Joint UKBTS / NIBSC Professional Advisory Committee (*)

UKBTS General Information 05

Shelf Life Of Red Cells In Additive Solution (SAG-M)

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Background

During liquid storage at 4°C, red cells undergo both reversible and irreversible changes which constitute the "storage lesion". Changes include decrease of sodium and increase of potassium, accumulation of lactate, decrease in ATP and 2,3 DPG content, and lowering of pH. The formulation of red cell additive solutions is designed to minimise these changes. In the UK, SAG-M is the additive solution in current use. Red cells in SAG-M carry a shelf life of 35 days according to UKBTS (Red Book) Guidelines, though elsewhere in the world a 42-day shelf life is routinely assigned to this component. Council of Europe guidelines and the EU Directive allow for extension of storage, depending on the anticoagulant / additive system, up to the approved limit of the system.

Extending the shelf life of OAS red cells would enable reduction of wastage due to outdating, and facilitate more efficient inventory management. The potential benefits of extending the shelf life must outweigh potential risks. Risks to be considered include:

- Reduction in red cell viability/↑ haemolysis leading to higher potassium load
- Possible increased risk from bacterial contamination
- Possible increase in TRALI risk (Silliman hypothesis)

1. Measurement of red cell viability

The primary indication for transfusion of red cells is to replace oxygen carrying capacity. Storage conditions should be such that viability and functional capacity of red cells remain as intact as possible. Council of Europe and Red Book Guidelines require that haemolysis should be <0.8% (<1.0% for AABB) of total haemoglobin at the end of the storage period, and that at least 75% of the mean of transfused cells at the end of their shelf-life remain in circulation 24 hours after transfusion, as measured by red cell recovery studies. Measurement of 2,3 DPG is not relevant to discussions about extension of shelf life beyond 35 days, as levels are undetectable beyond 21 days' storage.

In vivo recovery studies are, however, not always performed unless a pack system or additive solution is particularly novel. Hogman[13] and others have attempted to correlate the concentration of ATP in red cells with post-transfusion survival – it is thought that a concentration of about 50% starting value is necessary for the maintenance of RBC viability. ATP levels however do not always correlate with viability and it has been further suggested that the total adenine nucleotide level (ATP + ADP + AMP) may be a better indicator of viability[8].

In vitro and in-vivo studies

For any pack system, the critical factors which affect red cell viability after storage are the formulation of the additive solution, and the plastic of the pack in which the component is stored.

Table 1 summarises available data on red cell recovery, haemolysis and ATP levels (plus total adenine nucleotide levels where available) of red cells in SAG-M after 42 day storage (and 35 day storage where results are available). Where identified, the pack plastic in the system being investigated is noted.

Hogman[5] has reviewed the available literature on post-transfusion in-vivo recovery of red cells suspended in additive solutions, which includes red cells in SAG-M. He concluded "Numerous studies show that SAG-M-stored red cells qualify > 75% in vivo recovery (24 hour red cell survival) with a 42-day shelf life".

Although SAG-M is the additive solution used in all studies referred to in Table 1, pack types and processing systems vary. Some processes include leucodepletion and some not. Williamson et al[28] comment that filtration improved ATP maintenance in the two groups processed by the top/top method. In their study in addition bottom-and-top (BAT) processing was superior to top/top for ATP maintenance.

Not all studies assessed 24-hour in-vivo recovery. Of those, which did, all but one met the required standard of > 75% mean recovery. The authors of the study showing worse recovery concluded that a 35 day shelf life should be assigned to this process[12] (this pack type is not used in the UK).

For haemolysis, all studies but one met the standard of a mean of < 0.8%. However, in the study where the mean was found to be 0.84%, the 24 hour recovery was high at 85%[7], indicating that the cells were viable.

In none of the studies did mean ATP loss exceed 50%. In those where % reduction in total adenylates was recorded, this was found to be low or zero, indicating good in vitro measure of viability.

Effect of increasing length of storage on supernatant potassium in SAG-M red cells

An increase in the levels of supernatant potassium in red cells may be a concern, particularly in paediatric practice. Data from evaluation of Baxter RZ2000 packs performed in the NBS Components Development Laboratory are given in Table 2 (personal communication, Rebecca Cardigan). This shows a small (but probably not significant clinically) increase in mean potassium levels from 41 mmol/l at day 35 to 48 mmol/l at day 42.

Data from manufacturers of currently used packs, supporting 42 day shelf life

This data, as supplied by manufacturers of packs in current use, is tabulated in the Appendix. Data supplied is not comprehensive, and it is not always clear to which pack type it refers.

In summary, data provided by manufacturers generally shows compliance with specification for haemolysis, except for Macopharma data. However, the latter study was performed some years ago and may not be applicable to pack systems currently in use. In addition, the red cells were not leucodepleted, which is known to improve in vitro data. Reduction in ATP content was variable, but as stated above this does not necessarily equate with poor post-transfusion recovery. Total adenylate content was only provided for the evaluations from Fresenius.

Data from SNBTS on evaluations of filters currently in use from 3 manufacturers gives results within required specification for both haemolysis and ATP content.

Information on the plastic used in the final storage containers in current use by the UKBTS is provided at the end of the Appendix (all use PVC with DEHP as plasticiser).

Clinical significance of red cell age

Results of studies which have attempted to address the question of whether transfusion of older red cells has any clinical impact are conflicting. One investigated the effect of a 3-unit transfusion on oxygen kinetics in septic critically ill patients, and found no correlation between age of red cells and oxygen delivery, but noted on further analysis an association between age of blood and gastric intra-mucosal pH. This was interpreted as indicating worse gastric oxygenation with increasing age of blood [15]. A study in rats showed that 28-day old rat blood failed to improve systemic oxygen consumption [1]. Subsequent work, however, has shown that rat red cells deteriorate rapidly during storage and at 28 days only 5% remain viable. A retrospective analysis of coronary artery bypass graft patients found a positive correlation between transfusion of older red cells and post-operative pneumonia [24]. A later study from the same group, however, demonstrated no association between transfusion of 'old' red cells and increased morbidity in cardiac patients [25]. These studies were all performed using non-leucodepleted blood, and were not randomised controlled studies (RCT).

The one, although small, prospective RCT recently reported, which investigated the effect of age of red cells on tissue oxygenation, randomised ICU patients to receive leucodepleted blood either < 5 days old, or > 20 days. There was no change in PCO_2 gap, pHi or oxygenation index between the two groups [26].

A very recently published laboratory study [18], in which a rat model was exchange transfused with human red cells of different ages and oxygen delivery to the microvascular circulation was determined, concluded that at low haematocrit, the oxygen-delivering capacity of red cells stored for 5 – 6 weeks is reduced compared to that of fresh and intermediate-stored (2 – 3 weeks) red cells. The authors were not able to determine the mechanism for this, but excluded reduced 2,3 DPG content and lack of red cell deformability as possible causes. It is yet to be determined what relevance these findings have to clinical practice.

2. Bacterial contamination

Because red cell concentrates are stored at 4°C they provide an inhospitable culture environment for many bacteria. Yersinia enterocolitica and psychrophilic pseudomonads are exceptions which grow well at 4°C. For such organisms, in inoculation experiments a lag phase of about 2 weeks is followed by exponential growth, reaching a concentration of about 10^9 organisms/ml after 4 weeks. This is associated with a parallel rise in endotoxin [3]. Septic transfusion reactions associated with contaminated red cells usually occur with units that have been stored for more than 21 days. This is in keeping with the slow growth characteristics in the cold of most bacteria and the growth kinetics of those that grow well at 4°C. There is, therefore, a theoretical risk that bacterial contamination may increase if red cells are stored for a longer period.

In a recent rapid review of 18/21 transfusion-related fatalities from bacterial contamination of red cells (January 1985 – December 2004) the average storage duration was 26 days (range 7-41 days). Most of these cases were due to Yersinia enterocolitica with a few due to Pseudomonas and Serratia species (Jay Epstein, FDA, personal communication). The FDA considered, but did not implement, two measures to reduce risk: a) limiting red cell storage to 25 days – this was not implemented due to the significant impact it would have on the blood supply and b) the screening of prospective donors for mild gastrointestinal illness – 12% of donors may report such symptoms in the month prior to donation but only 40% of donors linked with Y. enterocolitica transmission report symptoms. Inoculation studies have shown that leucocyte reduction results in decreased growth of Y. enterocolitica although data are less conclusive with other strains of bacteria.[4]. Data from SHOT (Serious Hazards of Transfusion) shows 4 clinical reports of bacterial contamination from red cell transfusions (1 October 1995 – 31 December 2003). In ¾ cases where the storage period was known this was 23, 32 and 33 days, and the implicated organisms were coagulase negative staphylococcus, Staph. Epidermidis and Y. enterocolitica (fatal) respectively [23].

There is little published data on the routine culturing of stored red cells to detect bacterial contamination, however, available recent evidence suggests that extended storage does not result in increased risk. The Paul-Ehrlich institute in Germany routinely culture a proportion of red cell units at the end of their shelf life (which varies from 35 to 49 days between different blood facilities, 80% of those tested being 42 days or greater). The contamination rate was found to be 0.16% of those tested in 1998, and 0.1% in 2001. The corresponding results from the NBS National Bacteriology Laboratory (after 35 days' storage) were 10 positive cultures identified out of 10,890 tested between 1999 and 2004, i.e. 0.1%. Interestingly, bacterial contamination of red cells does not appear to have been studied when determining the shelf life of red cells in the US, validation being limited to red cell recovery studies. However, in 1985, the FDA reduced shelf life from 49 to 42 days because of a drop off in survival time. (personal communication, Jay Epstein).

3. TRALI risk

Silliman[22] has suggested that some cases of TRALI may be caused by the neutrophil priming action of biologically active lipids present in stored cellular components. He describes TRALI

as occurring more after transfusion of older platelets, but suggests also that the priming activity may be present in stored red cells. Webert and Blajchman[27] note that the evidence for this as a cause of TRALI comes mostly from experimental animal studies, and suggest that further work is needed before recommending the use of red cells < 10 days old for patients at risk of TRALI. A recent retrospective study looking at acute lung injury in mechanically ventilated patients concluded that age of red cell units transfused was not associated with the development of ALI in these patients, in contrast to Silliman's hypothesis[2].

Most reports of TRALI, however, including those from SHOT, indicate that TRALI is more common after transfusion of plasma-rich components (e.g. FFP) rather than red cells[14]. There is therefore at the present time no convincing evidence that increasing the storage period of red cells would increase the risk of TRALI from this component.

Conclusions

Data from the literature show that, where evaluated, mean 24 hour in-vivo red cell recovery is maintained at > 75% after storage of red cells in SAGM for 42 days, supporting the extension of the shelf life of this component. Studies that have not evaluated red cell recovery have shown levels of haemolysis and ATP within specified limits, which is considered to be an in-vitro marker of viability. Supernatant potassium levels are slightly higher at 42 days compared to 35, but this is unlikely to be of clinical importance. There is no convincing clinical evidence to suggest that transfusion of 'older' red cells has any adverse effects.

There appears to be no increase in bacterial contamination rate of 42 day red cells compared with 35 day storage. There is no convincing clinical evidence that increasing the age of red cells will increase TRALI risk.

Manufacturers whose blood pack systems are in use in the UK have stated that their packs are suitable for storing red cells up to 42 days. Other Blood Services, for example the Netherlands and the majority of German Blood facilities (accounting for approximately 80% of red cell components in Germany) routinely store red cells in additive solution for 42 days (49 days for 15% of German red cell components), which is in accordance with CoE guidelines.

Recommendations

1. **Allowable red cell shelf life of red cells in additive solution should be extended to 42 days, provided that data is available for a specific process to show either mean red cell recovery at 24 hours is > 75% or ATP depletion is no more than 50%, and mean haemolysis is < 0.8%.**

2. **All future blood pack system evaluations should include evaluation of data up to this period.**

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APPENDIX - Data supplied by manufacturers of currently used packs

Fresenius / NPBI

Data has been received of work performed in CLB, Amsterdam (unpublished).

1. 1994, T/T quad system with BC removal (no day 42 data provided).

	Day 35	Day 49
% reduction ATP	43	62
% red total A	14	33
% haemolysis	0.5	0.8

2. 1995, T/T quad system, buffy coat removal

	Day 35	Day 49
% reduction ATP	29	49
% red total A	3	22
% haemolysis	0.4	0.7

3. 2001, BAT ?LD

	Day 35	Day 42
% reduction ATP	30	45
% haemolysis	0.10	0.172

4. 2003, BAT quad system ?LD

	Day 35	Day 42
% reduction ATP	33	43
% haemolysis	0.15	0.21

Baxter

Data from evaluation of soft housing filter for red cells (NBS, Bristol, 1990) Storage pack PL146. Compared filtration at RT < 8 hrs after collection, and filtration after up to 48 hrs storage at 4^0C.

	Day 42, 2-8 hrs, RT	Day 42, 48 hrs 4^0C
% reduction ATP	35	57
% haemolysis	0.2	0.18

Pall

Specification sheet stating blood components produced from the Pall Leucotrap WB system are suitable for storage for up to 42 days.

24-hour recovery data provided for AS3 additive solution only.

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Macopharma

Data provided from study in Regional Transfusion Centre, Lille, 1993 on RCC prepared from Macopharma quintuple packs, SAGM additive solution. Red cells were not leucodepleted.

	Day 36	Day 42
% reduction ATP	73	81
% haemolysis	1.1	1.6

Data provided to SACBC Technical Group from SNBTS on LD filter evaluations

Filter manufacturer /type	% haemolysis Mean (range)		% ATP loss Mean (range)	
	Day 35	Day 42	Day 35	Day 42
NPBI RCC	0.20 (0.09–0.23)	0.29 (0.18– 0.59)	32 (21 – 49)	43 (41 – 67)
NPBI WB	0.18 (0.12-0.28)	0.27 (0.16– 0.59)	39 (28 – 50)	49 (39 – 63)
Pall WB (WBF2)	0.54 (0.16-1.78)	0.63 (0.22– 1.74)	32 (21 – 46)	37 (27 – 47)
Pall RCC (RCM1)	0.2 (0.15-0.26)	0.26 (0.17– 0.44)	36 (29 – 46)	40 (38 – 48)
Baxter WB	0.22 (0.12-0.45)	0.35 (0.14 – 0.6)	24 (12 – 37)	34 (20 – 44)
Baxter RCC	0.19 (0.13-0.25)	0.27 (0.17– 0.35)	22 (13 – 35)	32 (23 – 42)

Plastic used in final storage containers in current use in UKBTS

Manufacturer	Plastic	Plasticiser
Baxter	PVC (PL146)	DEHP
MacoPharma	PVC	DEHP
NPBI	PVC	DEHP
Pall	PVC	DEHP

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Table 1: Red cell storage studies performed on CPD/SAGM units after 42-day storage (day 35 results in brackets where available)

Author/ Ref	Year	24 hour in-vivo survival (%)	Haemolysis (%)	% ↓ in ATP content	% ↓ in total adenylates	Pack type / Comments
Hogman [6]	1983	77.4 +/- 4.7 (83.5 +/- 5.3)				Travenol DEHP-PVC PL146
Hogman [9]	1985	77.4 +/- 4.7	0.49 +/- 0.15	42	12	Fenwal DEHP-PVC PL146 – investigated effect of rejuvenating solution (data from unrejuvenated)
Hogman [12]	1985	73.3 +/- 6.6	0.63 +/- 0.32	40		Teruflex DEHP-PVC (Terumo). Recommended 35 day shelf life only
Hogman [7]	1987	85.3 +/- 2.9	0.84 +/- 0.44			Terumo BAT (investigated effect of warming unit – data from unwarmed)
Hogman [11]	1988	84.2 +/- 4.2 (87.5 +/- 4.1)	0.39 +/- 0.2	40	0	Baxter BAT – DEHP-PVC
Hogman [10]	1991	83.2 +/- 5.1	0.55 (0.37)	39 (29)	0	BTHC-PVC PL2209, Fenwal
Rogers[21]	1995	80.9 +/- 5.9 (@35 days only)	0.21 (0.15)	34 (31)		BAT – PL146
Rider[20]	1996	82.3 +/- 6.0	0.2 (0.15)	22 (11)		Baxter Optipack – PL146
Muller-Steinhardt [17]	1997		<0.27 T/T < 0.53 BAT	7 – 31 depending on pack type		Baxter PL146 triple (T/T), PL2209 BAT
Rapaille[19]	1997		<0.25 (<0.20) filtered 0.33 (0.23) non-filtered	40 (36)		Baxter PL146 in-line filter with non-filter control
Williamson [28]	1999		<0.2 T/T filtered 0.3-0.6 unfiltered < 0.3 BAT (filt & non-filt)	40 top/top 25 BAT		Baxter PL146 triple (T/T) and PL2209 BAT Comparison between filtered and control (non-filtered)
Muller-Steinhardt [16]	2002		< 0.3 (0.2 mean)	37 (20)		Baxter and Macopharma soft-body filters

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Table 2: Supernatant potassium levels in SAG-M red cells to day 42 of storage (n=21)

Day 0/1	Day 7	Day 35	Day 42
1 (1-2)	13 (9-26)	41 (37-52)	48 (42-55)

Data is median with 97% CI and is expressed in mmol/l. Data from evaluation of Baxter RZ2000 packs.

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