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## ***In Vitro* Evaluation of Pathogen Inactivated RBC Using the S-303 Treatment System**

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# In Vitro Evaluation of Pathogen Inactivated RBC Using the S-303 Treatment System



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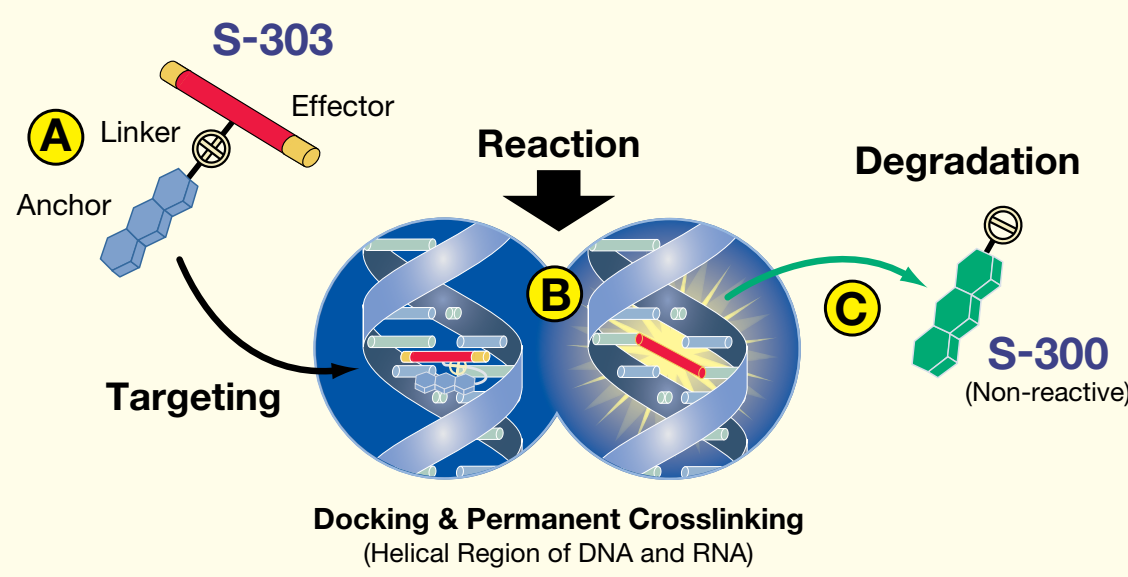
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## Background

A second generation S-303 treatment system for red blood cells (RBC) has been developed using S-303 to crosslink nucleic acids and prevent replication of contaminating pathogens and leukocytes (Figure 1). Glutathione (GSH) is included to quench nonspecific reactions and a Diluent facilitates pathogen inactivation (PI). This system has shown robust inactivation of a variety of pathogens including Gram-negative and Gram-positive bacteria, and enveloped and non-enveloped viruses. A recent Phase 1 clinical study successfully met the primary endpoint of 24-hour recovery per the FDA criteria. Subsequent development studies have focused on expanding the S-303 treatment process by optimization of the disposable sets to demonstrate compatibility with US and EU input RBCs.

### Figure 1: S-303 Treatment Process Mechanism of Action

S-303 is a modular compound with three components: an acridine anchor, an effector and a linker (A). The anchor selectively targets nucleic acids where it intercalates and reversibly binds to the helical regions of the molecule. The effector then irreversibly cross-links the nucleic acids at guanine bases thereby preventing nucleic acid replication or transcription (B). The linker is hydrolyzed to release S-300, a nonreactive degradant resulting from the reaction (C).



## Methods

One day post donation whole blood (450mL), stored overnight at 4°C, was leukocyte-depleted and separated into platelet poor plasma and RBCs. The RBC concentrate was suspended in SAG-M; ABO matched pairs of SAG-M RBC were combined and split into units of 247-312mL (N=6). For each replicate, Control units were stored at 4°C, and Test units were treated with the S-303 treatment system. Each Test unit was treated with the S-303 treatment process by combining the RBCs in SAG-M with GSH and Diluent followed by addition of S-303 to the RBC mixture (Figure 2, Step 1). The final concentration of GSH and S-303 is dependent on the volume of SAG-M RBC input; the approximate final concentrations of GSH and S-303 are 20mM GSH/0.2mM S-303 for a 280mL RBC input. After an 18h room temperature (RT) hold (Figure 2, Step 2), S-303 treated RBCs were centrifuged and the bulk of the treatment solution (containing SAG-M, Diluent, GSH, and process degradants) was removed, using a plasma press, and replaced with fresh SAG-M (Figure 2, Step 3). All RBC units were stored at 4°C for 35 days (Figure 2, Step 4) and sampled post S-303 treatment (Day 2) and

on Days 7,14, 21, 29, and 35 for evaluation of the parameters listed in Table 1. On Days 35-36, ATP, 2,3-DPG and p50 were measured on aliquots of Test and Control which had been rejuvenated using PIPA (1.11g/dL pyruvate, 2.68 g/dL inosine, 1 g/dL pH 7 phosphate, 68 g/mL adenine)<sup>1</sup>, washed in saline, and resuspended in SAG-M.

Repeated measures ANOVA (SAS statistical software) was used to examine the impact of time and treatment on various RBC test outcomes. Assays performed on Test and Control samples but not repeated throughout storage were evaluated using Student's paired t test (Microsoft Excel). For all statistical comparisons a p-value less than 0.05 is considered statistically significant.

### Figure 2: S-303 Treatment System for RBC

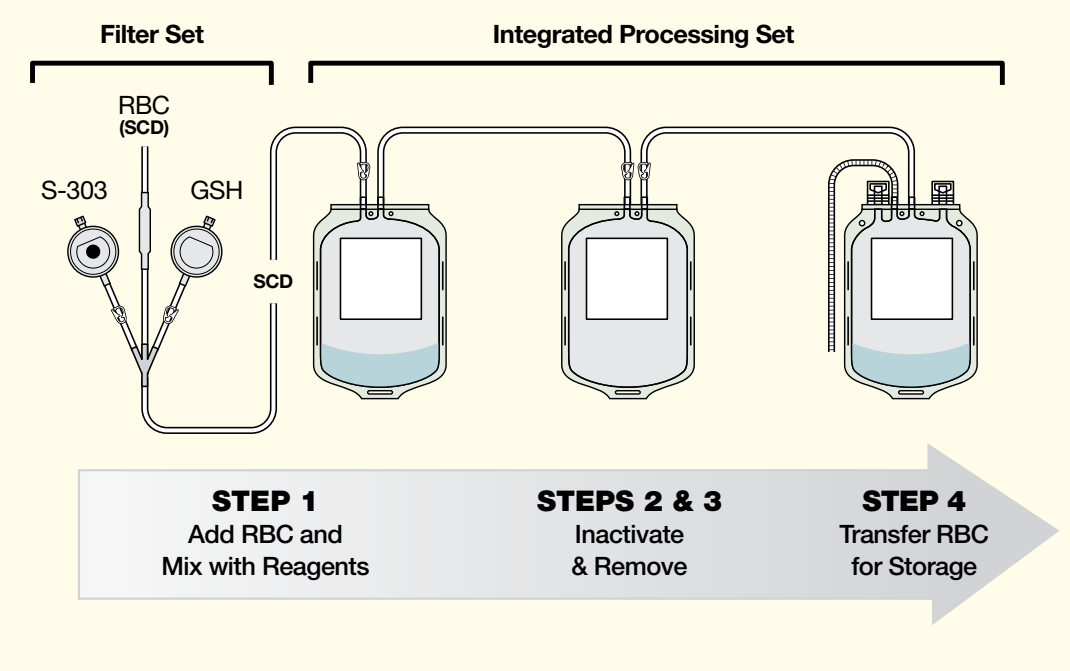


Table 1: Methodology for Measurement of Quality Parameters

Parameter	Equipment/Methodology
pH (37°C)	238 Blood Gas Analyzer (Siemens)
MCV, MCHC, Hematocrit (Hct), Hemoglobin (Hb)	Advia 120 Hematology System (Siemens)
Plasma Free Hb	Hemacue Low
ATP	Luciferase assay
2,3 DPG	Roche Diagnostics, (Indianapolis, IN)
Total Protein	Pierce® 660 nM Protein Assay Reagent (Rockford, IL)
Glucose	Hexokinase assay
Lactate	Lactate dehydrogenase assay
Na <sup>+</sup> and K <sup>+</sup>	Ion Selective Electrode
p50	Oxygen-dissociation curve

## Aims

The purpose of this study was to demonstrate the quality of stored S-303 treated RBCs using the optimized disposable set design.

## Results

After S-303 treatment, Test RBCs had 52.2±2.1 g of Hb reflecting a loss of 1.7±1.5% of Hb during PI processing. The S-303 treatment process reduced the extracellular protein concentration 20-fold in Test (37±10 mg/dL) compared to Control (743±123 mg/dL). After 35 days of storage ATP, K<sup>+</sup>, Na<sup>+</sup>, MCHC and MCV were not different between Test and Control (Table 2). Hct was significantly lower in Control, whereas hemolysis was marginally higher in Control (Table 2). Test units had lower pH, glucose, lactate, and MCF compared to Control (Table 2).

The statistically significant (p<0.001) difference between Test and Control Hct is a result of the slightly higher RBC input volume for Test units compared to Control. Hct increased in both study arms during storage (p=0.0003) and the rate of that increase was not different between Test and Control (p=0.0737; Figure 3A).

Hemolysis, a key indicator of RBC function, increased in both study arms during storage (Figure 3B), with Control units having consistently greater hemolysis than Test units (p=0.0536; Table 2) and a faster rate of increase in hemolysis than Test units (p=0.0185).

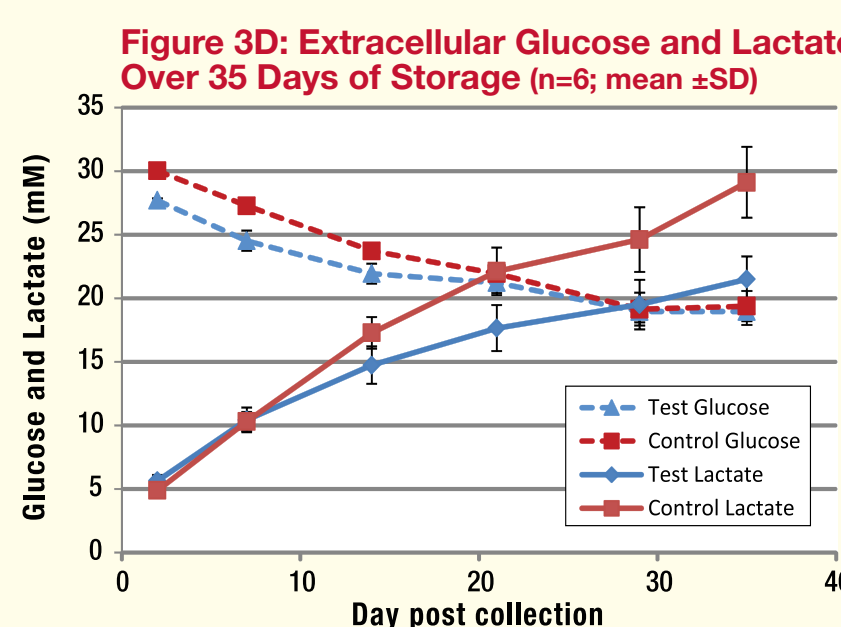
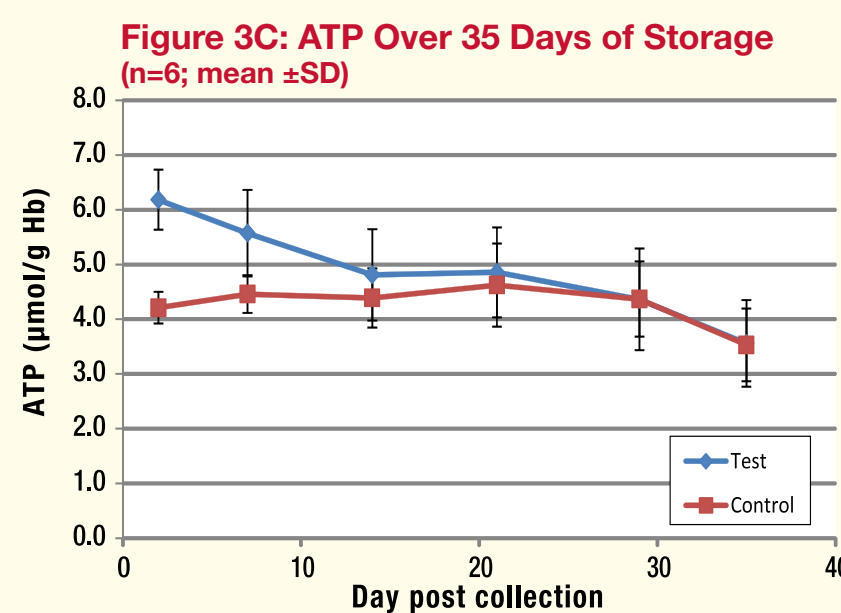
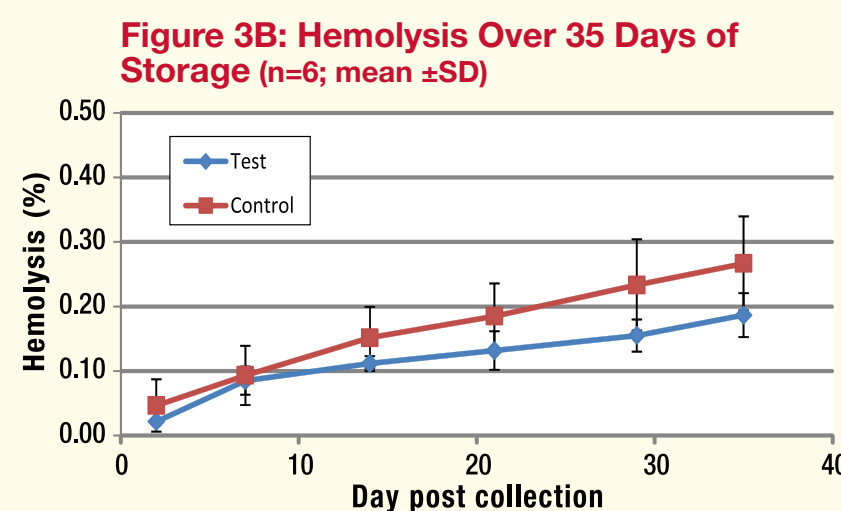
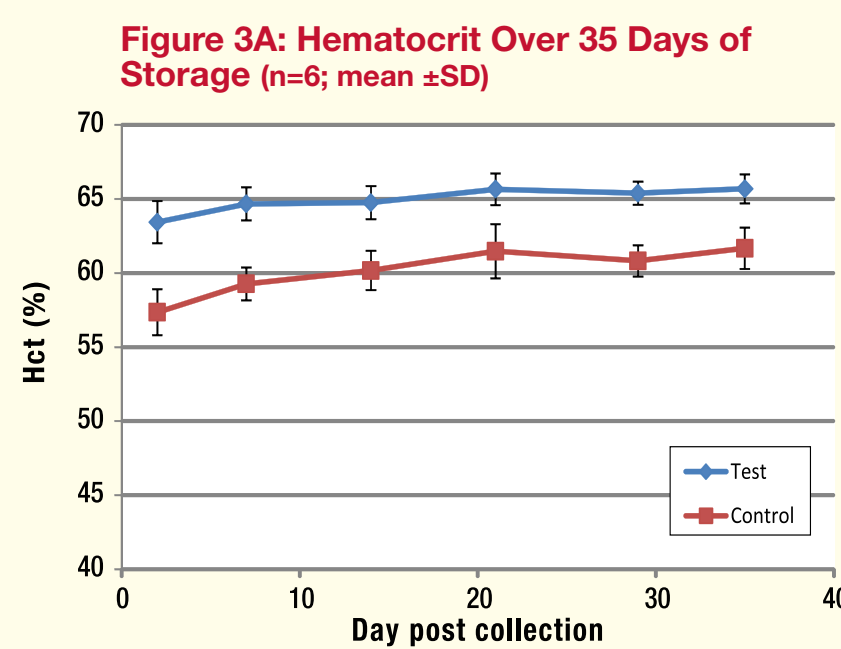
The ATP content of RBC units prepared for transfusion is an established measurement of red blood cell quality. Test RBCs contained a higher concentration of ATP than did Control RBCs on Day 2, and although the rate of decrease in ATP was greater for Test units than for Control units (p = 0.0147), the overall mean ATP concentration in Test RBCs was not significantly different from Control during storage (p=0.1286; Table 2, Figure 3C). On Day 2, post PI, 2,3 DPG was significantly higher in Control (11.16±1.49 μmol/g Hb) versus Test (1.09±0.66 μmol/g Hb) due to the room temperature hold time for the PI process and reduced pH in Test.

The rate of pH reduction during storage was faster for Control than for Test units (p=0.0291) which may be attributed to the increased lactate production. However, the Test units had an overall lower pH as compared to Control (p<0.0001, Figure 4) possibly due to the removal of the buffering capacity of plasma during the removal. However, the pH for both Test and Control RBCs throughout the storage period was within a range (6.3 – 7.0), as typical for RBCs stored in SAG-M.<sup>1</sup>

RBCs metabolize glucose and produce lactate during storage. Glucose levels in Test units were lower than Control units during storage (p=0.0022) due to replacement of Test unit treatment solution (which contained residual plasma glucose) with SAG-M during the removal step. During 35 days of storage the rate of glucose consumption in Test units was slower than that of Control units (Figure 3D) but the difference was not statistically significant (p=0.0911). Overall mean lactate concentration in Control units was greater than that in Test units (p=0.0032). The rate of lactate production in Test units during storage was slower than that in Control units (p=0.0003, Figure 3D). Differences in the rates of glucose consumption and lactate production may be an effect of the slightly lower pH of Test RBC units on glycolytic enzymes.

Indices of RBC hydration showed that MCHC and MCV were similar between Test and Control, and the MCF was slightly reduced in Test.

Evaluation of rejuvenated Test and Control, a method used to model clinical transfusion, showed that Test and Control were able to generate 2,3 DPG and ATP and had equivalent p50 values. (Table 3).



## Conclusions

All S-303 treated RBC units met the EU guidelines for leukocyte depleted RBCs in additive solution.

- Hematocrit was within 50-70%.
- Hemoglobin content was >40 g per unit.
- Hemolysis was <0.8% at end of storage.

ATP levels in S-303 pathogen inactivated RBCs correspond to levels which predict acceptable *in vivo* RBC viability.

Rejuvenation of RBCs after 35 days of storage resulted in measurable 2,3-DPG and similar p50 values between Test and Control RBC indicative of acceptable post-transfusion RBC quality.

All measured *in vitro* parameters of S-303 treated RBCs indicate suitability for transfusion.

## References

- 1) Högman CF, Meryman HT. 1999. Storage Parameters Affecting Red Blood Cell Survival and Function after Transfusion. *Transf Med Rev* 13:275-96.

Table 2: Day 35 RBC Quality (mean ±SD, n=6)

Parameter	Test	Control	p-value
Hematocrit (Hct, %)	65.7 ±1.0	61.7 ±1.4	<0.0001
Hemolysis (%)	0.19 ±0.03	0.27 ±0.07	0.0536
pH (37°C)	6.381 ±0.042	6.473 ±0.040	<0.0001
Total ATP (μmol/g Hb)	3.56 ±0.79	3.53 ±0.66	0.1286
Extracellular potassium (K <sup>+</sup> , mM)	48.57 ±2.75	46.58 ±2.34	0.9304
Extracellular sodium (Na <sup>+</sup> , mM)	103.2 ±3.7	108.2 ±3.4	0.2529
Extracellular glucose (mM)	19.0 ±1.0	19.4 ±1.2	0.0022
Extracellular lactate (mM)	21.5 ±1.8	29.1 ±2.8	0.0032
Mean corpuscular hemoglobin concentration (MCHC, g/dL)	28.7 ±0.7	28.5 ±0.6	0.2720
Mean cell volume (MCV, fL)	95.6 ±5.1	96.8 ±5.1	0.8944
Median corpuscular fragility (MCF, mOsm)	147 ±4	151 ±4	0.0056

Table 3: Day 35-36 RBC Post Rejuvenation (mean ±SD, n=6)

Parameter	Test	Control
2,3-DPG (μmol/g Hb)	6.48 ±1.53	8.69 ±1.68
Total ATP (μmol/g Hb)	7.93 ±0.61	7.31 ±0.36
P50 (mm Hg)*	26.5 ±1.4	27.0 ±1.4

\* Filled tubes were shipped on ice to the testing laboratory, where p50 analysis was performed approximately 24h post-rejuvenation using a Hemox-Analyzer (TCS Scientific Corporation, New Hope, PA).

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