Joint UKBTS Professional Advisory Committee

UKBTS General Information 03

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

June 2016

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

There are various points in the blood supply chain where red cells may be temporarily stored out with their designated storage temperature (2-6°C). This could include transport of red cells between the blood centre and hospital, but also from a hospital transfusion department to a satellite fridge or another external site, or when red cells are issued and collected for transfusion to a patient. This document summarises data on component quality and bacterial risk when red cells are stored at ambient temperature for multiple short periods and makes recommendations for changes to the current guidance base on the available evidence.

a) Current regulatory requirements for storage and transport temperature of red cells

The current requirement in the UK and Europe is that red cell components must be stored with their core temperature in the range 2 to 6°C, whereas AABB Standards state 1-6°C [1-4].

Exceptionally, it is allowed that the core temperature may extend from 1 to 10°C, providing that this deviation has happened on one occasion only, and that the duration is no longer than five hours [2]. In addition, the UK Guidelines allow surface temperatures up to 10°C for up to 12 hours during transport, although currently it is not stated on how many occasions. The Council of Europe Guidelines [3] allow up to 10°C for 24 hours during transit. The AABB Standards [4] and AABB Technical Manual state that blood storage and transit temperature should not exceed 10°C but no time limit is stated. None of the published guidelines on transport of red cells state on how many occasions during the shelf-life of a red cell this may occur, and are unclear about whether these recommendations relate to blood centres or hospitals or both.

The EU Directive and Blood and Safety Quality Regulations state that ‘transport and distribution of blood and blood components at all stages of the transfusion chain must be under conditions that maintain the integrity of the product’.

These storage and transportation regulatory requirements are in place to a) inhibit the growth of any bacteria introduced into the bag at the point of collection, processing, or storage; and b) to preserve red cell quality.

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 do not give any guidance on how long blood can be out of controlled temperature before transfusion is commenced, the British Committee for Standardisation in Haematology (BCSH) guidelines [1] state that 'If red cell units are out of temperature controlled storage for more than 30 minutes they should not be put back into storage for re-issue. If an IT tracking system is being used it should be able to immediately highlight to laboratory staff the presence of any returned units that need withdrawal from stock.' The BCSH guidelines also recommend that transfusions are completed within 4 hours of removal from a controlled temperature, apart from neonatal transfusions which can be up to 4.5 hours to allow for 4 hours for the transfusion itself in order to allow for transfusions up to 20mls/kg at a rate of 5 ml/kg/hr.

It is likely that the 30 minute rule originated as a result of the 1971 publication of Pick and Fabijanic [5] 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 the core temperature took 45 to 60 minutes to reach 10°C. Thirty minutes thus would appear to be a reasonable cut-off to ensure that the core temperature did not rise above 10°C. Since this original work, there have been a number of studies that have confirmed the rate of warming, in increasingly sophisticated ways [6-9]. It is not clear why Pick and Fabijanic chose 10°C as the upper limit, it may have been on the basis of data published by Hughes-Jones [10] that showed reduced, but acceptable, recovery of red cells following transfusion when stored at 10°C for 34 days. In addition, 10°C may have been chosen as a practical limit based on the wet ice type of transit containers that were available at that time. The relevance of short-term exposures to 10°C, and thus the relevance of the 30 minute rule, is therefore worthy of review.

The 30 minute rule can result in wastage of red cells in two respects:

a) If a patient is not ready to receive a planned transfusion, and red cells are out of controlled storage for more than 30 minutes they cannot be returned to stock for issue to that or another patient.
b) Red cells sent to a location remote from a blood refrigerator or off-site in case a transfusion is needed, cannot be returned to stock if not transfused within 30 minutes of removal from controlled storage.

There is general concern among blood services and hospitals that a considerable number of RCC are lost unnecessarily as a result of the 30-minute rule. Data from the UK Blood Stocks Management Scheme (BSMS) repeatedly shows approximately 10, 000 RCC are discarded every year due to out of temperature control excursions outside of the laboratory, and this represents almost one quarter of all red cell wastage [11]. In a recent survey of hospitals by the BSMS, over 96% of respondents indicated that extending the 30 minute rule to 60 minutes would enable most of their out of temperature control units to be re-issued.

A systematic review published by Brunskill *et al* in 2011 [12], concluded that "It is possible that the 30-minute rule could be extended to a "60-minute rule," but the main concern remains bacterial growth in any contaminated units that are returned to clinical stock… and further studies are required before that question is answered." The papers reviewed by Brunskill *et al* are summarised below, along with those subsequently published and recent reports from NHSBT in response to the call for "robust, modern studies using multiple combinations of blood, current anticoagulant and additive solutions, and with defined temperatures and times of exposure that are relevant to current clinical practice."

2. Impact of changes in the temperature of red cells on their quality

a) 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 is 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 four hours 2,3 DPG will have fallen to 35% of the initial concentration, and it will be totally depleted within 18 hours [13].

Several workers have assessed the effect of intermittent and repeated warming on red cell metabolism. Strauss *et al* [14] stored ACD anticoagulated blood at different temperatures, and on the basis of changes to 2,3 DPG, ATP, pH, extracellular K^+ and Hb concentration, concluded that acceptable shelf-life was nine days at 10°C, six days at 15°C and three days at 20-25°C. If adenine and guanosine were added, the storage times were increased to 20, 10 and five/four days respectively.

Shields stored units of plasma reduced-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 haemoglobin (Hb), supernatant potassium (K^+), haematocrit (Hct) or osmotic fragility [15]. This provides some evidence to support the upper limit of 10°C being acceptable with respect to red cell metabolism, but the extensive range of laboratory assays that would have been employed currently were not available at that time. Most notably, the authors are not aware of any study that has assessed ATP levels in red cells exposed at 10°C for multiple or prolonged periods.

Shields [15] also exposed plasma-reduced 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^+, 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* compared CPDA-1 anticoagulated packed red cell units warmed to 25°C for 24 hours at either day 6 or day 20 with control units [16]. The warmed units had lower concentrations of glucose, higher lactate, and lower pH than controls. The rates of decrease in adenosine triphosphate (ATP) were greater in the warmed units during the week after warming compared with controls. There was no statistically significant increase in plasma Hb, and haemolysis did not exceed 1% in any of the units. 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 two hour exposure to ambient temperature might be expected to reduce the storage life of a unit by one day.

Reid *et al* studied red cells in additive solution (AS-5). Units were warmed to 25°C for 24 hours on day 14 or day 28 [17]. 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 performed a similar study, but exposed units to ambient temperature for a shorter length of time [18]. 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 considered necessary for adequate post-transfusion survival of >2 µmol/g Hb. There was no significant difference between the groups for lactate, glucose, sodium (Na^+) or K^+, and haemolysis was < 0.5%.

Hancock and colleagues at NHSBT's Component Development Laboratory (CDL) studied the effect of the storage and transport deviations permitted by the UK Guidelines (10°C for 5 hours and 12 hours, as described in the introduction) on red cells stored in SAGM and found no significant effect on any *in vitro* red cell parameters tested (including haemolysis, ATP, K^+) [19].

In a second study Thomas and colleagues studied red cells in SAGM subjected to repeated short term exposures to 21°C, and showed that three exposures of three hours on days 3,8 and 15 of storage plus one exposure of five hours at 21°C (to model the transfusion itself) did not have a negative effect on *in vitro* markers of red cell quality (including haemolysis, ATP, K^+) [20].

The concluding study from Thomas and colleagues at CDL investigated the effect of multiple 30 minute or 60 minute exposures to warm (30°C) temperatures (up to three exposures on each of three separate days of storage, days 15, 17 and 21) [21]. The study design also included the 5 and 12 hour exposures to 10°C and a five hour transfusion period (also at 30°C), and used both adult-sized and paediatric-sized components in SAGM. No significant differences were seen in ATP or K^+, but increased haemolysis was seen in the units that had been subjected to multiple exposures (adult units subjected to at least two 60 minute exposures on each of three days and in paediatric units exposed for 60 minutes once on each of three days). The authors concluded that, on the basis of *in vitro* quality markers, an extension of the 30 minute rule to 60 minutes could be considered but only if the total number

of exposures was limited to no more than three, and that adequate time was allowed to cool the units properly in between exposures.

Ramirez-Arcos and colleagues from the Canadian Blood Service reported on two studies using red cells in SAGM. In the first study, a single five hour exposure to room temperature showed no immediately significant effects on the *in vitro* quality of the red cells, although six days after the exposure ATP and K^+ levels were significantly lower than in unexposed controls [22]. In the second study, units were exposed to room temperature for 30 minutes on each of five separate days, and no significant effects on *in vitro* red cell quality markers were reported [23].

de Grandmont and colleagues from Hema-Quebec studied red cells in SAGM and in AS-3, which were exposed to room temperature for 30 or 60 minutes at weekly intervals for five weeks. No significant differences were seen in the *in vitro* markers tested (including haemolysis, ATP, K^+) [24].

Wagner and colleagues in Austria studied red cells in SAGM that were stored constantly at one of three temperatures (1-6, 13 or 22°C) for 42 days or at 2-6°C in conjunction with five weekly exposures at 13 or 22°C for 24 h each time [25]. The data show that long term storage at 13 or 22°C results in increased haemolysis. Repeated exposure to 22°C for 24 hours also increased haemolysis which was not observed in units subjected to repeated exposure to 13°C. Due to lack of control data on ATP the effects on this variable are difficult to assess. Nonetheless, 24 hour exposures to 22°C appeared to have the expected effect of reducing ATP levels significantly.

Gulliksson and colleagues in Sweden have also assessed red cells in SAGM after transient warming to ambient temperature for 6h on multiple occasions or a single exposure to ambient temperature on day 5 or 21 for 6, 12, 18 or 24 hours [26]. Exposure to ambient for multiple periods of 6 hours appeared to have little effect on in vitro measures of red cell quality. Warming during the second half of shelf life led to more haemolysis than when exposed at day 5, but not to an excessive degree. As expected, exposure for longer periods, such as 18-24 hours, had a more pronounced effect on haemoylsis and reduction in ATP.

b) In-vivo studies

Strauss and Raderecht [27] 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 two different studies, exposure to ambient temperatures for 24 hours has been shown to reduce red cell recovery following transfusion to normal subjects. Shields warmed ACD packed red cells stored for 7, 21 or 28 days to 22°C for 24 hours immediately prior to transfusion [15]. They found that 24-hour recovery was reduced in 21 day old blood, and the difference was 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 storage at 4°C.

In the study of Reid *et al* [17] red cells in additive solution (AS-5) were warmed to 25°C for 24 hours on day 14 or day 28 and then stored to day 35, with control units kept at 4°C for 42 days. The 24 hour recovery of red cells at day 35 for units exposed to ambient temperature for 24 hours was similar to controls at day 42. The reduction in recovery was paralleled by a reduction in ATP, and therefore this appears to be a useful laboratory marker of the reduction in red cell recovery that may occur to due warming of red cells. The conclusion reached was that one day of storage at 25°C reduces the storage time by 12 days, but shorter exposures such as two hours would produce differences in viability and recovery that are too small to measure, and is consistent with laboratory studies showing little effect of multiple short-term exposures on ATP levels.

Hogman [28] warmed 42-day old SAG-M red cells for one hour at 37°C, immediately prior to transfusion. There were no observed differences in haemolysis, K^+, 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.

A limitation of the data to date is that studies have assessed basic measures of red cell quality. More recent concepts such as oxidative changes during red cell storage have not been assessed.

Although studies by NHSBT have included paediatric-sized units that will warm up more quickly when removed from controlled storage, components that have undergone secondary processing such as irradiation, washing, or red cells for exchange transfusion have not been assessed. In terms of bacterial risk, exposure of irradiated or washed red cells to ambient temperature is unlikely to result in a higher risk than standard red cells, since they are stored in the same medium and for shorter duration. The shelf-life of both washed and irradiated red cells is restricted to 14 days due to the effects of both processes on red cell quality. It is not known whether there would be more of a detrimental effect on red cell quality of exposing cells that have already been subjected to additional stress such as washing and irradiation to periods at ambient temperature since these studies have not been performed. This highlights the importance of trying to restrict the amount of time that red cells are out of controlled temperature to a minimum.

c) Conclusions on red cell quality

The current recommended red cell component storage temperature of 2-6°C is correct. Storage of red cells out with their recommended temperature should be kept to a minimum.

The current upper limit of surface temperature of 10°C for a single period no longer than 12 hours for transportation between blood centres and hospitals and to cover refrigerator breakdown (a single occurrence no longer than 5 hours) is acceptable.

UKBTS General Information 03

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

June 2016

Periods of 24 hours or more of warming to ambient temperature have been shown to accelerate metabolism and ageing of red cells, resulting in reduced red cell recovery in vivo and therefore 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.

In terms of component quality, the 30 minute rule could be extended to 60 minutes, but a limitation is required on the total number of exposures, as increased haemolysis has been seen in adult and paediatric units exposed more than once on each of three occasions, and sufficient time must be allowed in between multiple exposures for the red cells to return to 2-6°C.

3. Impact of changes in temperature of red cells on bacterial contamination

a) Incidents of bacterial contamination in red cells

The most significant concern is that the rate of growth of any bacteria that have entered the unit, either from the donor skin or blood-borne, may increase when red cells are removed from the cold environment. Contamination of red cells with bacteria is a very rare occurrence and usually involves Gram-negative species which are able to survive and/or multiply in cold storage. Data compiled by NBL on organisms implicated in bacterial transmissions from RCC between 1995 and 2014 identified six incidents reported to NHSBT from approximately 40 million units, giving an incidence of contamination of 1 in 6 million (see Table 1).

Table 1 – Bacterial infections transmitted by transfusion of red cells (1995 – 2014)

	Organism	Frequency	Potential Source	Patient Outcome
Gram negative	*Pseudomonas putida*	2	Environment	Death (1) Morbidity (1)
	*Yersinia enterocolitica**	1	Gut	Death
	Enterobacter cloacae	1	Gut	Morbidity
	Serratia liquefaciens	1	Gut	Morbidity
Gram positive	*Staphylococcus* sp.	1	Skin	Morbidity

* prior to introduction of universal LD in the UK. Since this organism is leucocyte-associated LD is expected to reduce this risk and no cases have been reported since the introduction of universal LD in1999

b) In vitro studies

In 1990, Hamill and colleagues reviewed the literature and performed experimental studies on a panel of various organisms, showing that exposure of spiked units of non-leucodepleted red cells in AS-1 additive solution to 26°C for two hours on two occasions had no significant effect on the rates of bacterial multiplication [29, 30]. The authors therefore called for extension of the 30 minute rule to two hours. However, Brunskill's systematic review concluded that whilst overall there was no evident increased risk of bacterial contamination outside of the rule, further studies were called for, using a more representative range of microbes, and more current anticoagulants and additive solutions, to resolve the issue [12].

NBL investigated the currently permitted storage and transport temperature conditions, with spiking experiments used to determine bacterial growth. Four groups of RCCs were spiked separately with six species and stored to reflect deviations in refrigeration due to a) equipment breakdown (10°C for 8h on day 2-equating to a core temperature of 10°C for 5 h), b) transport (surface temperature 10°C for 12 h on day 3), c) a combination of both a and b and were compared to units stored at 4°C throughout. Samples were taken over the shelf life of the unit. The species tested included *Pseudomonas putida, Bacillus cereus, Enterobacter cloacae, Serratia liquefaciens, Yersinia enterocolitica* and *Staphylococcus epidermidis*. Of these, only the cold tolerant *P. putida* exhibited a statistically significant increase in numbers (>1 log) compared with controls over all temperature deviations, reaching counts of 10^7 to 10^8 cfu/ml by day 21.

There are no comparable studies in the literature exploring the growth response of *P. putida* in similar storage and temperature deviations. Ramirez-Arcos and colleagues [23] investigated the growth of bacteria in RCC held out of storage temperature for more than four hours but utilised single strains of *S. liquefaciens* and *S. marcescens*. *S. liquefaciens* grew at a constant rate in refrigerated units and reached *c.* 10^6 cfu/ml within one week of storage. By contrast *S. marcescens* grew markedly slower and required 21 days to reach a comparable count. *S. liquefaciens* was further tested in a single five hour exposure to room temperature, three days post spiking of the unit, and exhibited a significant increase in viable count at three hours of exposure. At five hours the mean count was 3.4×10^3 cfu/ml.

The level of risk due to the current storage and transport conditions must therefore be considered to be very low given the rarity of contamination events. None of the six bacterial transmissions since 1995 are known to have been associated with deviations in storage temperatures of the implicated units.

The debate over the 30 minute rule was further advanced by Ramirez-Arcos *et al* of the Canadian Blood Service, whose experiments with the cold tolerant species *Serratia marcescens*, showed a statistically significant difference in bacterial numbers in spiked RBC units exposed to room temperature on five occasions for 30 minutes compared with non exposed controls [23]. The range of species was extended to include *Yersinina enterocolitica*, *Escherichia coli* and *Staphylococcus epidermidis* in a follow up study [22]. This showed that *E. coli* and *S. epidermidis* failed to grow in control or ambient temperature exposed RCC. *Y. enterocolitica* grew to similar levels in tests and controls but *S. marcescens*

grew to numbers of 1-log higher (10^4 cfu) in units exposed for 30 or 60 minutes on multiple occasions (once each on day 7, 14, 21, 23, 28 and 35 post collection). However, there was no significant difference in counts between units exposed for 30 or 60 minutes and the authors concluded that an extension of the 30 minute rule to 60 minutes was therefore 'reasonable'.

More recent data from a separate but similar study from Hema-Quebec using RCC spiked with 1-5 cfu/unit each of reference strains of *S. epidermidis*, *S. marcescens* and *S. liquefaciens*, in two additive solutions, also showed that 30 and 60 minute exposures to room temperature did not result in significantly increased bacterial growth with the exception of S.marcesens [24]. However, the latter was not significantly different between red cell units exposed for 30 or 60 minutes. An interesting observation was that *S. marcescens* failed to grow in additive AS-3 while *S. liquefaciens* grew to similar levels in this and SAGM solutions. This finding is difficult to explain as the significant differences between AS-3 and SAGM with regard to growth promotion is the presence of citrate in the former and mannitol in the latter; both species are able to utilize these compounds for growth.

NBL performed a study that modelled similar multiple warm exposures of RCC in SAGM (adult and paediatric packs) as used by Thomas *et al* at CDL [21], with the units spiked with six clinically relevant isolates (*Pseudomonas putida, Bacillus cereus, Enterobacter cloacae, Serratia liquefaciens, Yersinia enterocolitica* and *Staphylococcus epidermidis)*. The units were exposed to 30°C either once on three separate days, or on three occasions on the same day, for either 30 or 60 minutes. Units were sampled before and after each deviation and viable counts compared with control units maintained in refrigeration. An increase in bacterial numbers of ≥ 1-log in test over controls was considered evidence of significant multiplication of the test organism in view of the variability in measuring these endpoints. The key finding was that none of the exposure conditions tested had a significant impact on bacterial counts of five of the six species tested. The exception was *P. putida* which showed a > 1-log difference in count from the negative control in an adult pack following three exposures for 60 minutes at 30°C on the same day (day 15). Other exposures of adult or paediatric packs to three 30- and 60- minute temperature deviation cycles showed no significant difference in counts of *P. putida*. Since the difference between control and test units in the adult unit study was only observed when tested on day 15 (the same day as exposure to 30°C), and not in the same units later during storage. On repeat experiment, this difference was not confirmed and counts did not differ significantly from the control units suggesting that the earlier finding was an artefact. Based on these results up to three exposures of 30 minutes at 30°C on the same or separate days and three exposures of 60 minutes on separate days do not appear to constitute an increase in risk for the component. It is noteworthy that with the exception of *S. epidermidis* in control adult units, which declined in count below the limit of detection, all of the species tested were culturable from both control pack types at day 35, underlining their ability to survive, albeit in low numbers, for the duration of cold storage.

A limitation of the studies conducted by NBL is that for S.liquafaciens and Y.enterocolitica, growth of the bacteria had reached stationary phase by day 15 when the units were first exposed to deviations in temperature. Further work was undertaken using lower spiking

inocula to ensure that growth on day 15 was in exponential phase. For S. liquefaciens, counts of test and control units were closely similar for all units and confirmed that the stated deviations had no effect on the growth response of this organism. Repeating of these experiments for Y. enterocolitica with a lower initial inoculum resulted in exponential growth as desired and no differences were observed between tests and controls for paediatric units. Considerable fluctuation in test and control counts was evident in adult units with pre temperature deviation counts on day 15 exceeding the 1 log difference threshold. However, by the end of the deviations no further increase in counts between tests and controls was observed. These findings indicate that repeated excursions of adult units out of temperature control for greater than 30 minutes did not result in a significant acceleration of growth of this organism.

A notable outcome from the second Ramirez-Arcos study was the demonstration of elevated and clinically significant levels of endotoxin in both *Y. enterocolitica* and *S. marcescens* spiked RCC. Concentrations of endotoxin (> 1000 EU/ml) for *Y. enterocolitica* in tests and controls were evident by day 20 post collection and correspondingly by day 35 for *S. marcescens* [22], but were not significantly different in units exposed to 30 or 60 minutes at ambient temperature compared to controls stored at 4°C throughout. However, the accurate measurement of endotoxin in whole blood is difficult due to non specific protein binding and technical factors such as the high haematocrit.

c) Discussion

The in depth review by Brunskill *et al* in 2012 concluded that RCC temperature excursions did not result in significant bacterial growth, but insufficient studies had been published [12]. However, this view has been underlined by studies published since then and the current investigations by NBL.

Few bacterial species are able to multiply at 4°C and thus be classified as obligate psychrophiles although several species (mesophiles) are tolerant of cold and may survive storage due to selective shut down of metabolic systems. The problematic species for RCC contamination are mainly those groups able to survive and multiply, albeit slowly, in cold storage such as the Gram-negative organisms *Pseudomonas putida/fluorescens* and *Serratia marcescens/liquefaciens*. Of the organisms identified by NBL from incidents of contamination, only *P. putida*, *Y. enterocolitica*, and *S. liquefaciens* are associated with growth at refrigerated temperatures but this property is likely to vary with the origin of the strain from environmental and human sources [31]. *E. cloacae* is an environmental saprophyte and could be expected to survive in the cold, while the recovery of *Staphylococcus* sp. most likely represents initial gross contamination from the skin at venupuncture.

Serratia spp., are environmental bacteria which have long been associated with contamination of blood components. Few Gram-negative species are able to withstand the bactericidal effect of plasma and killing by phagocytes in the reduced oxygen concentration in a blood bag. The *Serratia* group are facultative anaerobes, which means that they are able to survive and grow in an atmosphere severely limited in oxygen. Some work has shown that

a strain of *S. marcescens* isolated from a contaminated blood unit was able to grow in deionised water supplemented with materials derived from the plastic bag, and in deionised water alone, and growth was greatest under anaerobic conditions [32]. This apparent intolerance of oxygen in nutrient limited conditions was considered to be a consequence of growth promoting compounds leaching out of the blood bag materials [33]. Both *S. marcescens* and *S. liquefaciens* produce potent haemolysins, and proteolytic enzymes that degrade complement, and other defence related plasma proteins thus enhancing their survival in blood. Indeed, it has been demonstrated that *S. liquefaciens* after inoculation into plasma from standard blood donations multiplied 22 million times greater than *E. coli* and 11,000 times more so than *P. fluorescens* [34]. Skin carriage of *Serratia* is believed to be rare in the healthy but screening data are limited and mostly anecdotal. If derived from the decontaminated skin of a donor, initial counts are most likely to be low ($< 10^2$) but by the end of the 35 day shelf life of a unit of RCC counts could reach $\geq 10^8$ /ml and thus constitute a significant risk to a recipient. However, both the Canadian studies and data from NBL show that when compared to refrigerated control units, counts of *S. liquifaciens* were not significantly greater in units exposed to warm temperatures for up to 60 minutes. By contrast in the Canadian study, the cold tolerant species *S. marcescens*, showed an increased in count of 1 Log over controls in units exposed multiple times to room temperature for 30 or 60 minutes but the increase was not considered to be of clinical significance for this species as counts fell below the threshold of 10^5 cfu/ml advocated by Jacobs *et al* in platelet component transfusions and was not different between those units exposed for 30 or 60 minutes.

Pseudomonas putida, and its near relative *P. fluorescens*, are primarily pathogenic for plants, being ubiquitous in the natural environment. As a consequence of a general ability to thrive in refrigerated temperatures and high proteolytic activity, they are commonly isolated as food and dairy spoilage agents. *Pseudomonas* spp. were found colonizing the arms of approximately 1% of blood donors, with *P. fluorescens*, specifically, being present in 0.3% of donors [35]. These species are rarely found in clinical specimens and are often of doubtful significance outside of immunosuppressed individuals (see Appendix).

Nevertheless, initially low numbers of either *Pseudomonas* or *Serratia* spp., in a red cell unit, even if rapidly refrigerated post collection, could proliferate over time to constitute a significant microbial hazard to a susceptible recipient and this could be exacerbated by repeated and/or prolonged excursions from refrigeration in excess of those validated.

d) Conclusions on bacterial risk

- Sepsis following transfusion of RCC is exceedingly rare (*c.* 1 in 6 million).

- The level of risk due to the current storage and transport conditions is considered to be very low given the rarity of contamination events. None of the six bacterial transmissions since 1995 are known to have been associated with deviations in storage temperatures of the implicated units.

- Although based in antiquity and on little evidence, the 30-minute rule has served to reduce the risk of bacterial proliferation in units contaminated with the cold-tolerant species *Pseudomonas* and *Serratia* spp., which could constitute a significant clinical hazard to recipients.

- Published research and studies by NBL suggest that a reasonable case can be made to extend the out of temperature control rule to 60 minutes for no more than three exposures to ambient temperature when red cells have been returned to 2-6°C between exposures. This is based on the finding that the increase in bacterial counts following each exposure did not exceed the threshold limit of 1log difference.

4. Current practice in UK hospitals

In response to data demonstrating a continued significant wastage of red cells in hospitals being 'out of temperature control', the Blood Stocks Management Scheme (BSMS) undertook a survey of at the end of 2014 to better understand the issue and how it could be improved. A total of 130 hospitals responded to questions relating to temperature control of areas of the supply chain from Blood Centre to patient.

- The movement of red cells from blood supplier to hospital is a controlled process, both in terms of temperature and also inability for units to be tampered with, and there was no evidence that this area causes significant problems.
- Transfer of red cells from receipt to stock refrigerator in the hospital transfusion laboratory and from the transfusion laboratory to the issue refrigerator is also very well controlled. For transit times of >15 minutes from the transfusion laboratory within the same hospital location, 98% of respondents use boxes validated to keep red cells between 2-6°C, this figure reduces to 72% for 5-15 minutes and 48% for <5 minutes.
- For transfer from the transfusion laboratory to off-site locations such as hospices, all respondents indicated that where this is applicable validated transport boxes are used with (13%)/without (87%) temperature monitoring.
- Temporary storage of red cells in validated, storage portable containers for periods between 2 and 24 hours (mainly less than 4) was more common than expected for transfusions at home/hospices and use on haematology wards, trauma packs and air ambulances. The portable containers appear to be validated appropriately but few (13%), had temperature monitoring devices and 27% of respondents allowed access to the container to check the contents.
- The greatest wastage of red cells out of a controlled environment occurs in the transfer from an issues refrigerator to the location where the patient is to be transfused; 59% of respondents do not use validated transport containers when collecting blood for a patient. 32% use validated transport boxes, of which 3% are temperature monitored.

- The majority of respondents (94%) follow the 30 minute rule and discard units that either exceed this, or where there is uncertainty that the time out of controlled storage was <30 minutes. A minority (3%) reduce this to 15 minutes, and 3% extend this to 60 minutes if they check the temperature of the units.

The storage of red cells for significant durations in portable containers, and also when issued to patients appears to be areas where improvements in practice could be achieved.

5. Conclusions and recommendations

- The current recommended red cell component storage temperature of 2-6°C is appropriate to ensure red cell quality is maintained and the risk of bacterial proliferation is minimised.

- The current upper limit of 10°C surface temperature for <12 hours of transportation between blood centres and hospitals is acceptable, but it should be clarified that this is on a single occasion since bacterial studies where red cells have undergone prolonged or multiple shorter deviations in storage temperature due to transportation in combination with deviations that may occur later in shelf-life (i.e representing the 30 minute rule) have not been conducted.

- The current upper limit of a core temperature of 10°C in the case of refrigerator breakdown for a maximum of <5 hours on a single occasion is acceptable. If this occurs in the hospital setting, then hospitals must ensure this is the only excursion due to equipment failure that has occurred through discussion with their Blood Supplier.

- We advocate minimising the time that red cells are stored out with 2-6°C in order to optimise red cell quality and reduce bacterial risk as far as possible, by using blood transport containers that are validated to keep red cells at 2-6°C, especially for transit times that will exceed 15 minutes.

- For occasions when removing red cells from 2-6°C controlled storage is unavoidable then:

    - It is best to restrict time out of a controlled temperature environment to <30 minutes.
    - If 30 minutes is exceeded the unit should not be returned to the issue location in the refrigerator, but returned to the transfusion department or quarantined remotely using electronic blood tracking.
    - Up to 60 minutes out of controlled temperature is acceptable, provided the unit is quarantined, by placing in a secure refrigerator for at least 6 hours, to allow the unit to return to 2-6°C, prior to reissue.
    - Hospitals will need to identify these units so that they are not subject to out of controlled temperature storage for >30 to <60 minutes on more than three occasions.

UKBTS General Information 03

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

June 2016

- At this time, there is insufficient evidence to extend the 4 hour rule covering the maximum time that may be taken for transfusion.

- A change notification is written to cover the recommendations in this paper and include them into specifications of red cell components in the Red Book (note added following completion of this paper, the exact wording and draft change notification was approved by JPAC 23 June 2016 and can be found at appendix 2).

UKBTS General Information 03

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

June 2016

Appendix 1. Hazard profiles of cold-tolerant bacteria from contaminated RCC

P. putida/*P. fluorescens*

Both species are primarily plant pathogens and common in soil, water. They are cold tolerant, metabolically versatile, and readily isolated from the general environment as well as transient colonists of human skin. They are among the most common species recovered from contaminated blood products and infusion solutions. They have an intrinsic ability to form biofilms on inanimate surfaces including plastics and this may contribute to survival in blood collection bags. Although low grade pathogens for man they produce high affinity iron-chelating compounds to acquire iron from haemoglobin to facilitate growth in blood. Transmission is through direct or indirect contact with contaminated inanimate surfaces or transfer from the skin to an adjacent body site, e.g. at venesection. The likelihood of infection is low except in patients with long term indwelling venous access devices and onset of infection is often delayed several days following exposure to a contaminated source. Some strains exhibit increased resistance to disinfectants and antiseptics and are often recovered from non sterile blood collection tubes where they contribute to false positive blood cultures or 'pseudobacteraemia.'

Their infectious dose is unknown but likely to be in excess of 10^4 organisms/ml if introduced directly into the blood stream. Similarly, there are scant data on the activity of the endotoxins produced by these species. In keeping with most pseudomonads, they exhibit relatively high levels of natural resistance to antibiotics but most strains should be susceptible to third and fourth generation cephalosporins, carbapenems and the newer fluoroquinolones.

Y. enterocolitica

This species is a common cause of infection in animals and less so in humans. It is widespread in nature owing to asymptomatic shedding from the animal gut. Relatively few strains are enteropathogenic in man and infection is usually a self-limiting diarrhoea, on occasion with fever. *Y. enterocolitica* is one of the most frequent contaminants of blood owing to its ability to thrive at refrigerated temperatures. Most strains are susceptible to a wide variety of antimicrobials with the exception of ampicillin and cephalothin.

In immunosuppressed individuals, the organisms can spread from the gut to liver and spleen and form abscesses. It is speculated that subclinical or mild episodes of gastroenteritis may result in extension of the bacteria from the gut to the blood stream leading to a transient bacteraemia in a donor. They are potent producers of siderophores which sequester iron from haemoglobin and individuals with haemochromatosis are particularly susceptible to infection. Infections are sometimes associated with inflammatory sequelae such as arthritis and Reiter's syndrome.

S. marcescens/S. liquefaciens

Both species are relatively widespread in nature and are transient but rare colonists of human skin. *S. marcescens* is the most clinically relevant species particularly as an

opportunist agent in neonates and the severely immunocompromised. The intrinsic pathogenicity of *S. liquefaciens* for man is debatable but it has been repeatedly cited as a cause of serious sepsis and death in recipients of contaminated RBC units. Both species survive well in refrigeration but *S. liquefaciens* is held to be more rapid growing in the cold than *S. marcescens* but evidence for this is scant. Strains are often susceptible to a variety of antimicrobials

Transmission of the organism most likely occurs at venesection and clinically significant numbers and possibly endotoxin accumulate steadily over storage time.

Joint UKBTS Professional Advisory Committee

UKBTS General Information 03

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

June 2016

Appendix 2 Change notification approved by JPAC 23rd June 2016

Joint UKBTS Professional Advisory Committee (1) Summary Sheet

1. Paper for the JPAC meeting on:	23 June 2016
2. Date submitted:	14 June 2016
3. Title (including version no.):	Inclusion of '30 minute rule' in component specifications
4. Author(s):	Dr Rebecca Cardigan on behalf of the SAC on Blood Components
5. Brief summary:	JPAC agreed at their meeting March 2016 that guidance relating to re-issue of blood components that have been removed from controlled temperature storage, agreed by JPAC in June 2015 (JPAC 15-54), should be included in the relevant component specifications in the Red Book as well as in the revised BCSH Guidelines on the Administration of Blood Components. The attached change notification has been drafted by SACBC. Although the studies previously reviewed by SACBC and JPAC to support the extension from 30 to 60 minutes have mainly used standard red cells or paediatric sized red cells in additive, we consider that it is reasonable to extrapolate these data to most red cell components since: 1) bacterial risk is unlikely to be significantly altered between red cell component types 2) The component quality is likely to be similar for red cells stored in SAGM or CPD/plasma. However washed, or frozen-washed red cells could conceivably be more adversely affected by exposure to ambient temperatures since they have already been exposed to additional stresses as part of the

	production process. There are no data to guide policy in this respect. We therefore recommend that the recommendations attached DO NOT apply to these components. Return and re-issue of these components following removal from a controlled environment should be subject to risk assessment and issue under clinical concession. This should be a rare occurrence since the number of these components issued is very small relative to standard red cells, and they are usually requested and transfused to specific patients. We have not included recommendations in relation to how many times blood can be out of controlled storage for <30 minutes as there is little evidence to base this on and limited data on current practice. Therefore the focus is on the extension of 30 to 60 minutes, on the assumption that <30 minutes is current practice and that bacterial sepsis associated with transfusion of red cells according to current practice is rare. However, this could be clarified further in the BCSH Guidelines to provide a more practical interpretation of this content. In order to be consistent with the recently revised specification relating to the use of thawed plasma, we have also included a statement in relation to the time within which transfusion must be completed. This is in accordance with the current BCSH Guidelines on the Administration of Blood Components and current practice. It has not been extended from 4 hours as SACBC/JPAC considered there was insufficient evidence to do so (summarised in JPAC 15-54).
6. ***Action required by JPAC:*** (What do you want JPAC to do in response to this paper?) e.g. • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within	Endorse the change notification.

UKBTS General Information 03

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

June 2016

the work plan • provide a steer on policy	
7. Any other relevant information:	JPAC 15-54

(1) **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices **Professional Advisory Committee**

UKBTS General Information 03

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

June 2016

Text for Change Notification

Removal of red cells from a controlled temperature environment

Applies to the Guidelines for the Blood Transfusion Services in the United Kingdom – 8th Edition 2013

Following a review of data from UKBTS and published studies, a number of changes are being made in relation to red cells as follows:

Guidance relating to re-issue of blood components that have been removed from controlled storage is currently given in the BCSH Guidelines on the Administration of Blood Components (2009) but not the Red Book. Following a review of data relating to the quality and safety of red cells removed from controlled storage, we are making a number of changes to the storage and transport sections of the specifications of red cell components in the Red Book. This includes giving guidance relating to removal from 2-6oC controlled storage within hospitals which will also be detailed in the revised BCSH guidelines (due for release 2016). A paper describing the rationale for these changes can be found on the JPAC website.

These changes apply to the following components:

- 7.5:Red Cells, Leucocyte Depleted
- 7.6:Red Cells in Additive Solution, Leucocyte Depleted
- 7.22: Red Cells for Intrauterine Transfusion (IUT), Leucocyte Depleted
- 7.24:Red Cells for Exchange Transfusion, Leucocyte Depleted
- 7.25:Red Cells for Neonates and Infants, Leucocyte Depleted
- 7.26:Red Cells in Additive Solution for Neonates and Infants, Leucocyte Depleted

These changes **DO NOT** apply to the following components

- 7.7:Red Cells, Washed, Leucocyte Depleted
- 7.8:Red Cells, Thawed and Washed, Leucocyte Depleted

The storage and transport sections of these specifications are changed as follows:

Storage

For general guidelines, see section 6.7.

- Variation from the core temperature of 4 ±2°C of the finished component must be kept to a minimum during storage at all stages of the blood supply chain and restricted to any short period necessary for examining, labelling or issuing the component.

- Exceptionally, i.e. due to equipment failure at a Blood Centre or hospital, for temperature excursions where the core temperature has not exceeded 10°C or fallen below 1°C, components may be released for transfusion provided that:
  - the component has been exposed to such a temperature change on one occasion only
  - the duration of the temperature excursion has not exceeded 5 hours
  - a documented system is available in each Blood Centre or hospital to cover such eventualities
  - adequate records of the incident are compiled and retained.

Transportation

For general guidelines, see section 6.11.

For red cell components, transit containers and packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 6°C during transportation. Additionally:

- the validation exercise should be repeated periodically

- if melting ice is used, it should not come into direct contact with the components

- dead air space in packaging containers should be minimised

- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components

- for transportation between blood supplier and hospital an upper limit of 10°C surface temperature is acceptable but should be limited to one occasion, not exceeding 12 hours.

In some instances it is necessary to issue red cell components from the blood supplier to hospitals that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

Removal from and return to 2-6°C controlled storage within hospitals [Entirely new section]

For occasions when red cells are removed from 2-6°C controlled storage (eg when issued to a clinical area immediately prior to transfusion) and returned then:

UKBTS General Information 03

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

June 2016

- If possible, time out of a controlled temperature environment should be restricted to under 30 minutes

- if 30 minutes is exceeded the unit should not be returned to the issue location in the refrigerator, but returned to the transfusion laboratory or quarantined remotely using electronic blood tracking

- up to 60 minutes out of controlled temperature is acceptable, provided the unit is then quarantined by placing in a secure refrigerator for at least 6 hours prior to reissue, to allow the unit to return to 2-6°C

- Hospitals will need to identify such units so that they are not subject to being out of controlled temperature storage for between 30 and 60 minutes on more than three occasions.

Transfusion should be completed within 4 hours of issue out of a controlled temperature environment.

6. References

(1) British Committee for Standards in Haematology: Guideline on the administration of blood.; in: 2009.

(2) Guidelines for the Blood Transfusion Services in the United Kingdom: ed 8th The Stationary Office, London, 2013.

(3) Guide to the Preparation, Use and Quality Assurance of Blood Components: ed 17th Council of Europe Publishing, Strasbourg, 2013.

(4) AABB Standards Program Committee: Standards for blood banks and transfusion services, ed 28. Bethesda, aaBB, 2012.

(5) Pick P, Fabijanic J: Temperature changes in donor blood under different storage conditions. Transfusion 1971; 11(4):213-215.

(6) Johnson V, Langeberg A, Taye-Makuria A, Sandler SG: Temperature-sensitive labels for containers of RBCs. Am J Clin Pathol 2006; 126(3):406-410.

(7) McLaughlin N, Sadek I, Anderson D: Bacterial growth in RBC units exposed to room temperature for greater than 30 minutes. Transfusion 2010; 50(Suppl 2):76a.

(8) Perry HE, Prasad P, Kirwan S, Huang YQ: Core temperature changes in resuspended red blood cells (RBCs) and pediatric RBCs removed from refrigerated storage. Transfusion 2010; 50(1):174-177.

(9) Reiter U, Reiter G, Wagner T, Kozma N, Roland J, Schollnast H, Ebner F, Lanzer G: Four-dimensional temperature distributions in red blood cells withdrawn from storage and exposed to ambient temperature: a magnetic resonance thermometry study. Transfusion 2013; 53(1):167-173.

(10) Hughes-Jones NC: Storage of red cells at temperatures between + 10 degrees C. and -20 degrees C. Br J Haematol 1958; 4(3):249-255.

(11) Blood Stocks Management Scheme: Annual report 2012-2014; in: 2015.

(12) Brunskill S, Thomas S, Whitmore E, McDonald CP, Doree C, Hopewell S, Staves J, Cardigan R, Murphy MF: What Is the Maximum Time That a Unit of Red Blood Cells Can Be Safely Left Out of Controlled Temperature Storage? Transfus Med Rev 2011.

(13) Hogman CF, Meryman HT: Storage parameters affecting red blood cell survival and function after transfusion. Transfus Med Rev 1999; 13(4):275-296.

(14) Strauss D, Tanzer S, Schmutzler F, Gulke L, Rettig HP, Roigas H, Raderecht HJ: [The effect of storage temperatures between 4 and 25 degrees centigrade on the usefulness of stored blood]. Folia Haematol Int Mag Klin Morphol Blutforsch 1974; 101(2):243-255.

(15) Shields CE: Studies on stored whole blood. IV. Effects of temperature and mechanical agitation on blood with and without plasma. Transfusion 1970; 10(4):155-162.

(16) Ruddell JP, Lippert LE, Babcock JG, Hess JR: Effect of 24-hour storage at 25 degrees C on the in vitro storage characteristics of CPDA-1 packed red cells. Transfusion 1998; 38(5):424-428.

(17) Reid TJ, Babcock JG, Derse-Anthony CP, Hill HR, Lippert LE, Hess JR: The viability of autologous human red cells stored in additive solution 5 and exposed to 25 degrees C for 24 hours. Transfusion 1999; 39(9):991-997.

(18) Ecker T, Hitzler WE: Effect of 6-hour exposure to 20 degrees C on the ATP content and other biochemical measures of CPDA-1 packed red cells. Clin Lab 2000; 46(5-6):291-293.

(19) Hancock V, Cardigan R, Thomas S: Red cell concentrate storage and transport temperature. Transfus Med 2011; 21(5):325-329.

(20) Thomas S, Hancock V, Cardigan R: Repeated short-term warming of red blood cell concentrates has minimal effect on their quality. Vox Sang 2012;10-0410.

(21) Thomas S, Hancock V, Cardigan R: The 30 minute rule for red blood cells: in vitro quality assessment after repeated exposure to 30 degrees C. Transfusion 2013; 53(6):1169-1177.

(22) Ramirez-Arcos S, Perkins H, Kou Y, Mastronardi C, Kumaran D, Taha M, Yi QL, McLaughlin N, Kahwash E, Lin Y, Acker J: Bacterial growth in red blood cell units exposed to uncontrolled temperatures: challenging the 30-minute rule. Vox Sang 2013; 105(2):100-107.

(23) Ramirez-Arcos S, Mastronardi C, Perkins H, Kou Y, Turner T, Mastronardi E, Hansen A, Yi QL, McLaughlin N, Kahwash E, Lin Y, Acker J: Evaluating the 4-hour and 30-minute rules: effects of room temperature exposure on red blood cell quality and bacterial growth. Transfusion 2013; 53(4):851-859.

(24) de Grandmont MJ, Ducas E, Girard M, Methot M, Brien M, Thibault L: Quality and safety of red blood cells stored in two additive solutions subjected to multiple room temperature exposures. Vox Sang 2014; 107(3):239-246.

(25) Wagner T PMLGRUKNLGHB: Impact of constant storage temperatures and multiple warming cycles on the quality of stored red blood cells. Vox Sang 2015; 106:45-54.

(26) Gulliksson H, Nordahl-Kallman AS: Effect of transient warming of red blood cells for up to 24 h: in vitro characteristics in CPD/saline-adenine-glucose-mannitol environment. Vox Sang 2014; 106(1):61-67.

(27) Strauss D, Raderecht HJ: [The effect of adenine and guanosine on the survival rate of erythrocytes after storage at temperatures between 4 and 25 degrees centigrade]. Folia Haematol Int Mag Klin Morphol Blutforsch 1974; 101(2):232-242.

(28) Hogman CF, de Verdier CH, Borgstrom L: Studies on the mechanism of human red cell loss of viability during storage at +4 degrees C. II. Relation between cellular morphology and viability. Vox Sang 1987; 52(1-2):20-23.

(29) Hamill TR: The 30-minute rule for reissuing blood: are we needlessly discarding units? Transfusion 1990; 30(1):58-62.

(30) Hamill TR, Hamill SG, Busch MP: Effects of room-temperature exposure on bacterial growth in stored red cells. Transfusion 1990; 30(4):302-306.

(31) Chapalain A, Rossignol G, Lesouhaitier O, Merieau A, Gruffaz C, Guerillon J, Meyer JM, Orange N, Feuilloley MG: Comparative study of 7 fluorescent pseudomonad clinical isolates. Can J Microbiol 2008; 54(1):19-27.

(32) Szewzyk U, Szewzyk R, Stenstrom TA: Growth and survival of Serratia marcescens under aerobic and anaerobic conditions in the presence of materials from blood bags. J Clin Microbiol 1993; 31(7):1826-1830.

(33) Heltberg O, Skov F, Gerner-Smidt P, Kolmos HJ, Dybkjaer E, Gutschik E, Jerne D, Jepsen OB, Weischer M, Frederiksen W, .: Nosocomial epidemic of Serratia marcescens septicemia ascribed to contaminated blood transfusion bags. Transfusion 1993; 33(3):221-227.

(34) Puckett A: Bacterial contamination of blood for transfusion: a study of the growth characteristics of four implicated organisms. Med Lab Sci 1986; 43(3):252-257.

(35) Puckett A, Davison G, Entwistle CC, Barbara JA: Post transfusion septicaemia 1980-1989: importance of donor arm cleansing. J Clin Pathol 1992; 45(2):155-157.