

# Process Robustness of the S-303 Pathogen Inactivation System for Red Blood Cells



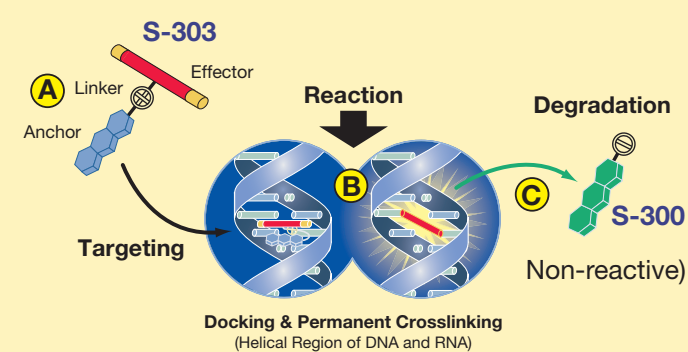
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## Background

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 nonspecific reactions and a diluent facilitates 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 recently completed Phase 1 clinical study successfully met the FDA's recommended criteria for 24-hour recovery 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 significantly different at the 0.05 level (Test 87.9%, Control 89.8%;  $p=0.31$ ). The input for the S-303 PI system used in the Phase 1 clinical study was leukocyte-depleted RBC's in AS-5, prepared following US practices. The RBC input volume ranged from 266 to 284 mL. 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.

**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).

## Results

**Study 1.** The mean RBC input volume was 219.8±0.0 mL for the 220 mL arm and 339.4±0.5 mL for the 340 mL arm. After treatment with the S-303 PI process, the extracellular protein concentrations were 25.73±1.72 mg/dL (220 mL) and 76.79±6.81 (340 mL) mg/dL. After 35 days of storage ATP, pH, mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were not statistically different between 220mL and 340mL input volumes. Hematocrit (Hct), potassium (K<sup>+</sup>) and lactate were higher in 340mL volume, whereas hemolysis, lactate and glucose were lower.

**Table 1: Study 1; In Vitro Parameters Over 35 days of Storage (mean±SD), n=4**

Attribute	RBC input volume (mL)	Day 1 (RBC input)	Day 2	Day 14	Day 35
Hematocrit (%)	220	65.2±1.0	60.8± 0.9	61.7± 0.2	62.5± 0.9
	340		69.5± 0.5	70.1± 0.8	70.7± 1.5
Free hemoglobin (mg/dL)	220	7.5± 5.0	10.0± 0.0	45.0± 5.8	77.5± 12.6
	340		12.5± 5.0	55.0± 10.0	82.5± 22.2
Hemolysis (%)	220	0.00± 0.00	0.02± 0.00	0.09± 0.01	0.16± 0.03
	340		0.02± 0.01	0.08± 0.01	0.12± 0.03
ATP (µmol/g Hb)	220	4.79± 0.66	6.76± 0.47	6.07± 0.70	4.06± 0.69
	340		6.43± 0.63	5.94± 0.64	4.10± 0.69
MCHC (g/dL)	220	30.2±0.7	30.4±0.7	30.6±0.6	29.3±0.7
	340		30.2±0.5	30.1±0.8	29.6±0.6
MCV (fL)	220	98.2±1.8	99.1±1.8	101.1±1.4	102.5±1.5
	340		99.2±1.8	101.1±1.4	101.8±1.3
pH (37°C)	220	Not Measured	6.79± 0.01	6.58± 0.01	6.40± 0.03
	340		6.78± 0.01	6.58± 0.01	6.40± 0.02
Glucose (mM)	220	28.8±0.8	27.5± 0.3	23.5± 0.6	19.4± 0.9
	340		25.6± 0.4	21.0± 1.2	16.5± 1.0
Lactate (mM)	220	3.8±0.3	3.6± 0.5	12.2± 0.5	19.4± 1.7
	340		5.0± 0.3	15.4± 0.9	23.7± 1.3
K <sup>+</sup> (mM)	220	1.30± 0.07	0.5± 0.0	21.3± 1.5	39.8± 2.3
	340		0.8± 0.0	28.6± 2.2	51.5± 3.0

## Aims

These studies were designed to assess robustness of the S-303 PI system over a range of RBC input volumes (220-340 mL). The quality of stored S-303 treated RBCs was assessed for transfusion suitability as well as PI efficacy.

## Methods

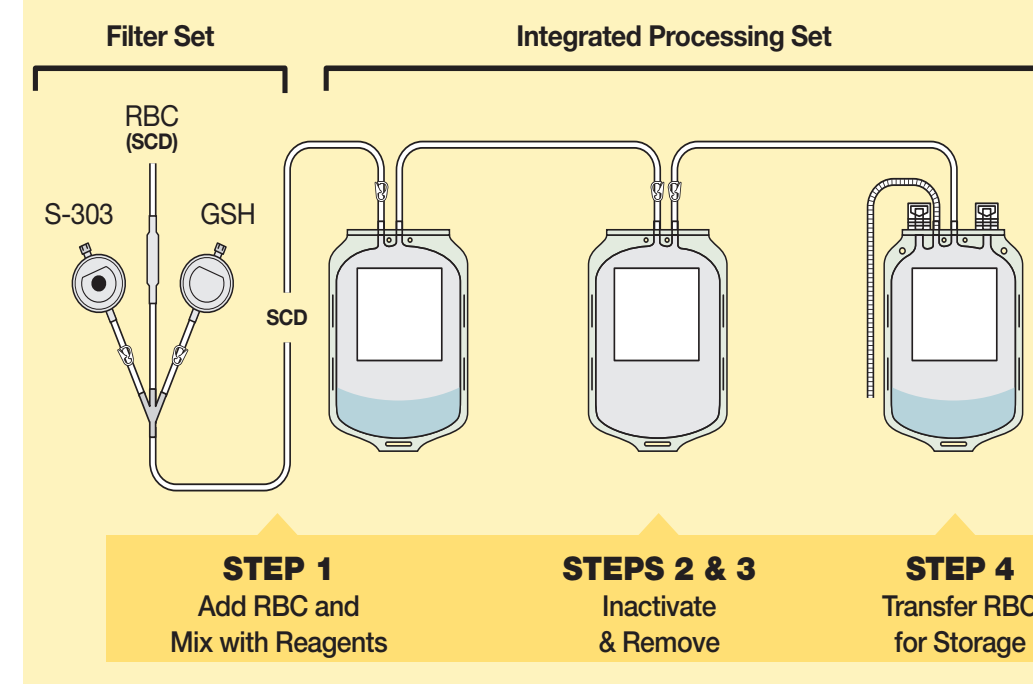
Leukocyte depleted (LD) SAG-M RBCs were prepared from whole blood (WB, 450 - 500 mL) held overnight at 4°C (Study 1) or at RT (Study 2) and separated without platelet recovery. For each replicate, ABO matched RBCs were pooled and divided into matched pairs of 220mL and 340mL for Study 1 (n=4) and 280 mL and 340mL for Study 2. Units were 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, Steps 1 and 2). 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 26mM GSH/0.26mM S-303 for a 220 mL input, 20mM GSH/0.2mM S-303, for a 280mL RBC input and 17mM GSH/0.17mM S-303 for a 340 mL RBC input.

**Study 1:** After an 18h room temperature (RT) hold, S-303 PI RBC 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, Steps 3 and 4). RBC units were stored at 1-6°C for 35 days. RBC quality was assessed on RBC inputs (Day 1), post PI treatment (Day 2) and after 14, 22, and 35 days of storage. Cell-free supernatants were prepared to evaluate extracellular hemoglobin (Hb), total protein, potassium (K<sup>+</sup>), sodium (Na<sup>+</sup>), glucose, and lactate.

**Study 2:** Paired RBC units of approximately 280mL and 340mL were inoculated with approximately 10<sup>6</sup> bacteria/mL before treatment. The larger, 340 mL RBC input, units result in lower concentrations of

treatment components and, thus, represent worst case conditions for PI efficacy. A sample of 5mL was removed before addition of S-303 and immediately assayed for viable bacteria to establish the baseline (pre-treatment) titer. The units were incubated for 3 hours at room temperature after S-303 addition, before being assayed for viable bacteria. Bacteria were quantified by growth on Luria-Bertani, Miller, agar plates.

**Figure 2: S-303 PI System for RBC**



## Conclusions

- All S-303 PI RBC units met the AABB guidelines for LD RBCs; hemolysis was <1% at end of storage.
- ATP levels in pathogen inactivated RBC's were greater than 4 µmol/g Hb throughout storage and correspond to levels which predict acceptable *in vivo* RBC viability.
- *In vitro* characteristics support the use of RBCs with an input volume of 220mL to 340mL.
- PI effectiveness was retained across 220-340 mL RBC input volumes.

**Table 2: Study 2; Mean Log Reduction<sup>a</sup> of Bacteria following RBC PI Treatment in 280 mL or 340 mL RBC units**

Bacteria	n	280 mL	340 mL
<i>S. aureus</i>	7	4.2 ± 1.1	3.7 ± 1.0
<i>Y. enterocolitica</i>	4	5.7 ± 0.2	4.9 ± 0.9
<i>E. coli</i>	3	6.0 ± 0.5	5.8 ± 0.2

<sup>a</sup>Log reduction is calculated as log(pre-treatment titer/post treatment titer), where titer is expressed as 10<sup>6</sup> organisms/mL.

**Study 2.** Table 2 shows the log inactivation of *Staphylococcus aureus* (n=7), *Yersinia enterocolitica* (n=4), and *Escherichia coli* (n=3) in RBC units containing 280 mL compared to RBC units containing 340 mL. Although there is a decrease in inactivation of some bacteria in the higher volume (lower S-303 concentrations), in all cases efficacy remains adequate for inactivation of the levels of bacteria expected in recently collected units.

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