**

Strauss and Raderecht [14] tested the in-vivo recovery of WB collected into either ACD or ACD with added adenine and guanosine (ACD-AG) and stored at temperatures ranging from 4 °C to 25 °C. 24-hour recovery of the cold-stored ACD-AG blood was 83%. This declined to “unacceptably low values” between 20 – 27 days at 10 °C, 10 – 14 days at 15 °C, and 4 - 5 days at 25 °C. Time of acceptable storage was lower for warmed ACD blood.

In the study of Reid et al [12] red cells in additive solution (AS-5) were warmed to 25 °C for 24 hours on day 14 or day 28. In vitro biochemical markers showed an accelerated decline as described above, but RBC recovery and survival showed no significant difference between the groups. The conclusion reached was that one day of storage at 25 °C reduces the storage time by 12 days, but shorter exposures such as 2 hours would produce differences in viability and recovery that are too small to measure.

In support of this, Hogman [15] warmed 42-day old SAG-M red cells for 1 hour at 37 °C, immediately prior to transfusion. There were no observed differences in haemolysis, K<sup>+</sup>, glucose, lactate, or 24-hour recovery. The ATP concentration decreased slightly, but there was no difference in adenylate energy charge. Warming was noted to improve the cellular shape significantly.

However, longer exposure to warm temperatures has been shown to affect post transfusion recovery. Shields [10] warmed ACD whole blood to 22 °C for 24 hours prior to transfusion and found that 24-hour recovery was reduced in 21 day old blood, and the difference was

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statistically significant with 28 day old blood (75% compared to 62%). This was considered to be an equivalent loss of viability to that seen with an additional week of cold storage.

#### **4. Bacterial contamination**

A major concern about warming of red cells is that any bacteria that have entered the unit, either from the donor skin or blood-borne, may increase their rate of growth when removed from the cold environment. Hamill [16] reviewed the literature and discusses which bacteria are known to be responsible for contaminating red cell components – most common are gram-positive cocci, which survive cold incubation poorly. Gram-negative bacteria, although less common, are more likely to thrive in cold environments and are of more concern with respect to refrigerated blood components, but do not necessarily grow rapidly on exposure to a warm environment.

Hamill et al also investigated non-leucodepleted RCC in AS-1 additive solution, spiked with various bacteria. They compared units which had been exposed to 26 °C for 2 hours on two occasions, with refrigerated units [16]. There was no significant increase in bacterial growth in the units that were warmed compared to the control units. He noted a lag phase in bacterial growth after removal from the refrigerator, during which it is thought bacteria have to re-adapt to the new temperature before they can begin an accelerated growth rate – this may last for up to 4 hours. This data supported Hamill's earlier conclusion that "it appears that the time period that a unit may be at room temperature and still be reissued could be extended to 2 hours without increased risk to the recipient" [16].

Saxena and colleagues [17] performed bacterial culture on samples from 333 red cell and 63 platelet components. The red cell units had been removed from the refrigerator for 2 periods of 6 hours during their storage. Only one positive culture was found, but after further investigation this was thought to be due to laboratory contamination.

#### **5. Conclusions**

- The current recommended red cell component storage temperature of 2 – 6 °C is correct, but an upper limit of 10 °C may be acceptable for storage periods up to one week.
- Periods of 24 hours or more of warming to ambient temperature have been shown to accelerate metabolism and ageing of red cells, and to potentially shorten the shelf life of the component by 10 – 12 days per 24 hours of exposure.
- Shorter periods of warming are less likely to cause the same effect, but the critical duration has not been determined. In vitro markers appear satisfactory after six hours at 20 °C, and in vivo results were acceptable after 1 hour of warming to 22 °C but poor after 24 hours at 37 °C.

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- Studies on bacterial growth in blood suggest that there is no accelerated growth for several hours after removing units from the cold [16, 18]. However, these studies were not performed with leucodepleted components or current additive solutions.

**6. Recommendation**

- The acceptable time that a unit can be out of the refrigerator prior to commencement of transfusion could be increased to 1 hour. This may be stipulated as such, or simply guidance that the transfusion should be completed within 4 hours of removal from the refrigerator would be sufficient to ensure that no adverse effects will ensue.
- The current recommendation for units that have been removed from the refrigerator for more than 30 minutes but transfusion has been delayed is that they should be discarded. The available evidence suggests that no significant increase in red cell metabolism or bacterial growth would ensue if this period were extended to one hour on one occasion only. However, few blood banks are likely to have systems in place which could record such events throughout the shelf life of the component. It is therefore recommended that the time allowed out of controlled temperature if the unit is to be returned to the blood bank continues to be 30 minutes maximum. Blood banks should, however, be encouraged to use controlled temperature boxes when issuing blood which could extend this period and minimise wastage.
- Further in vitro, in vivo, and bacterial studies on current red cell components exposed to realistic storage temperature deviations are warranted.

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