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

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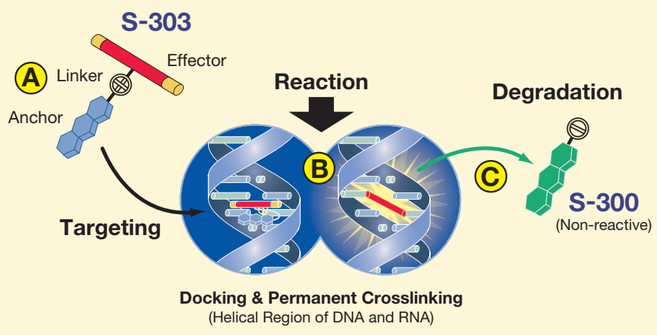
Background

A second generation pathogen inactivation (PI) system for red blood cells (RBCs) 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 non-specific reactions. 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 recently completed Phase 1 clinical study successfully met the FDA's recommended criteria for 24-hour recovery studies based on the absolute recovery and variability in the measurement of 24-hour recovery; 24-hour post-transfusion recovery between Test and Control RBCs, stored for 35 days, was not significant at the 0.05 level (Test 87.9%, Control 89.8%; p=0.31).

Leukocyte-depleted RBCs in additive solution, prepared following US practices, were used as the input for the S-303 PI system used in the Phase 1 clinical study and in early development of the processing set; whole blood was separated into components on the day of donation or held overnight at 1-6°C prior to separation. Subsequent development studies have focused on expanding the compatibility of the S-303 PI process with different RBC inputs and optimization of the processing set design. Studies conducted in collaboration with the DRK-Blutspendedienst Baden-Württemberg - Hessen evaluated the quality of S-303 treated RBCs to ensure that the second generation process is also compatible with RBCs prepared following standard EU practices.

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).



Aims

This study was conducted to evaluate the compatibility of the second generation S-303 PI system with leukocyte-depleted RBCs in additive solution produced according to standard EU blood bank practices (whole blood held overnight at 20-24°C and separated using the buffy coat method). The quality of stored S-303 treated RBCs was assessed to verify suitability for transfusion.

Methods

Leukocyte-depleted SAGM RBCs were prepared from whole blood units (500mL) that had been held overnight at room temperature (RT) then buffy coat depleted. For each replicate, two units of ABO matched RBCs were pooled and divided into units of approximately 280 mL. One of the units was stored at 2-6°C (Control) and the other was treated with the S-303 PI system (Test). Three replicates were performed.

The S-303 treatment process involved combining RBCs in SAG-M with GSH and Diluent followed by addition of S-303 to the RBC mixture for a final concentration of 20mM GSH and 0.2mM S-303. After an 18h RT hold, Test units were centrifuged and the bulk of the treatment solution (containing SAGM, Diluent, GSH, and process degradants) was removed, using a plasma press, and replaced with fresh SAG-M (**Figure 2**). RBC units were stored at 2-6°C for 35 days and sampled at days 2 (post-PI process), 14, 21, 28, and 35 post-collection.

RBC Quality Parameters

The methods used to assess RBC quality are described in **Table 1**. The red cell indices; mean cellular volume (MCV) and mean corpuscular hemoglobin content (MCHC) were used to evaluate RBC hydration. Cell-free supernatants were used to evaluate extracellular hemoglobin (Hb), potassium (K^+), glucose, and lactate.

Figure 2: S-303 PI System for RBC

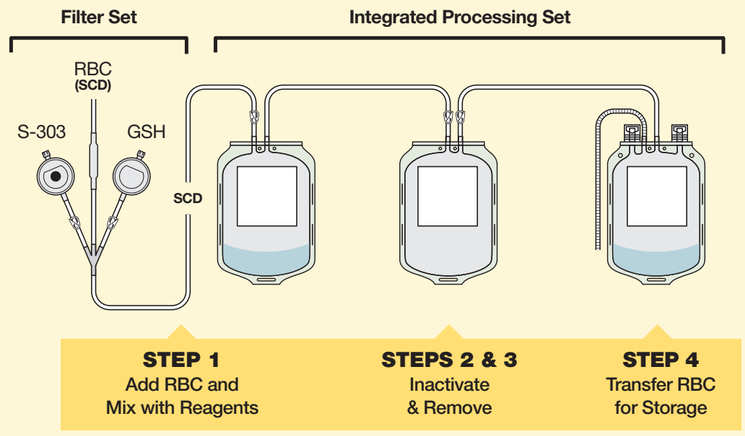


Table 1: Methodology for Measurement of Quality Parameters

Assay	Method
pH (37°C)	ABL700
MCV, MCHC, Hematocrit (Hct), Hemoglobin (Hb)	Sysmex XT 1800i
ATP	Luciferase assay
Glucose	Hexokinase assay
Lactate	Lactate dehydrogenase assay
K^+	Ion Selective Electrode

Results

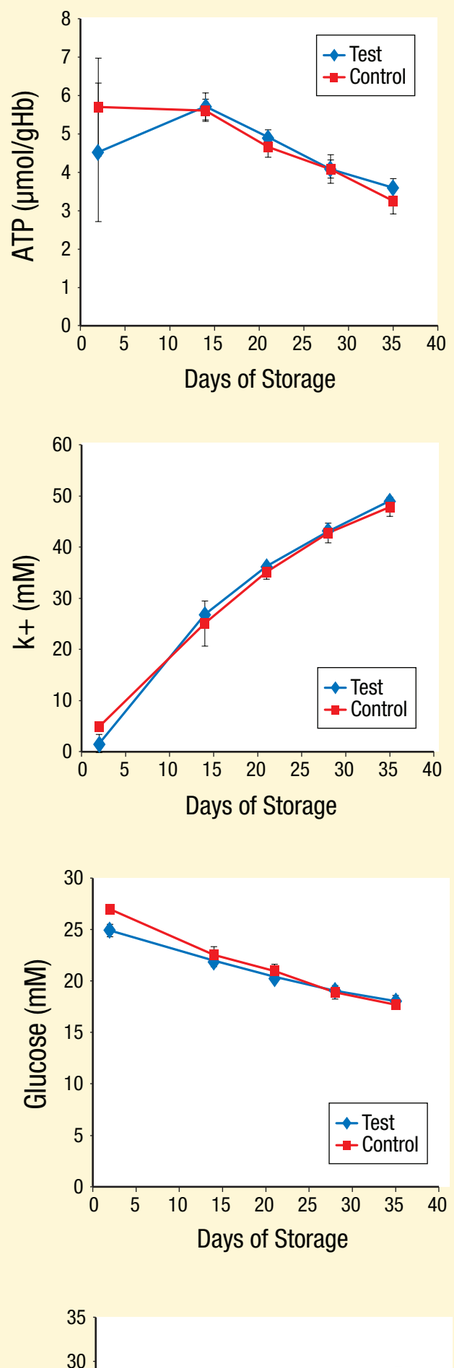
All Test units had >50 g Hb/unit at completion of the S-303 PI process; Control units also had >50 g Hb/unit. Hydration status of the RBCs was measured using MCHC as this provides a direct measure of intracellular water. The MCHC of the S-303 treated Test units was comparable to that of the Control units after 35 days of storage (**Table 2**). The MCV and Hct were also similar between Test and Control. Although the S-303 treatment process resulted in reduced pH (37°C) throughout storage the pH of Test units was within a range (6.3 – 7.0) reported as typical for RBCs stored in SAG-M (Högman 1999, *Transf Med Rev 13:275-96*). This decrease is due to the addition of the reagents (**Figure 2, step 1**) as well as the removal of the treatment solution which removes plasma and its buffering capacity (**Figure 2, Steps 3 and 4**).

After 35 days of storage both Test and Control have ATP levels predictive of acceptable *in vivo* RBC viability. ATP levels are comparable between Test and Control units over 35 days of storage (**Figure 3a**). Accumulation of extracellular K^+ during storage is similar between Test and Control (**Figure 3b**). Although glucose consumption is similar during storage (**Table 2, Figure 3c**), the rate of lactate production is slower in Test units throughout storage (**Figure 3d**). Glycolytic enzymes can be inhibited by the slightly lower pH in Test units resulting in a decreased rate of lactate production during storage. Hemolysis is slightly higher in Test units compared to Control after 35 days of storage (**Table 2; Figure 3e**).

Table 2: RBC Quality After 35 Days of Storage (n=3, mean±SD)

Parameter	Test	Control
MCHC (g/dL)	32.3±0.5	31.6±0.4
MCV (fL)	93.3±2.6	95.6.±2.0
Hct (%)	63.9±2.0	62.2±1.8
pH (37°C)	6.35±0.01	6.43±0.01
ATP (µmol/gHb)	3.59±0.24	3.25±0.34
K^+ (mM)	49.0±1.5	47.9±1.9
Glucose (mM)	18.0±0.6	17.7±0.4
Lactate (mM)	19.9±0.7	28.4±0.7
Hemolysis (%)	0.34±0.11	0.25±0.08

Figure 3: S-303 Treated RBC vs. Control Over 35 Days Storage (mean±SD)



Conclusions

- **All treated units met the EU guidelines for Hct (50-70%), hemoglobin (>40 g per unit) and hemolysis (<0.8% at end of storage) for leukocyte-depleted RBCs.**
- **ATP levels in S-303 treated RBCs were greater than 3 µmol/g Hb throughout storage and correspond to levels which predict RBC viability in vitro.**
- **The in vitro quality of S-303 treated RBCs is comparable to that of Control (untreated) RBCs and verifies that the second generation PI process is compatible with input RBCs prepared following standard EU blood bank practices.**
- **In vitro characteristics indicate that S-303 treated RBCs are suitable for transfusion.**

This work was partially supported by the US Army Medical Research and Materiel Command under Contract No W81XWH-10-1-0857.