JPAC 08-79

REPORT

Red cell concentrate quality

Re-manufacture of RCC-CPD into RCC-SAGM

Additional work II

Components Development Laboratory,
National Blood Service, England & North Wales (NBS)

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Personnel responsible for project	Mike Wiltshire
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2 Declarations

We accept responsibility for the conduct of this study, which was performed in compliance with specification SPN/DDR/CD/001.

Mike Wiltshire
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Stephen Thomas
CDL Manager

I agree with the conclusions of this report and have made the CSG aware of its findings.

Rebecca Cardigan
Head of Components Development

I agree with the conclusions of this report.

Sheila MacLennan
Interim Clinical Director (Products)

3 Glossary of terms

2,3-DPG	2,3-Diphosphoglycerate
ATP	Adenosine Triphosphate
CDL	Components Development Laboratory
CPD	Citrate Phosphate Dextrose
CSG	Components Strategy Group
ExTx	Exchange transfusion
Hb	Haemoglobin
NBS	National Blood Service
NHSBT	NHS Blood and Transplant
RCC	Red Cell Concentrates
Re-MFR	Re-manufacture (units processed into ExTx units, and stored at 4 °C. Units were then converted into SAGM red cell concentrates on day 6)
SACBC	UK Standing Advisory Committee on Blood Components
SAGM	Saline Adenine Glucose and Mannitol
TRALI	Transfusion Related Acute Lung Injury
vCJD	Variant Creutzfeldt-Jakob Disease
WB	Whole Blood
WBS	Welsh Blood Service

4 Introduction

Following the initial work on the re-manufacture of plasma reduced (exchange transfusion) red cell concentrates into red cells in SAGM (Study C; EVAL/CD/2006/44a), the Standing Advisory Committee on Blood Components (SACBC) have requested data on the re-manufacture of units on day 6 to maximise the hold period, i.e. to the end of day 5. The Welsh Blood Service (WBS) has studied units re-manufactured on day 6 and investigated volume, haemoglobin content, haemolysis and supernatant potassium throughout storage (Rainbird, 2006). This additional study aims to add to this data by measuring ATP, as well as haemolysis and supernatant potassium levels, at days 35 and 42 post donation, in day 6 re-manufactured units.

5 Methods

5.1 Study design

This project required 10 units of whole blood collected into CPD anticoagulant in Pall WBT436CEU collection/storage packs.

- Study F: Plasma reduced red cells for exchange transfusion (CPD) held until day 6 and re-manufactured into SAGM red cells.

10 whole blood units collected into CPD were either leucocyte depleted as whole blood and plasma reduced on day of collection (day 0), or placed at 4 ?C and held overnight for day 1 leucodepletion and plasma reduction, depending on processing capability.

Red cells in plasma (CPD) were stored at 4 ?C and sampled on day 6. Following sampling, the exchange transfusion units were returned to the processing department for re-manufacturing into SAGM red cell concentrates (RCC). Note: the plasma expressed during the primary processing was retained as there is a need to add this back to facilitate re-manufacture. These units were then stored at 4 ?C and tested on days 35 and 42. Units were assayed at each time point for volume, haematocrit haemoglobin content, haemolysis, supernatant potassium and cellular ATP.

Results were compared to data from EVAL/CD/2006/44a, data from the WBS study (Rainbird, 2006) and laboratory reference data where appropriate.

5.2 Deviations from protocol

There were no deviations from the protocol.

6 Results

6.1 Red cell concentrate content

- All of the units re-manufactured on day 6 met UK and local specifications for volume, haemoglobin content and haematocrit (Table 1; Figure 1).
- The volume of the re-manufactured units was significantly lower than for units manufactured using standard processing techniques (reference data), ($p<0.05$) (Table 1; Figure 1a).
- The median amount of haemoglobin per unit was lower in re-manufactured units than in control RCC although the difference was not significant (Table 1; Figure 1b). However, the reduced haemoglobin amount is due mainly to the starting units containing a lower amount of haemoglobin (Median: 56.3 gHb), while the secondary processing (re-manufacturing) step, resulted in a loss of approximately 1.9 gHb.
- Re-manufactured units had haematocrit values that were significantly lower than those of reference (control) units ($p<0.001$) but all units were within specification (Table 1; Figure 1c).

Red cell Component	Volume (ml)	Haemoglobin (g/unit)	Haematocrit (%)
Post Re-MFR (SAGM)[1]	271 (257-315)[2]	55 (49-69)[2]	61 (59-65)[3]
Re-MFR Reference[4]	270 (240-299)[2]	54 (40-68.5)[2]	58 (53-63)[3]
SAGM Reference[5]	298 (266-339)	59 (49-72)[2]	62 (56-68)
Spec (SAGM)[6]	220-340	> 40	50-70

Table 1. Red cell concentrate content

Data are given as median with range, Re-MFR n=10, Re-MFR Reference n=20, SAGM Reference n=125.

[1] Values measured on day 35

[2] Values corrected for sampling.

[3] Haematocrit values are uncorrected and will have been affected by sampling.

[4] Re-Mfr reference data (day 5) generated in EVAL/CD/2006/44a.

[5] SAGM reference data generated in EVAL/CD/2005/35.

[6] UK or local NHSBT specification.

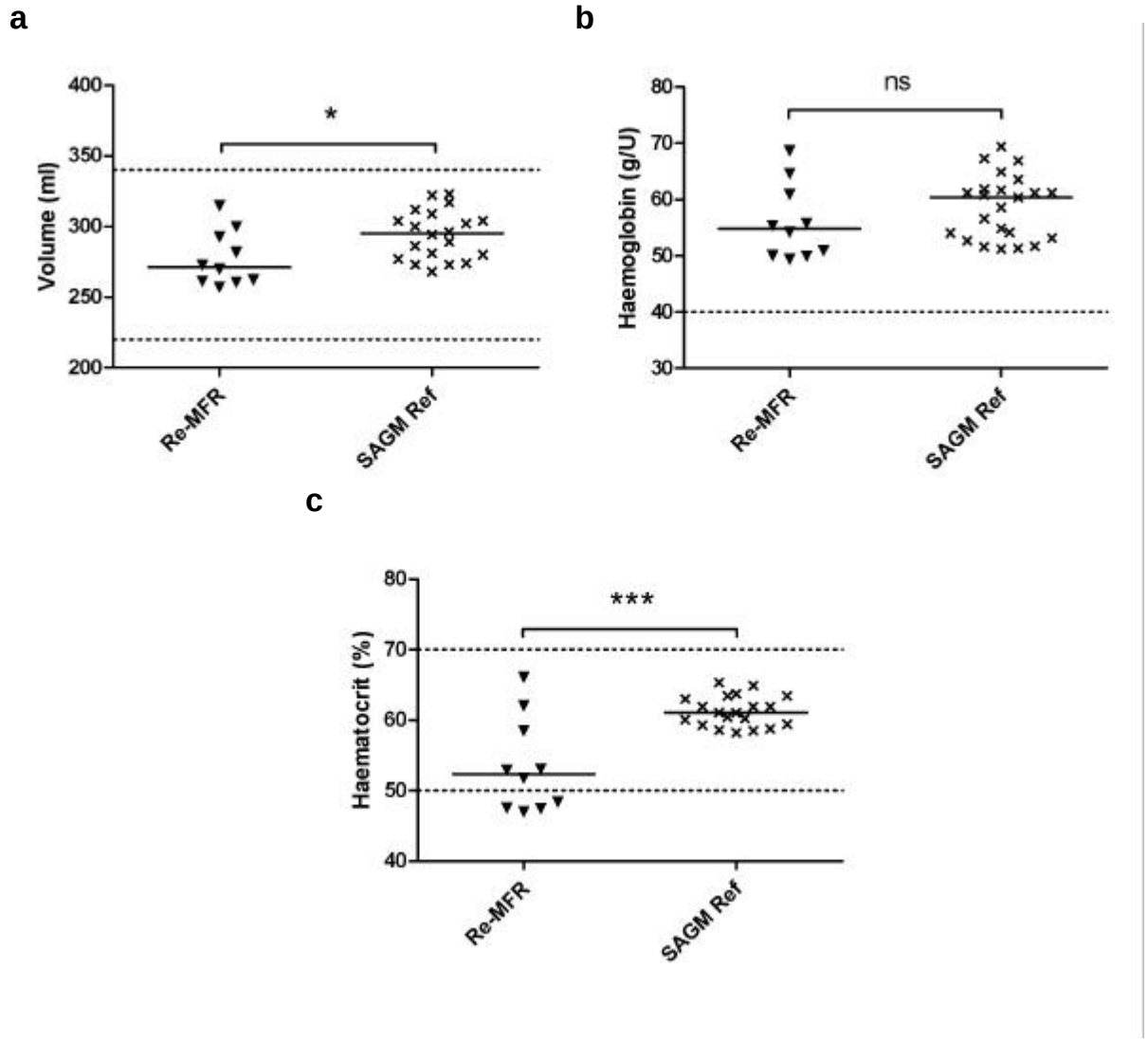

Figure 1. Red cell concentrate content

a: RCC volume; **b**: RCC haemoglobin content; **c**: RCC haematocrit. Graphs show values on the day of component manufacture (—— Median value). ns not significant, *p<0.05, *** p<0.001

---------- UK or local NHSBT specification.

6.2 Haemolysis and supernatant potassium

- Haemolysis increased steadily throughout storage, but at a greater rate in units re-manufactured on day 6 compared with control SAGM units. Consequently, at days 35 and 42, re-manufactured units were significantly more haemolysed than control RCC ($p<0.05$ and <0.01 respectively) (Figure 2b).
- 100 % of re-manufactured units had haemolysis values at day 42 of <0.8 % and therefore met the UK specification at day 42 for standard red cell components (>75 % of units with haemolysis <0.8 %).
- The re-manufacturing step which occurred on day 6 resulted in the removal of supernatant and therefore of supernatant potassium. Consequently, re-manufactured units contained significantly less supernatant potassium (mmol/L and mmol/U) than laboratory reference (control) units at days 35 and 42 ($p<0.001$) (Figure 2c,e).

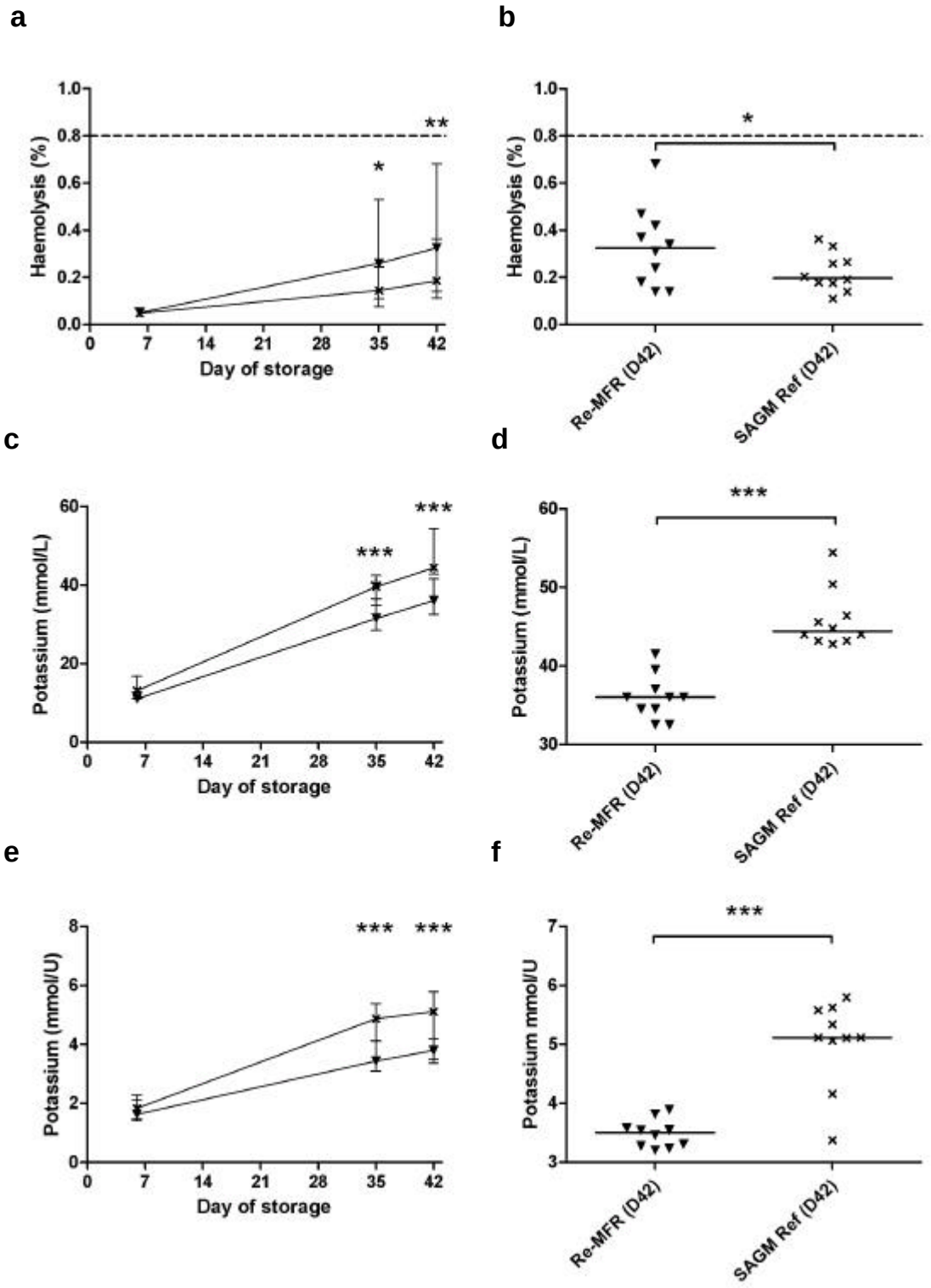

Figure 2. Haemolysis and supernatant potassium

a: RCC haemolysis; **b**: RCC haemolysis at end of shelf-life; **c**: RCC supernatant potassium concentration; **d**: RCC supernatant potassium concentration at end of shelf-life; **e**: RCC supernatant potassium; **f**: RCC supernatant potassium at end of shelf-life. ? Re-manufacture, **x** SAGM reference data. Graphs b, d, f show median values (——). SAGM reference data generated in EVAL/CD/2004/29 (n=10). * p>0.05; ** p>0.01, *** p>0.001.

---------- UK or local NHSBT specification.

6.3 ATP

- Following storage to day 6 in CPD/plasma, red cells contained significantly lower levels of ATP than red cells stored for a comparable amount of time in SAGM (reference data) ($p < 0.05$) (Figure 3a).
- ATP levels decreased during storage and the rates of decline were similar in units re-manufactured on day 6 and laboratory reference (control) units produced using standard processing techniques (Figure 3a).
- At day 42 (end of shelf-life), re-manufactured units contained similar amounts of ATP, as reference units (Figure 3b).

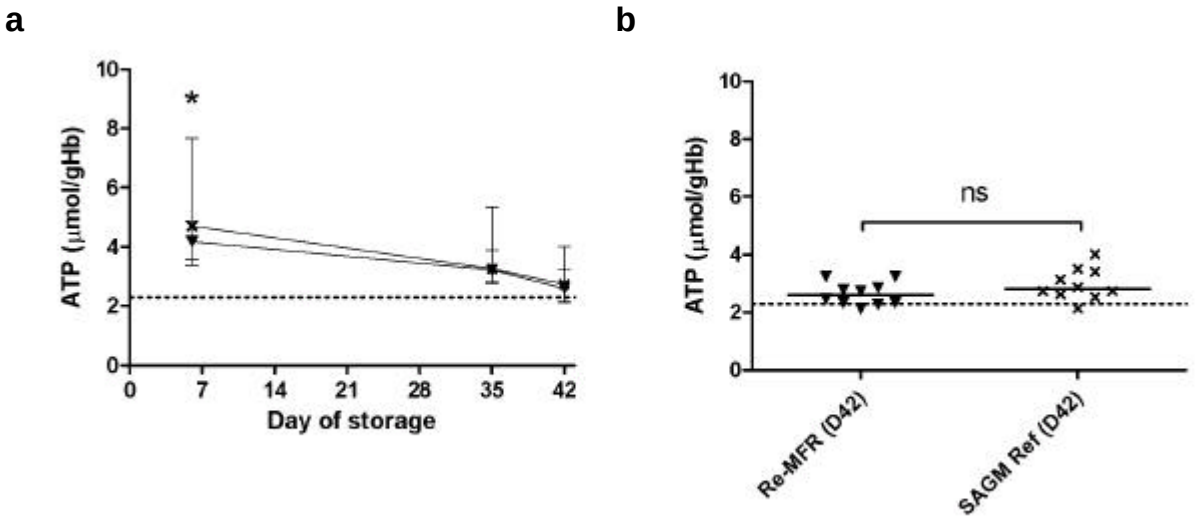

Figure 3. ATP and 2,3-DPG

a: RCC ATP; **b**: RCC ATP at end of shelf-life. ? Re-manufacture, **x** SAGM reference data. Graphs 3b show median values (——). ns not significant, * $p<0.05$.

..... 2.30 µmol/gHb, ATP level below which recovery and survival may be adversely affected (Heaton, 1992).

7 Discussion

Red cell concentrates re-manufactured on day 6 from ExTx units met current UK and local specification for standard red cells in SAGM, but had significantly lower volume and haematocrit. Although re-manufactured units contained slightly less haemoglobin per unit than reference units produced using standard processing, the re-manufacture step only resulted in a loss of approximately 1.5 gHb. Haemolysis was greater in re-manufactured units than reference units, although all units had < 0.8 % haemolysis at day 42. However, the removal of supernatant during the re-manufacture step reduced the amount of supernatant potassium and so re-manufactured units contained significantly lower levels of potassium than reference units. Finally, ATP levels in re-manufactured units declined during storage as expected and were comparable to reference units at day 35 and 42. Importantly, 70 % of units had ATP values above 2.3 µmol/gHb (minimum 2.1 µmol/gHb) suggesting acceptable red cell recovery in vivo (Heaton, 1992).

- SAGM red cells re-manufactured from RCC units for exchange transfusion on Day 6 are of comparable quality to standard SAGM units, with the exception of higher haemolysis values at day 35 and 42.

7.1 Re-manufactured units manufactured on day 5 versus day 6

In study F, re-manufacture was performed on day 6 to maximise the period when plasma reduced red cells are available for use in foetal or neonatal exchange transfusion i.e. to the end of day 5. Units re-manufactured on day 6 had comparable volume, haemoglobin and haematocrit to those produced previously with day 5 re-manufacture (studies C and E). Furthermore, the loss of haemoglobin during the secondary processing (re-manufacture) was similar (EVAL/CD/2006/44a,b). Levels of haemolysis in day 6 re-manufactured units were lower at day 42 than those measured in units re-manufactured on day 5, although this difference was not statistically significant (Figure 4a). Importantly, all 10 units in this study had haemolysis values of <0.8 % at days 35 and 42, compared with only 8 and 7 respectively following day 5 re-manufacturing. The reason for this difference is not known, but could be due to donor variation. Supernatant potassium levels in day 6 re-manufactured units were lower than that seen with day 5 re-manufactured units ($p < 0.001$; Figure 4b,c) and probably reflect the removal of plasma on day 6 as well as the reduction in haemolysis. ATP levels in the day 6 re-manufactured units were slightly lower than those seen in day 5 re-manufactured units, although this difference was not significant ($p > 0.05$, Figure 4d) and levels suggest acceptable red cell recovery in vivo, with 70 % of units containing >2.3 µmol/gHb ATP (Heaton, 1992). 2,3-DPG levels were not measured during this study as there was no reason to suspect that levels would be significantly different from those measured in units re-manufactured on day 5. Furthermore, beyond day 14 of storage 2,3-DPG levels are generally undetectable for units stored in SAGM (historical laboratory data).

- Red cells re-manufactured on day 6 are of comparable quality to red cells re-manufactured on day 5 for all parameters measured.

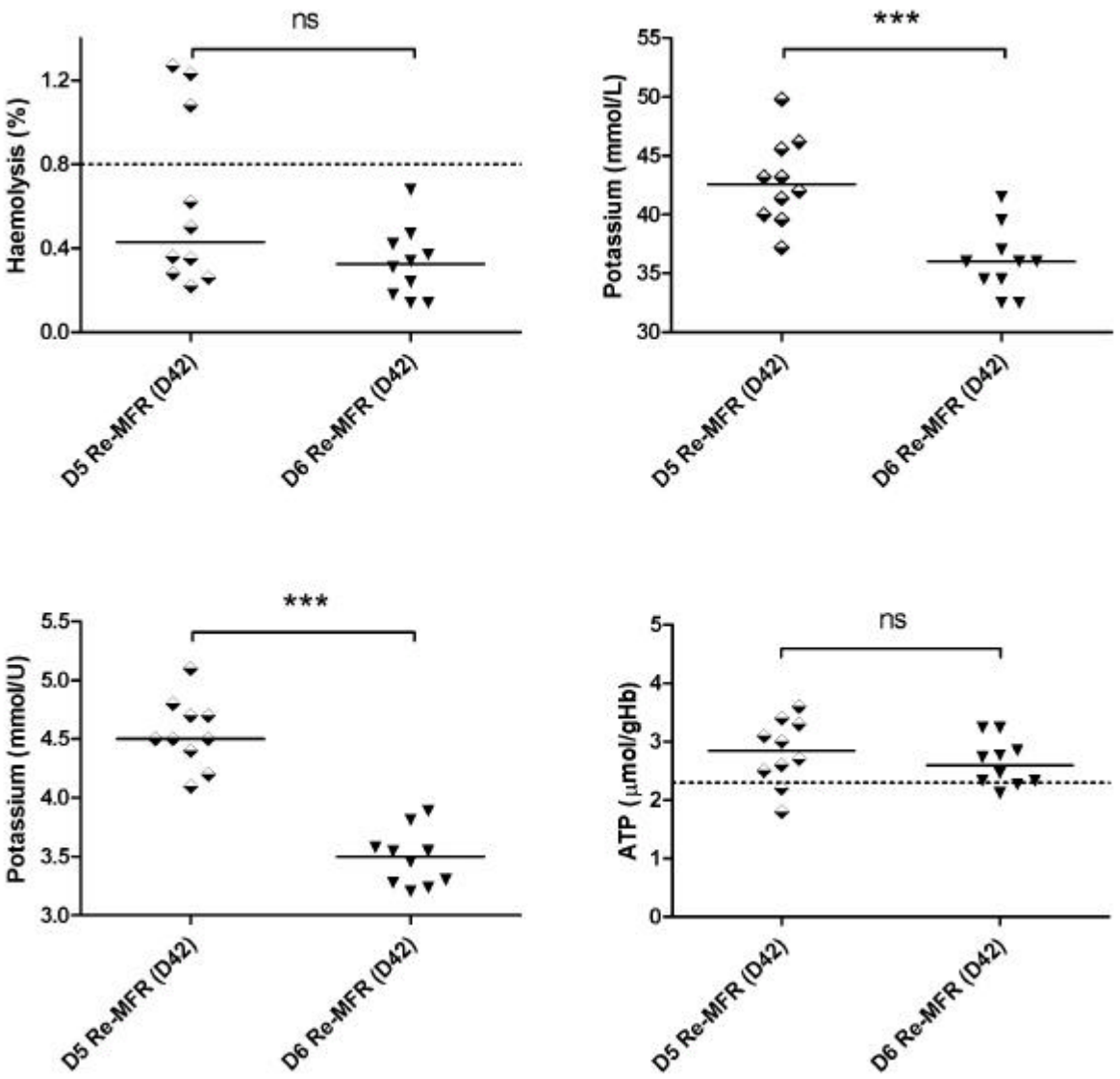

Figure 4. Re-manufactured units manufactured on day 5 versus day 6

a: RCC haemolysis at end of shelf-life; **b**: RCC supernatant potassium concentration at end of shelf-life; **c**:RCC supernatant potassium at end of shelf-life; **d**: RCC ATP at end of shelf-life. RCC Re-manufactured on day 5; ? RCC Re-manufactured on day 6. Graphs show median values (——). ns not significant; *** p>0.001.

---------- UK or local NHSBT specification.

7.2 Comparison of NHSBT and WBS data for units re-manufactured on day 6

The WBS re-manufactured 10 units of ExTX units (CPD) into SAGM RCC on day 6 (Rainbird, 2006, EVAL/CD/2006/44a,b report Appendix 12.2). Units produced by the WBS had comparable volume and haemoglobin content to units from this study but lower haematocrit (median 61 % versus 55 %). Haemolysis and supernatant potassium values were similar in units re-manufactured by the WBS on day 5 and NHSBT on day 6. Re-manufactured units produced by NHSBT and the WBS all met current UK specifications for standard red cell components in SAGM.

- Re-manufactured units produced by NHSBT and the WBS were similar for all parameters tested with the exception of haematocrit which was lower in WBS units.

8 Summary

- All NHSBT re-manufactured units (n=30, EVAL/CD/2006/44a,b,c) met current volume, haemoglobin and haematocrit specifications for standard red cells in SAGM.
- Haemolysis levels were significantly elevated in re-manufactured components. However, 85 % of units (17/20) had haemolysis values at day 42 of <0.8 %. This component therefore met the UK specification for standard red cell components (? 75 % of units with haemolysis of < 0.8 %).
- At 42 days post donation, 80 % of re-manufactured units (16/20) had ATP values of > 2.30 µmol/gHb, suggesting acceptable red cell recovery post transfusion (Heaton, 1992).

9 Recommendations

- Plasma reduced red cell units which are not required for exchange transfusion can be re-manufactured into red cells in SAGM up to day 6. This step reduces the plasma content (and therefore the risk of vCJD and TRALI) and improves the quality of red cells at the end of shelf-life (EVAL/CD/2006/44a).
- Re-manufactured components should have the same specification for volume, Haemoglobin content and haematocrit as standard red cell components in SAGM.
- Re-manufactured RCC can be stored for a total of 42 days post donation.
- Irradiated re-manufactured RCC can be stored for a maximum of 28 days assuming units are irradiated on day 14 (EVAL/CD/2006/44b).

10 References

Heaton W. Evaluation of posttransfusion recovery and survival of transfused red cells. Transfus Med Rev. 1992; **3**:153-69.

Rainbird S. Trial report that investigated the effects of red cells bled into CPD packs, remaining without SAG-M for 5 days and then converted back into SAG-M red cells. Trial reference: 05/10. Welsh Blood Service. SACBC reference S266. 2006. (Appendix 12.2).

11 Quality Assurance

11. Record Maintenance

Research protocols, raw laboratory data, correspondence and a copy of the final report are archived off-site. These records will be retained for a period of 30 years.

11.2 Copy distribution

Copies of the final report are retained within CDL document library, and CSG library

12 Appendices

12.1 Raw data - RCC re-manufactured on day 6

Volume	Day 6	Day 35	Day 42
Re-MFR (D6) 1	307	270	258
Re-MFR (D6) 2	396	315	303
Re-MFR (D6) 3	290	262	251
Re-MFR (D6) 4	357	300	289
Re-MFR (D6) 5	298	261	249
Re-MFR (D6) 6	289	257	245
Re-MFR (D6) 7	313	273	262
Re-MFR (D6) 8	290	260	250
Re-MFR (D6) 9	355	292	282
Re-MFR (D6) 10	330	282	271
Median	310	271	260
Min	289	257	245
Max	396	315	303

Haemoglobin	Day 6	Day 35	Day 42
Re-MFR (D6) 1	58	53	51
Re-MFR (D6) 2	66	66	66
Re-MFR (D6) 3	52	47	45
Re-MFR (D6) 4	66	62	60
Re-MFR (D6) 5	52	47	46
Re-MFR (D6) 6	53	48	46
Re-MFR (D6) 7	57	52	51
Re-MFR (D6) 8	52	48	47
Re-MFR (D6) 9	63	58	58
Re-MFR (D6) 10	56	53	52
Median	56	52	51
Min	52	47	45
Max	66	66	66

Haematocrit	Day 6	Day 35	Day 42
Re-MFR (D6) 1	57	62	62
Re-MFR (D6) 2	49	65	70
Re-MFR (D6) 3	56	59	60
Re-MFR (D6) 4	55	65	66
Re-MFR (D6) 5	53	59	62
Re-MFR (D6) 6	56	60	63
Re-MFR (D6) 7	53	59	62
Re-MFR (D6) 8	56	60	61
Re-MFR (D6) 9	54	64	66
Re-MFR (D6) 10	55	62	64
Median	55	61	62
Min	49	59	60
Max	57	65	70

Haemolysis	Day 6	Day 35	Day 42
Re-MFR (D6) 1	0.05	0.53	0.68
Re-MFR (D6) 2	0.04	0.38	0.47
Re-MFR (D6) 3	0.04	0.18	0.24
Re-MFR (D6) 4	0.07	0.33	0.42
Re-MFR (D6) 5	0.04	0.12	0.14
Re-MFR (D6) 6	0.05	0.28	0.34
Re-MFR (D6) 7	0.06	0.32	0.37
Re-MFR (D6) 8	0.05	0.11	0.14
Re-MFR (D6) 9	0.04	0.15	0.18
Re-MFR (D6) 10	0.06	0.24	0.31
Median	0.05	0.26	0.33
Min	0.04	0.11	0.14
Max	0.07	0.53	0.68

Potassium per L	Day 6	Day 35	Day 42
Re-MFR (D6) 1	11.6	31.0	36.0
Re-MFR (D6) 2	10.6	36.5	41.5
Re-MFR (D6) 3	11.0	30.0	34.5
Re-MFR (D6) 4	11.0	34.0	39.5
Re-MFR (D6) 5	11.0	30.5	34.5
Re-MFR (D6) 6	12.8	32.0	36.0
Re-MFR (D6) 7	11.2	29.5	32.5
Re-MFR (D6) 8	11.0	28.5	32.5
Re-MFR (D6) 9	10.0	33.5	37.0
Re-MFR (D6) 10	11.4	33.0	36.0
Median	11.0	31.5	36.0
Min	10.0	28.5	32.5
Max	12.8	36.5	41.5

Potassium per U	Day 6	Day 35	Day 42
Re-MFR (D6) 1	1.5	3.3	3.9
Re-MFR (D6) 2	2.1	4.1	4.1
Re-MFR (D6) 3	1.4	3.3	3.8
Re-MFR (D6) 4	1.8	3.7	4.2
Re-MFR (D6) 5	1.5	3.4	3.6
Re-MFR (D6) 6	1.6	3.5	3.6
Re-MFR (D6) 7	1.7	3.4	3.5
Re-MFR (D6) 8	1.4	3.1	3.5
Re-MFR (D6) 9	1.6	3.7	3.8
Re-MFR (D6) 10	1.7	3.7	3.8
Median	1.6	3.4	3.8
Min	1.4	3.1	3.5
Max	2.1	4.1	4.2

ATP	Day 6	Day 35	Day 42
Re-MFR (D6) 1	4.88	3.88	3.24
Re-MFR (D6) 2	3.56	2.86	2.12
Re-MFR (D6) 3	3.38	2.89	2.33
Re-MFR (D6) 4	4.57	3.28	2.73
Re-MFR (D6) 5	4.32	3.45	2.77
Re-MFR (D6) 6	4.52	3.74	2.85
Re-MFR (D6) 7	4.03	2.92	2.46
Re-MFR (D6) 8	4.39	3.66	3.24
Re-MFR (D6) 9	3.88	3.17	2.33
Re-MFR (D6) 10	3.64	2.76	2.27
Median	4.18	3.23	2.60
Min	3.38	2.76	2.12
Max	4.88	3.88	3.24