Evaluation of red blood cell stability during immersion blood warming

LN Nienaber *MBChB;FCA;MMed*
Department of Anaesthesiology, University of Pretoria, South Africa

ABSTRACT

Introduction: *The practice of warming blood for transfusion by immersion into a waterbath has been investigated.* ***Objective:*** *To find the maximum waterbath temperature at which blood can be heated effectively without effecting the red blood cell functional and structural integrity.* ***Method:*** *Blood, three days after donation (fresh blood), with CPD anticoagulant, was warmed at 37℃, 43℃, 45℃, 47℃, 50℃ and 55℃ for 10, 20, 30 and 60 minutes and analysed for haemolysis. In addition, the biochemical markers were done on the blood after 34 days of storage at 4℃ (old blood). Temperature increase within the blood unit being warmed by immersion in warm water is non-uniform, with the outer part showing the largest temperature increases. This was examined at waterbath temperatures of 45℃ and 47℃ and represented graphically. Temperature decrease in a stainless steel bucket filled with 10 liters of warm water (45℃ and 47℃), commonly used in theatre to heat blood, was analyzed and is graphically displayed. Results and discussion: Only minor biochemical changes resulted from warming up to 47℃ for 1 hour. The values of the free haemoglobin in old blood corresponded to the significant changes of fresh blood after being heated at 50℃ for 30 minutes ($p<0.001$). The change of the haematocrit in old unheated blood was much higher than the significant changes caused by heating the fresh blood at 55℃. The value of potassium of old blood corresponded to the value after heating fresh blood at 55℃ for 30 minutes, even though the potassium already increased significantly at 50℃ after 60 minutes ($p<0.01$). The value of lactate hydrogenase in old blood corresponded to the significant increase after heating fresh blood at 55℃ for 60 minutes, while this marker already increased significantly at 50℃ after 20 minutes exposure ($p<0.001$). The results suggest that immersion warming of fresh blood for 1 hour at 47℃ and below do not cause red cell damage. A limitation of immersion warming is that warming of the blood within the unit is not uniform, with a temperature gradient as big as 5℃ to 10℃ between the inner and outer parts of the unit. There is also a lag time of 30 to 40 minutes between the inner and outer parts to reach the same temperature in a thermostatically controlled waterbath. The temperature of the bucket of warm water in theatre decreased rapidly.* ***Conclusion:*** *It is safe to heat blood in a bucket of warmed water in theatre at a confirmed initial temperature of 47℃, as only the outer part of the unit is exposed to this temperature for a short while.*

Introduction

The practice of active blood warming arises from conflicting needs. On the one hand, the safety and preservation of blood requires refrigeration at 4°C up to the moment of transfusion. On the other hand, very rapid transfusion of cold blood causes hypothermia which leads to immune suppression, coagulapathy and arrhythmias.

LITERATURE REVIEW

Ideally, blood should be heated to reach the body at normal body temperature (37°C). The current maximum recommended temperature for intravenous fluids is 40^0C. This temperature may be insufficient because of heat loss from intravenous tubing prior to infusion. Heat loss occurs from intravenous tubing to the theatre environment, up to the point of reaching the body. This results in infusion of fluids at or below 37,5°C. Perhaps, this upper limit on warmed intravenous fluid should be reconsidered, as fluids warmed to higher temperatures (40 – 46°C) may significantly increase the amount of heat delivered to the patient.

At present, controversy exists about the upper limit to which blood can be safely warmed. The maximum operating temperature for blood warmers is 39°C (range 37°C to 39°C). The temperature in blood warmers is currently limited to a maximum of 42°C by the American Association of Blood Banks.

Landois demonstrated, as early as 1875, that blood is damaged at temperatures above 50°C. Chalmers & Russell (1974)

Correspondence :
DR LN Nienaber
e-mail: grant@askafrika.co.za

found no significant haemolysis at 45˚C for one hour. Van der Walt & Russell (1978) have showed that incubation of stored blood at 46˚C for one hour, left the osmotic fragility unchanged. Marks et.al. (1985) found only trivial biochemical and morphological changes in blood warmed at 45˚C for up to two hours. Utoh & Harasaki (1992) demonstrated that heating blood at 48˚C for up to 4 hours did not cause any significant haemolysis or increase in osmotic fragility. Linko & Palosaari (1979) suggested that warming of blood in a water bath should not cause haemolysis at temperatures below 45˚C.

It is more convenient to warm blood units before transfusion than to use in line blood warmers when rapid replacement is needed (Linko & Palosaari, 1979). Electromagnetic blood warmers have been designed for this purpose. However, probably because of the high price of these devices and the complications reported with the microwave oven (Arens & Leonard, 1971), blood units are still commonly warmed in a container of hot water or in a stirred and thermostated water bath. This kind of warming is believed to be slow and to cause considerable wastage of blood, because all units warmed may not be transfused and there is a risk of contamination (Braude et.al., 1952). There is also a risk of the blood cooling before the transfusion when prewarmed blood is used: blood warmed to 37˚C cools down by 3˚C in 5 minutes, and 5˚C in 15 minutes at an ambient theatre temperature of 21˚C (Linko & Palosaari, 1979). In addition, cooling occurs in the transfusion set, especially at low flow rates. Blood initially warmed to 37˚C, cooled to 32˚C at the end of the transfusion set, at a flow rate of 85 g/min at room temperature (21˚C). Linko & Palosaari (1979) suggested a bath temperature of 39˚C – 42˚C for rapid warming and to compensate for cooling of blood at ambient theatre temperature, during transfusion.

A possible drawback of immersion warming is that warming may not be uniform throughout the blood unit with the outer temperature being higher than the core temperature (Marks et.al., 1985). However, when blood units are warmed in a water bath, there is no risk of local overheating as with electromagnetic ovens. Accidental overheating is a hazard with any technique. Overheating can occur if a thermostat mechanism fails. Immersion warming offers the advantage that the temperature of the blood is actually felt before transfusion.

Transfusions may be given faster if blood is prewarmed by immersion, rather than by using an in line warming coil. Flow through a coil is impeded by the long, thin tubing and by the high viscosity of cold blood. Furthermore, warming coils are costly and increase the dead space.

Inexpensive, but safe, effective and user friendly methods are therefore sought to warm blood.

The changes in red blood cell integrity were measured in the following way:-

METHOD AND STATISTICS

One unit of whole blood was obtained from each of 11 volunteers by means of the standard blood donation practice into Fenwal bags containing citrate-phosphate-dexstrose (CPD) anticoagulant. The final volume was controlled by weighing the blood units. The volunteers where healthy persons aged 21-42, on no medication. A full blood count (FBC) and haemoglobin electrophoresis were done on the blood of each volunteer to ensure that no abnormal haemoglobin was present. The blood was stored at 4˚C until used 3 days after donation. This was regarded as “fresh blood”.

Biochemical indicators for measurement of haemolysis of red blood cells:

Haematocrit (Hkt), plasma free haemoglobin (Fhb), potassium (K+) and lactate dehydrogenase (Ldh) ; the day four values were taken as baseline values.

Test 1

Water was heated and maintained at a specific temperature measured with a standard mercury thermometer in a thermostated waterbath. The varying water bath temperatures were 37˚C, 43˚C, 45˚C, 47˚C, 50˚C and 55˚C. Room temperature was 23˚C. Before testing, the blood unit was gently agitated for 30 seconds when first taken from the refrigerator and for a further 30 seconds before taking samples, to ensure a uniform haematocrit. The blood was returned to the refrigerator between sampling.

For each temperature batch, the blood was allowed to flow freely from the pack into four 10 ml centrifuge tubes. A temperature batch consisted of the four tubes heated for varying periods at a single temperature for each unit. The centrifuge tubes were covered with parafilm. Seven milliliters whole blood (temperature 4˚C) in a test tube in the heated water bath was allowed to warm up to the temperature of the water bath. Temperature of the blood was monitored by a standard mercury thermometer.

Blood analysis for markers of haemolysis were taken after 10, 20, 30 and 60 minutes continuous exposure at the designated temperature (with minimal temperature loss from removal of heat exposure to biochemical measurement).

The test tubes were centrifuged at 3500 revolutions per minute for 7 minutes after which the plasma was decanted. The test was repeated on each of the blood units.

Plasma Fhb was measured spectrophotometrically.

After 34 days of storage at 4˚C the blood of each volunteer was analysed again for the markers of haemolysis, without being heated.

Test 2

Temperature gain within the blood unit heated from 4˚C, is nonuniform, with the outer parts of the unit being exposed to the largest temperature increases. A temperature to time graph was plotted from data obtained by using an outdated blood unit in a heated waterbath with thermometers measuring temperature at the inner and outer part of the unit in waterbaths

maintained at 45˚C and 47˚C. Room temperature was 23˚C. Temperature increase was measured with a digital, calibrated thermocouple thermometer every 15 seconds.

Test 3

Blood is commonly warmed in a 10 liter stainless steel bucket of water in theatre. Analysis of temperature loss from 10 liters of warm water (45˚C) in this stainless steel bucket to ambient theatre temperature (21˚C) was done. Temperature decrease was measured by the thermocouple thermometer every 30 seconds. The test was repeated with water heated to 47˚C. A temperature to time graph was plotted from the data obtained.

Data analysis

The coefficient of variation was calculated to assess the reliability of the spectrophotometrical measurement of the plasma Fhb. A ratio of less than 10% was considered to be a reliable estimation of plasma Fhb.

Statistical analysis of the changes in the blood indicators of haemolysis with heating at the different temperatures was done by using analysis of variance (ANOVA) for repeated measurements. Post hoc paired t-tests were performed to identify significant differences.

In addition to the above analyses, linear regression and a parabolic analysis were performed to determine if the relationship between time and temperature decline of water in a stainless steel bucket in theatre is linear. The coefficient of determination (R2) is reported.

RESULTS

Test 1

The coefficient of variation of the spectrophotometrical method used to estimate the plasma Fhb, was measured on 4 aliquots and was calculated as less than 7%. This ratio was less than the critical value of 10%.

The biochemical results of the blood on day four of storage at 4˚C and after 10, 20, 30 and 60 minutes at the different waterbath temperatures, namely 37˚C, 43˚C, 45˚C, 47˚C, 50˚C and 55˚C is shown in Figure 1 to 4. Statistical significance is indicated as follows: $p<0.01$ by * and $p<0.001$ as **.

Figure 1 shows the significant decrease in haematocrit at 30 and 60 minutes at 55˚C. Figure 2 shows a linear increase in the Fhb at 50˚C with continuous exposure. The increase in the Fhb was so marked at 55˚C, that it could not be plotted on the same graph. Figure 3 shows a linear increase in the potassium at 55˚C. The significant increase in Ldh after 20 minutes at 50˚C is seen in Figure 4, with the marked increase at 55˚C omitted for clarity purposes.

Blood in Fenwal packs containing CPDA anticoagulant stored at 4˚C expire on day 35. The changes in biochemical markers in old blood are compared to the baseline markers on fresh blood (Table 1). All markers differ significantly from

Figure 1: Changes in Hkt after exposure to 37ºC, 43ºC, 45ºC, 47ºC, 50ºC and 55ºC for 1 hour

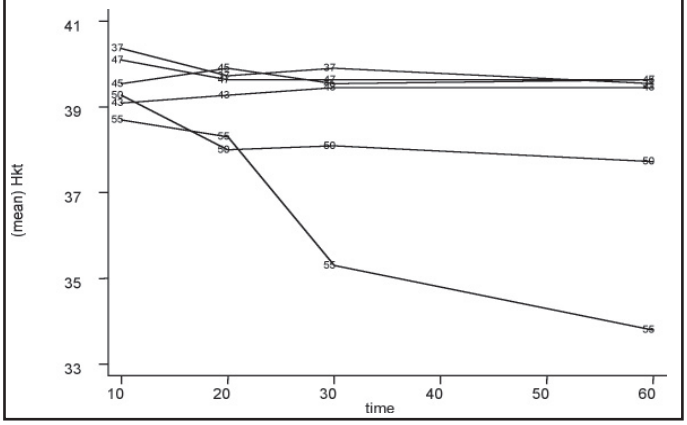


Figure 2: Changes in Fhb after exposure to 37ºC, 43ºC, 45ºC, 47ºC, 50ºC and 55ºC for 1 hour

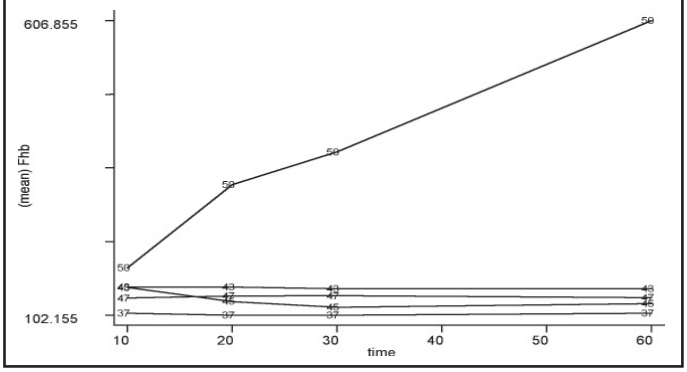


Figure 3: Changes in K+ after exposure to 37ºC, 43ºC, 45ºC, 47ºC, 50ºC and 55ºC for 1 hour

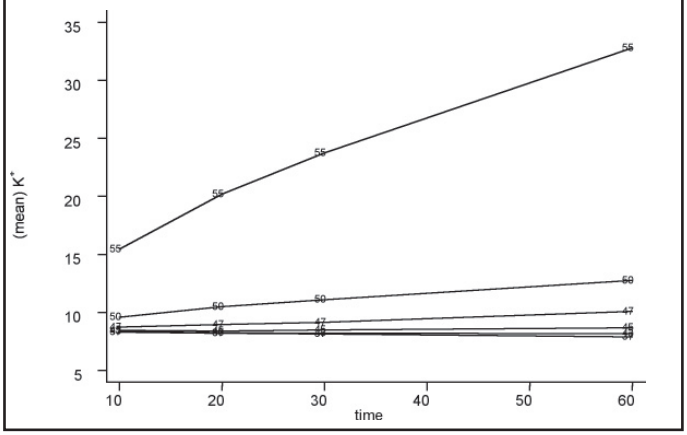


Figure 4: Changes in Ldh after exposure to 37ºC, 43ºC, 45ºC, 47ºC, 50ºC and 55ºC for 1 hour

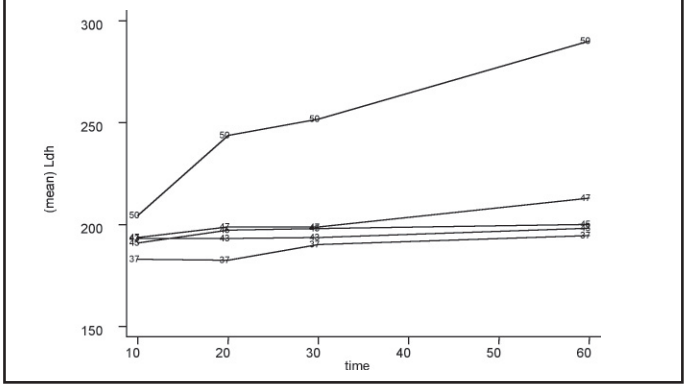
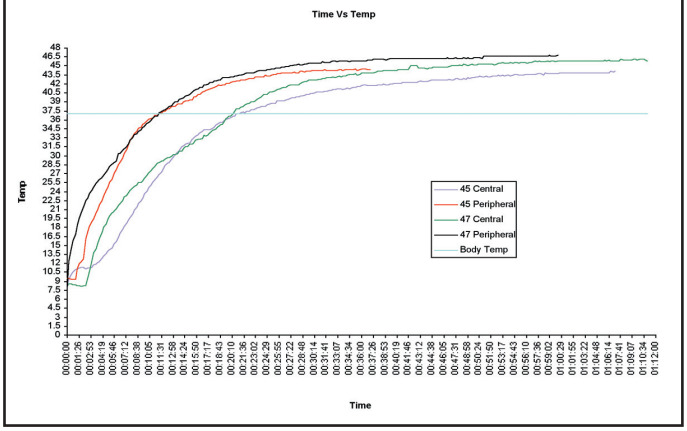


Table 1: Difference between baseline measurement on fresh blood (BD4) and old blood (D34)[the table may read easier if columns 2 & 3 are swopped

	Old blood	Fresh blood	Difference
Hkt[are the yellow highlighted values in the next two columns correct? They are not consistent with the discussion or logic]	41.46 (3.30)	39.73 (2.61)	1.73**
Fhb	361.80 (135.27)	122.80 (85.87)	238.99**
K+	21.06 (6.82)	8.53 (1.06)	12.54**
Ldh	698.73 (171.52)	162.82 (48.04)	535.91**

Significant differences are indicated by **p <0.001. Hkt, haemotocrit (%); Fhb, free haemoglobin (mg/l); K+, potassium (mmol/l); Ldh, lactate hydrogenase (IU/l). Results are given as mean (standard deviation).

Figure 5: Temperature increase at the central & peripheral parts of a blood unit at 45ºC & 47ºC



day 4 values. The day 34 values were compared to the significant changes in the markers after heating the fresh blood at 50˚C and 55˚C. The Fhb value in unheated old blood corresponds to the significant values after exposure of fresh blood at 50˚C for 30 minutes. The unheated change of the haematocrit in old blood was much higher than the significant changes caused by heating the fresh blood at 55˚C. The value of potassium of old blood corresponded to the value after heating fresh blood at 55˚C for 30 minutes, even though the potassium already increased significantly at 50˚ after 60 minutes. The value of lactate hydrogenase in old blood corresponded to the significant increase after heating fresh blood at 55˚C for 60 minutes, while this marker already increased significantly at 50˚C after 20 minutes exposure.

Test 2

Figure 5 shows the temperature increase at the central and peripheral parts of a blood unit at 4˚C in a waterbath at 45˚C or 47˚C. The peripheral part of the blood unit was heated to 37˚C at both waterbath temperatures after 11 minutes 15 seconds. A temperature of 37˚C was reached at the central part of the blood unit after 20 minutes 15 seconds for the 47˚C waterbath and after 20 minutes 45 seconds for the 45˚C waterbath. There was a temperature difference of 10.2˚C between the inner and outer parts of the unit for the 45˚C waterbath and 8.3˚C for the 47˚C waterbath after 11 minutes. There was a temperature difference of 5.5˚C for the 45˚C waterbath and 6.1˚C for the 47˚C waterbath after 20 minutes. A temperature of 44˚C was reached within the blood unit in the 45˚C waterbath after 67 minutes centrally and peripherally after 29 minutes. The blood unit in the 47˚C waterbath reached a temperature of 46˚C centrally after 68 minutes and peripherally after 37 minutes.

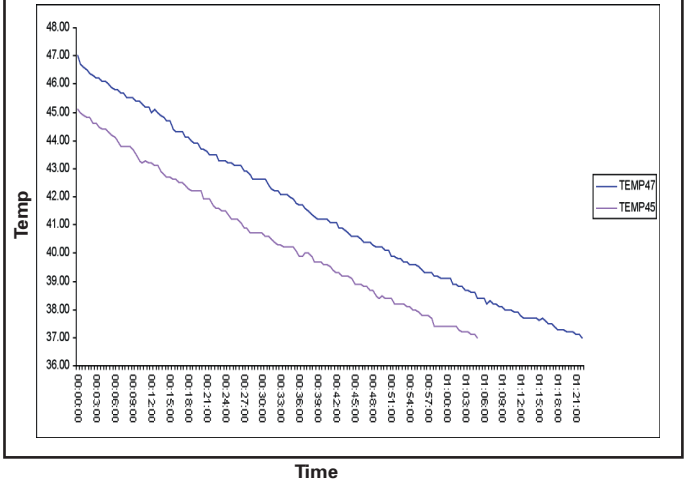
Test 3

Temperature decline of 10 liters warm water heating one unit of blood from 4˚C in a 10 liter stainless steel bucket in theatre at a temperature of 21˚C is shown in Figure 6. The 45˚C water cooled down to 37˚C after 65 minutes and the 47˚C water after 88 minutes. For both 45˚C and 47˚C water the temperature declined in a parabolic fashion and in particular for 45˚C the coefficients of determination (R2) was 0.9987 while for 47˚C it was 0.9994. However, for a linear fit these coefficients of determination were virtually identical to that of the parabolic fits, namely 0.9918 and 0.9923 respectively. From the linear fit for 45˚C it follows that the temperature decline is 0.123˚C per minute while for 47˚C it is 0.120˚C per minute.

DISCUSSION

It was demonstrated that the Fhb, potassium and Ldh are the most sensitive markers for haemolysis, with the Fhb and Ldh rising the earliest, namely after 20 minutes exposure at 50˚C (Table 1). Significant changes ($p<0.01$) in the biochemical markers occurred after heating fresh blood at 50˚C and 55˚C for 20 minutes but not after 60 minutes continuous exposure at 47˚C. Fresh blood can therefore safely be heated by immersion warming for an hour in a waterbath of 47˚C with no ad-

Figure 6: Temperature decline of water in a stainless steel bucket in theatre



verse effect on red blood cell integrity. These findings correspond to those of Chalmers & Russell (1974) and Utoh & Harasaki (1992).

The finding that markers in old blood correspond to, or are higher than the significant changes in the biochemical markers caused by heating fresh blood at 50˚C for 20 minutes and 55˚C at all time intervals, validates the above finding. Statistically insignificant changes were found after exposing fresh blood to 47˚C and below for one hour compared to the changes observed in unheated old blood ($p<0.001$). The question therefore arises whether blood stored for longer periods of time such as 30 to 34 days, should be exposed to heat prior to infusion at all, as storage causes a progressive acidaemia and hyperkalaemia, while heating causes a further rise in H+ and K+ concentrations. According to Marks et al (1985) these changes with heating are less pronounced in older blood that was stored for 4 weeks, than blood 3 days old; potassium increase in blood 3 days old was 1.9mmol/l when heated to 45˚C, compared to an increase of 0.5mmol/l in blood 2 weeks old and a decrease of 0.3mmol/l in blood 4 weeks old. It is possible that these markers were maximally released after being stored for a certain period and therefore displayed minimal increases with further heating (ceiling effect). Marks et al (1985) failed to demonstrate clinically significant changes after warming stored blood (3 days, 2 weeks and 4 weeks old) for up to 2 hours at 45˚C.

A limitation of immersion warming is that warming of the blood within the unit is not uniform, with a temperature gradient of 5˚C to 10˚C between the inner and outer parts of the unit. There is also a lag time of 30 to 40 minutes between the inner and outer parts to reach the same temperature as the waterbath temperature in a thermostatically controlled waterbath. Therefore, if blood was to be heated in the stainless steel bucket in theatre with an initial water temperature of 47˚C, only the outside of the blood unit would be exposed to this temperature for a short while. The inner or outer parts of the unit will never reach this temperature as the temperature of the water would not remain constant for 30 to 60 minutes. The water cools down rapidly in theatre (Figure 6). It would therefore be safe to heat blood at an initial water temperature of 47˚C.

From the above we can also gather that immersion heating of blood in a bucket of warm water is extremely uncontrolled, but safe. Due to the cost effectiveness of this method, it is still commonly utilised. If water temperatures in the stainless steel bucket are to be monitored by a simple mercury thermometer it would definitely be in the patients interest. We strongly recommend that at least the initial water temperature of 47˚C be confirmed, as temperatures higher than this can damage blood. At a temperature of 47˚C water is subjectively rather hot.

In conclusion, no significant changes of markers of haemolysis have been found after exposure to 47˚C for up to 1 hour. Evidence has been provided that heating a unit of blood through immersion warming in a bucket of water is nonuniform within the unit, with only the outer part of the unit being exposed to the hot water in direct contact with it. We recommend that in circumstances where blood is commonly heated in a bucket of water, blood can be warmed in warm water at 47˚C, provided that the initial temperature is confirmed.

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