

Background

The pathogen inactivation process (PI system) for Red Blood Cells (RBC), currently in clinical development, uses the small molecule S-303 to form covalent cross-links with nucleic acids and prevent replication of contaminating pathogens and leukocytes. S-303 is a modular compound (Figure 1A-C) designed to spontaneously decompose by hydrolysis to the non-reactive compound S-300 (Figure 1D); glutathione (GSH) is included to quench non-specific reactions.

A processing set, designed to be conducive to blood bank workflow, is being developed with Laboratories Grifols for routine implementation of the PI system (Figure 2). RBC prepared following US practices were used in early development of the processing set; collaborative studies were carried out with the DRK-Blutspendedienst Baden-Württemberg - Hessen to ensure that the RBC PI process is compatible with RBC processed using standard EU practices.

Aims

The purpose of these studies was to assess the quality of stored PI RBC prepared from whole blood (WB) derived RBC that were processed with an overnight (o/n) hold and buffy coat (BC) depletion or without platelet recovery using a prototype processing set manufactured by Grifols.

Methods

S-303 PI System for RBC

Leukodepleted SAGM RBC were prepared from CPD drawn WB (500mL) as follows: **Method 1** held (o/n) at room temperature (RT) and separated into a BC, RBC, and plasma or **Method 2** held o/n at 4°C and separated into RBC and plasma without platelet recovery. Pairs of ABO matched RBC SAGM concentrates were pooled and split into units of approximately 280mL. One unit was untreated and stored at 1-4°C (Control). The second unit was treated with 20mM GSH and 0.2mM S-303, followed by RT incubation, and an exchange step which replaced the treatment solution with SAGM (Test). SAGM RBC units were treated 1-3 days post collection for Method 1 and 1 day post collection for Method 2 (Figure 2). After treatment Test units were stored at 1-4°C. Samples for quality control were periodically removed during the 35 days of storage; parameters assessed are reported below.

RBC Quality Control Parameters

Methods for the RBC quality control parameters are described in Table 1. Cell-free supernatants were prepared to evaluate extracellular hemoglobin (Hb), potassium (K+), glucose, and lactate.

Figure 1: S-303 Treatment Process Mechanism of Action

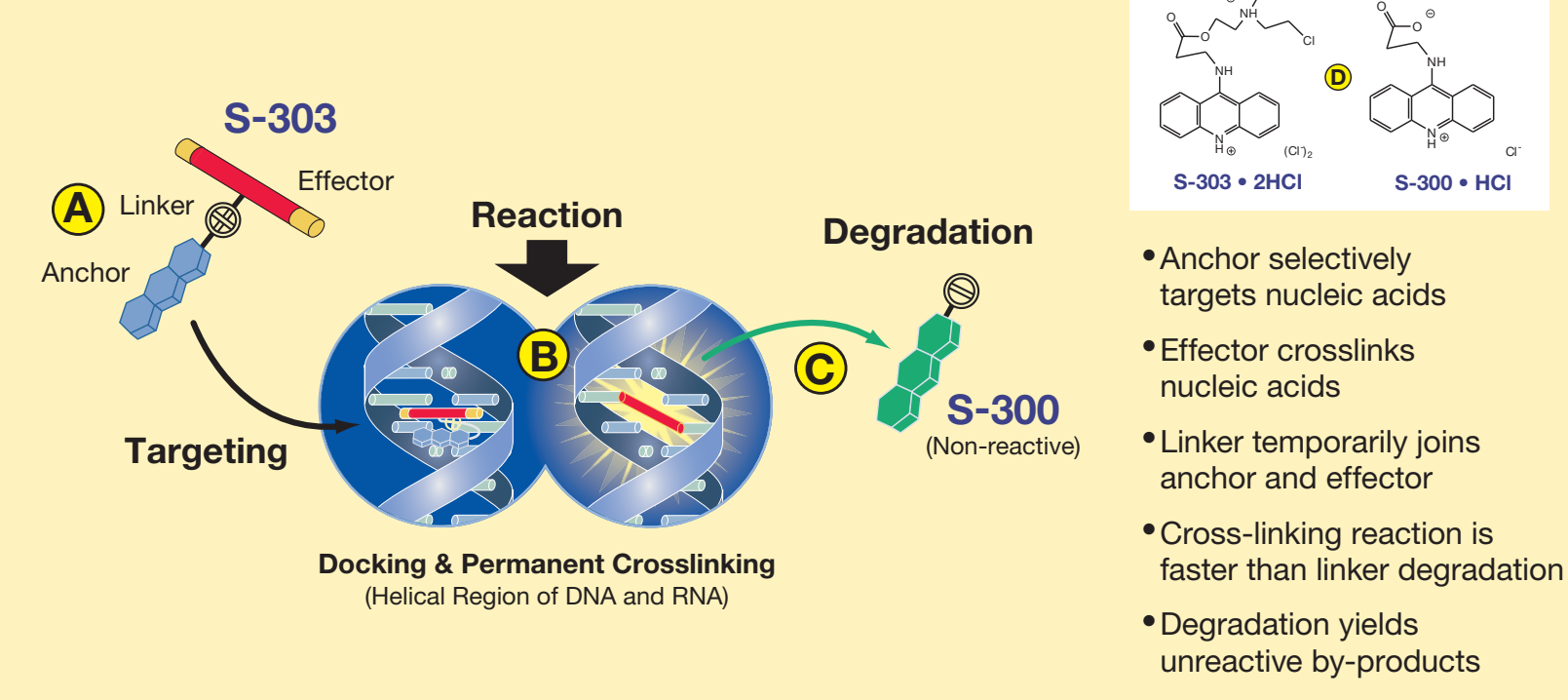


Figure 2: S-303 PI System for RBC

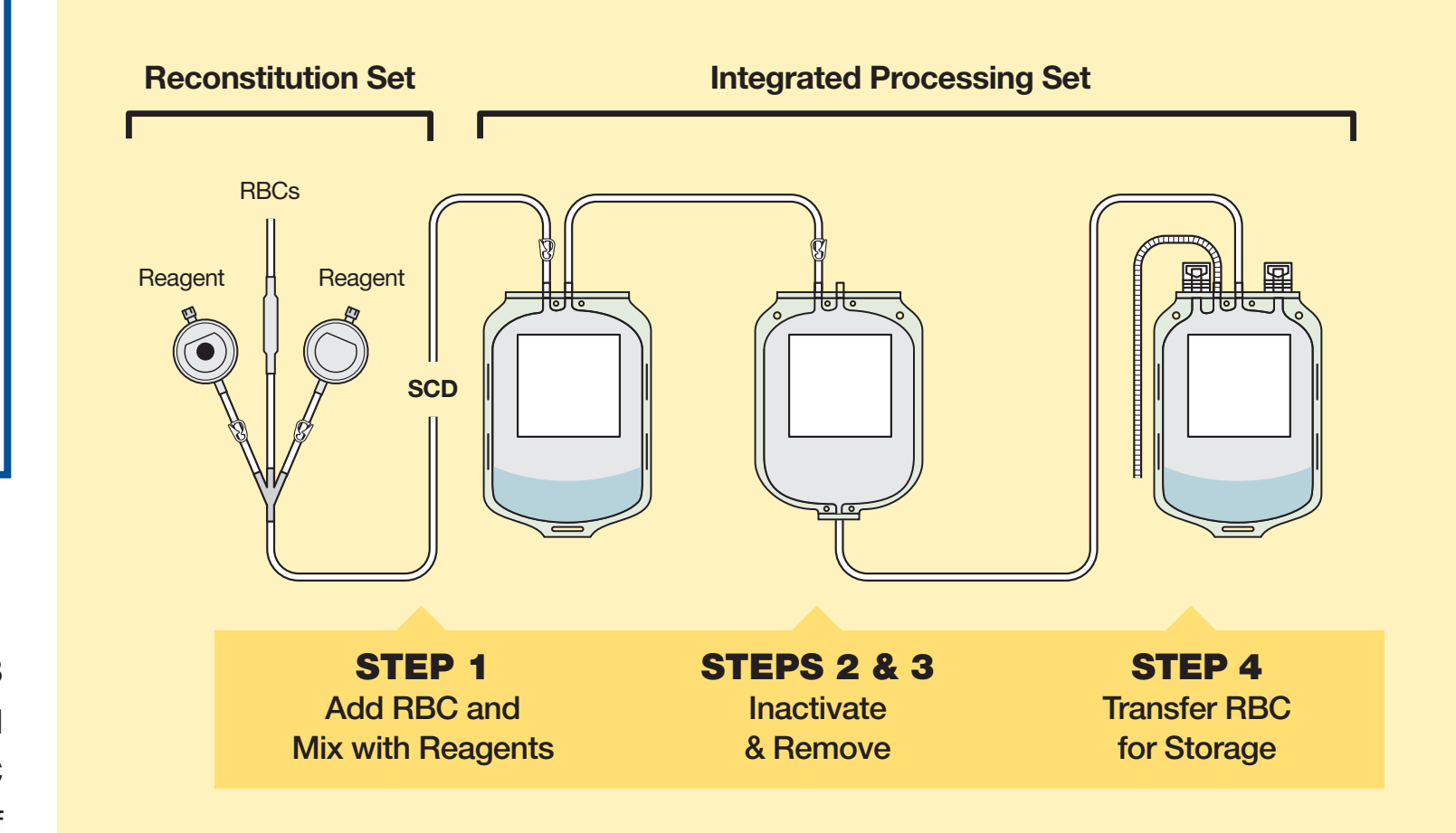


Table 1: Methodology for Measurement of Quality Control Parameters

Assay	Method 1	Method 2
pH (37°C)	ABL700	Siemens Rapidlab 248
MCV, MCHC, Hct, Hb	Sysmex XT 1800i	Advia® 120
ATP	Hexokinase assay	Luciferase assay
Glucose	Hexokinase assay	Hexokinase assay
Lactate	Lactate dehydrogenase assay	Lactate oxidase assay
K+	LEA 150	EasyLyte® Na/K

Results

All Test RBC units met Council of Europe guidelines for hemoglobin content and hematocrit, and met AABB guidelines for RBC quality after completion of the S-303 PI process. After PI, RBC prepared by Method 1 had a 5.4±1.0% loss in total Hb per unit compared to Control (Table 2). This reduction in total Hb is attributed to the manipulations of the treatment process. The loss of RBC is also reflected in the reduced hematocrit (Hct), seen in Test compared to Control for Methods 1 and 2; this decrease in Hct is also due to dilution inherent to the exchange step of the PI process when a standard volume (100mL) of SAGM is added to the RBC.

The Day 35 pH (37°C) was lower for Test RBC units; the lower pH of the Test units is attributed to the removal of the buffering capacity of plasma as a result of the replacement of treatment solution with SAGM. The MCV for Method 1 Test units was lower than that of Controls, whereas MCV values were comparable for Test and Control of Method 2. Since the Hct is affected by suspension volume of the RBC units, the MCHC measurement is a more precise indication of RBC hydration status. MCHC provides a direct measure of intracellular water. With the S-303 PI process, the MCHC of the Test units were comparable to Control for both Methods of RBC processing.

ATP levels of Test units were lower than those of the Control units at Day 35 for RBC prepared by Method 1, whereas Test units have higher ATP levels at Day 35 compared to Control with Method 2 (Figure 3). The ATP levels for all Test units remained above the critical threshold of 2 µmol/g Hb (Method 1: Test 2.4 ± 0.4 µmol/g Hb vs. Control 3.2 ± 1.2 µmol/g Hb; Method 2: Test 4.23 ± 0.74 µmol/g Hb vs. Control 3.48 ± 0.63 µmol/g Hb) believed to be a predictor of acceptable in vivo viability.

Extracellular glucose levels were similar between Test and Control units for both Methods (Figure 4), whereas, lactate concentrations of Test units were lower than those of Control units at Day 35 for both Methods (Figure 5). The differences in lactate production may be attributed to the slightly lower pH of Test RBC units.

During storage of RBC units prepared for transfusion, K+ released from cells accumulates in the extracellular fluid. At Day 35 the levels of K+ in units treated with the S-303 PI system were reduced by 8% (Method 1) and 15% (Method 2) compared to Controls (Figure 6, Table 2). The current standard of practice for inactivation

Table 2: Quality Control Parameters after 35 days of Storage in SAGM (mean±SD)

	Method 1 (n=4)		Method 2 (n=2)	
	Test	Control	Test	Control
Hb (g/unit) ^a	52.7 ± 2.5	55.7 ± 2.3	Not assayed	Not assayed
Hct (%)	55.9 ± 1.7	60.8 ± 1.6	63.1 ± 0.7	65.1 ± 0.1
pH (37°C)	6.389 ± 0.024	6.473 ± 0.015	6.444 ± 0.004	6.537 ± 0.003
MCV (fL)	88.8 ± 3.5	92.4 ± 3.3	96.8 ± 0.6	97.9 ± 1.1
MCHC (g/dL)	34.2 ± 0.5	33.1 ± 0.3	29.6 ± 0.6	29.0 ± 0.0
K+ (mM)	37.03 ± 17.14	40.25 ± 11.49	40.02 ± 3.68	46.97 ± 0.91
Hemolysis (%)	0.9 ± 0.2	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
	0.5 ± 0.1 (n=3) ^b	0.4 ± 0.1 (n=3)	0.6 ± 0.2 ^c (n=3)	0.5 ± 0.1 (n=3)

a. Post treatment. b. WB processed on the day of collection, optimized exchange step. c. WB held o/n at RT.

of residual leukocytes is gamma irradiation; when units are exposed to gamma irradiation at Day 14, they had 29% higher K+ than Control on Day 28 post collection (Transfusion 2009;49:75-80).

RBC processed with Method 1 displayed higher hemolysis after 35 days of storage for Test units compared to Control units, whereas Test and Control units had comparable hemolysis for Method 2. The Day 35 hemolysis for Control Method 1 (0.5 ± 0.1%) was higher than typically reported in standard SAGM QC units at Day 42 of storage (0.3 ± 0.4%, n=1332). The increased hemolysis for the Control may be attributed to either the additional manipulations during the pooling process, the repeated samplings, or the sampling technique.

An additional study was performed utilizing RBC prepared from WB which was held o/n at RT and separated as described for Method 2; Test Units had similar hemolysis compared to Controls at Day 35 (Test 0.6 ± 0.2% vs. Control 0.5 ± 0.1%, n=3; Table 2). Furthermore, optimization of the exchange step improved Day 35 hemolysis in units treated with the S-303 PI process when RBC were prepared by Method 1 on the day of collection; Test 0.5 ± 0.1% vs. Control 0.4 ± 0.1% (Table 2). Other measured RBC indexes were similar to those described above (data not shown). Units treated with the S-303 PI process for RBC met acceptable AABB and EU guidelines for hemolysis after 35 days of storage.

Figure 3: ATP for S-303 PI RBC vs. SAGM Control (Method 1 and Method 2) Over 5 Weeks Storage (mean ± SD)

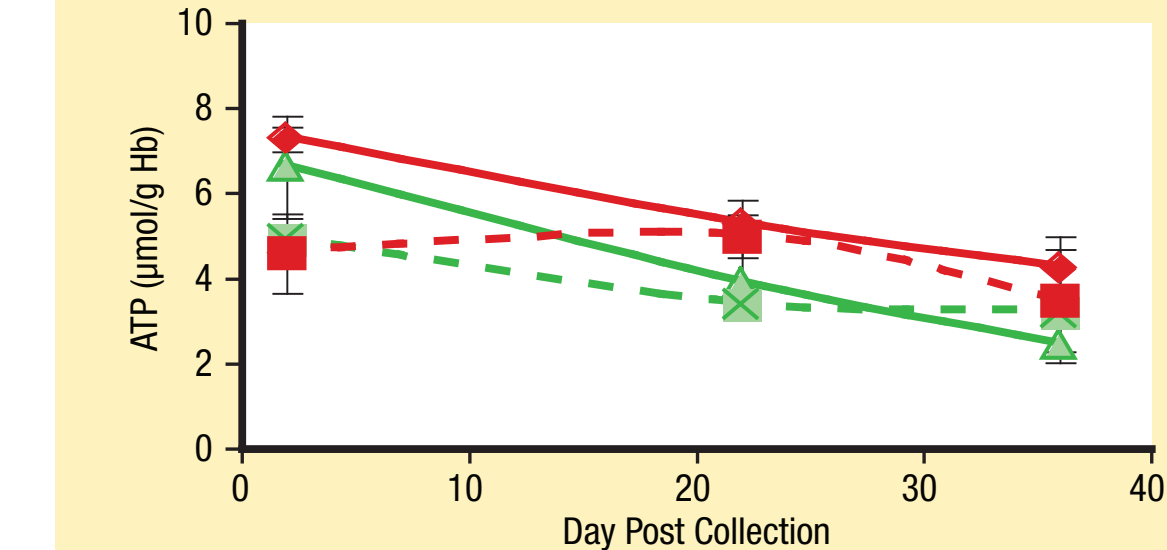


Figure 4: Extracellular Glucose for S-303 PI RBC vs. SAGM Control (Method 1 and Method 2) Over 5 Weeks Storage (mean ± SD)

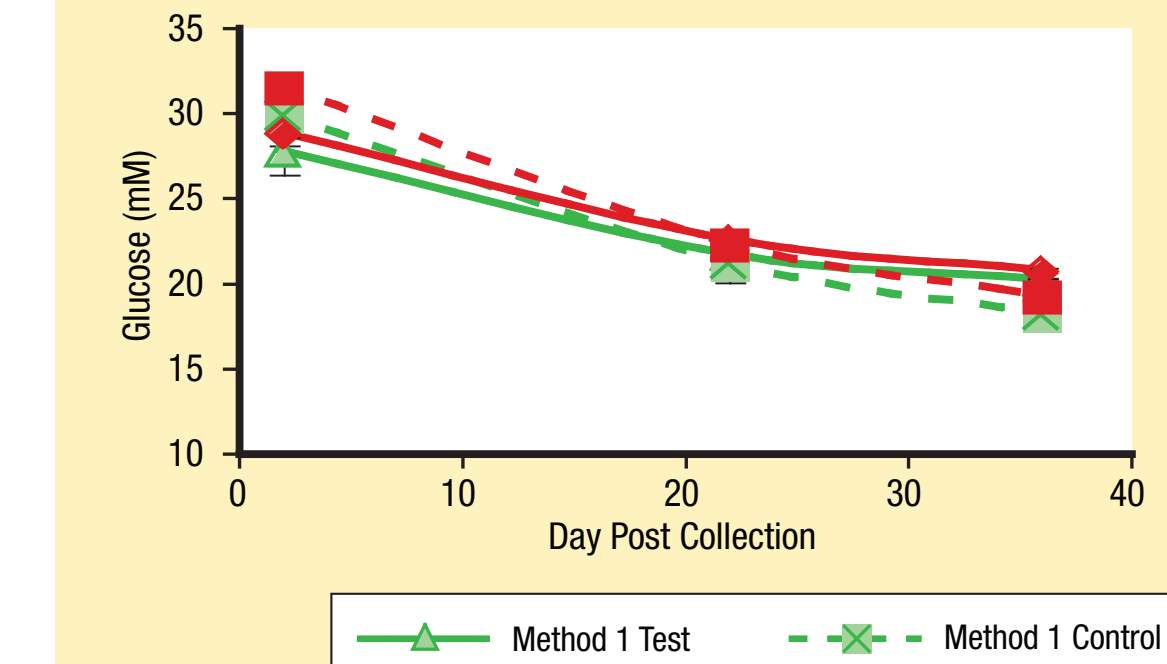


Figure 5: Extracellular Lactate for S-303 PI RBC vs. SAGM Control (Method 1 and Method 2) Over 5 Weeks Storage (mean ± SD)

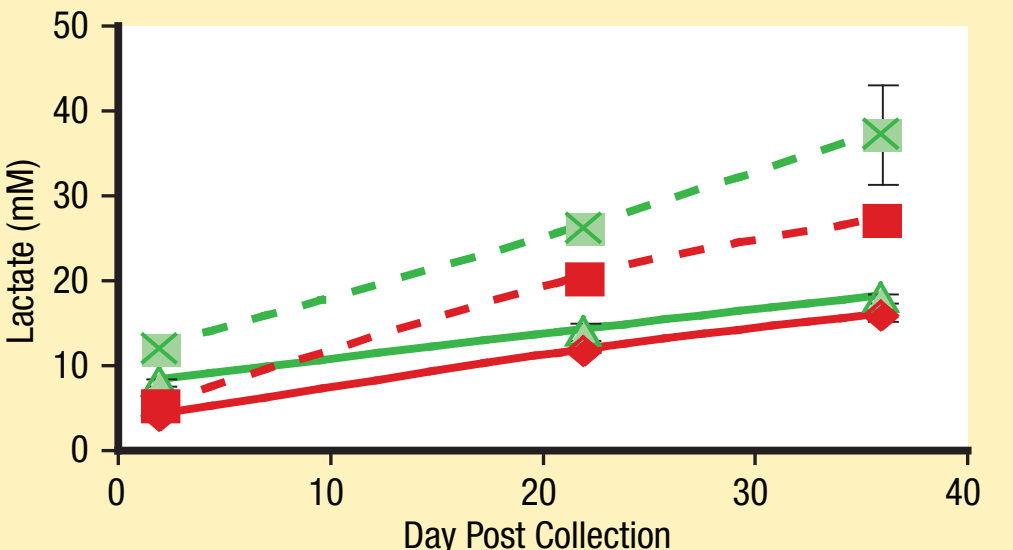
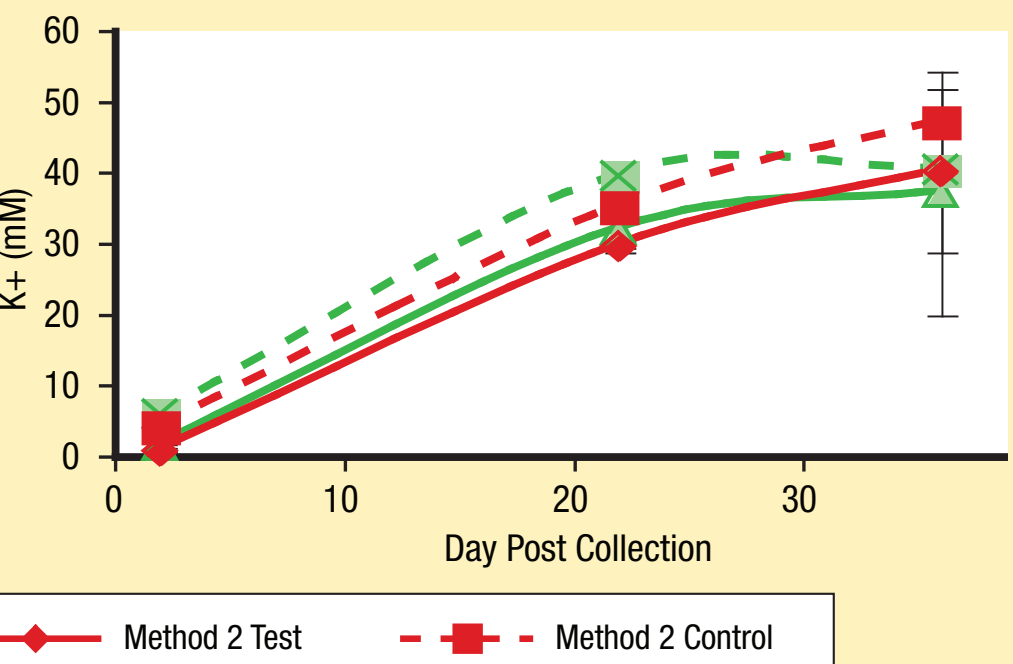


Figure 6: Extracellular K+ for S-303 PI RBC vs. SAGM Control (Method 1 and Method 2) Over 5 Weeks Storage (mean ± SD)



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Conclusions

- At Day 35 post collection ATP levels, indicative of acceptable in vivo viability, were achieved with the S-303 PI system
- The S-303 treatment process resulted in reduced extracellular potassium compared to Control
- RBC treated with the S-303 PI System for RBC met AABB and Council of Europe guidelines for leukoreduced RBC in additive solution