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

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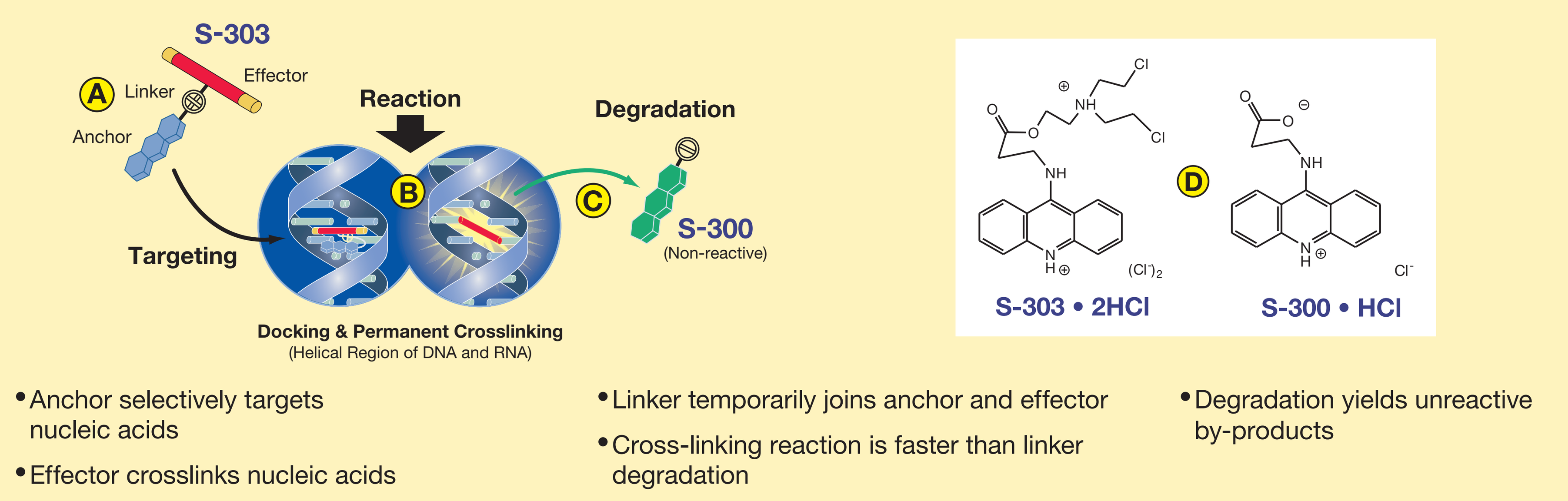
Deutsches Rotes Kreuz
Aus Liebe zum Menschen.

Background

A new pathogen inactivation (PI) process 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 non-specific reactions. A Phase 1 clinical study was recently completed and successfully met the primary endpoint of 24-hour recovery per the US FDA criteria. RBC prepared following US practices were used in early development of the processing set. More recently, collaborative studies were carried out with the DRK-Blutspendedienst Baden-Württemberg - Hessen to ensure that the new process is also compatible with standard EU practices for RBC production and to evaluate the quality of S-303 treated RBC.

Figure 1: S-303 Treatment Process Mechanism of Action



- Anchor selectively targets nucleic acids
- Effector crosslinks nucleic acids
- Linker temporarily joins anchor and effector
- Cross-linking reaction is faster than linker degradation
- Degradation yields unreactive by-products

Methods

S-303 PI System for RBC

Leukocyte depleted RBC in saline-adenine-glucose-mannitol (SAGM) were prepared from whole blood units (500mL) that had been held overnight (o/n) at room temperature (RT) then buffy coat depleted. For each replicate, three units of ABO matched RBC were pooled and divided into 280mL units. The PI process was performed on two units and the third unit was unprocessed (Control). Each study was performed in triplicate.

Study 1: Test RBC were combined with diluent solution, treated with 20mM GSH and 0.2mM S-303 and held for 18h at RT. The bulk of the treatment solution (containing SAGM, diluent, GSH, and S-303 degradants) was removed and replaced with fresh SAGM via a centrifugation step (**Figure 2**). Removal of the treatment solution was performed with either a plasma press (Test-Manual) or Compomat G4 (Test-Automated). **Study 2:** The PI process was performed as above except one Test unit was held at RT for 4h (Test–4h) and the other for 18h (Test–18h) prior to manual separation.

RBC units were stored at 4±2°C for 42 days and sampled at days 2 (post-PI process), 21, 35 and 42 post-collection.

For each study, repeated measures ANOVA was used to assess statistically significant differences (p-value <0.05) between Test and Control and Test conditions, allowing detection of small differences since assessment was carried out for four time points.

RBC Quality Parameters

Methods for the RBC quality parameters evaluated are listed in **Table 1**. Cell-free supernatants were prepared to evaluate extracellular hemoglobin (Hb), total protein, potassium (K^+), glucose, and lactate.

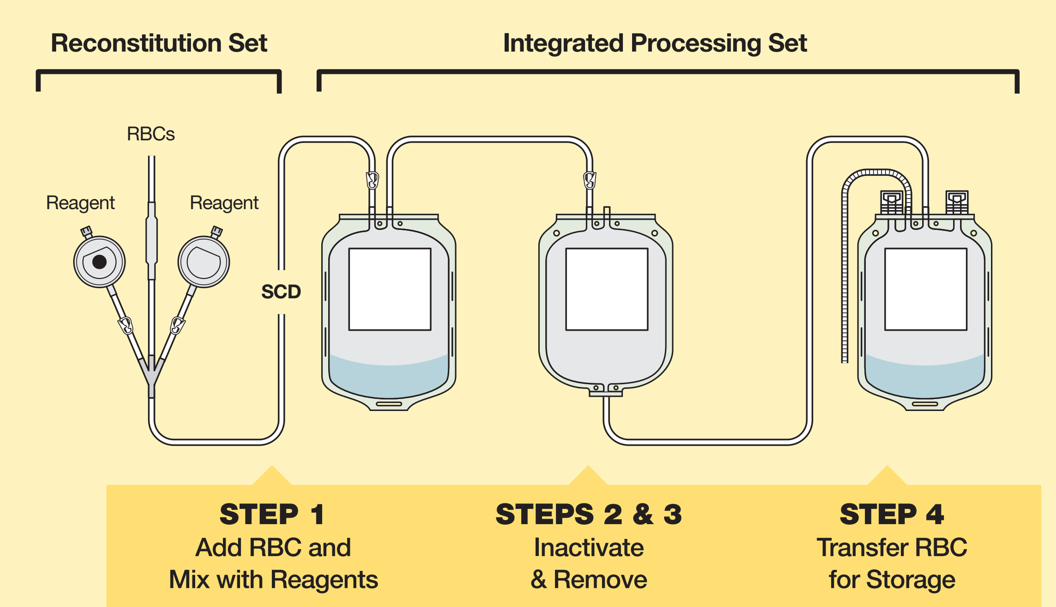
Table 1: Methodology for Measurement of RBC Parameters

Attribute	Instrument/Method
pH (37°C)	ABL700
Mean cell volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Hematocrity (Hct), Hemoglobin (Hb)	Sysmex XT 1800i
ATP	Luciferase assay
Glucose	Hexokinase assay
Lactate	Lactate dehydrogenase assay
Potassium (K+)	LEA 150

Aims

These studies were conducted to determine the compatibility of the new S-303 PI process with RBC produced according to standard EU blood bank practices and to measure *in vitro* properties of S-303 treated RBC which correlate with viability.

Figure 2: S-303 PI System for RBC



Results

All Test units (Study 1 and Study 2) had > 40 g Hb/unit at completion of the S-303 PI process; Control units also had >40 g Hb.

In Study 1, the removal step was either performed with a manual press (Test Manual) or the Compomat G4 (Test Automated). The methodology for the separation did not result in significant differences between Test units, however some statistically significant differences (p<0.05) were detected when comparing all Test units to Control (**Table 2**). Hct, pH, potassium (K^+), total protein and lactate were significantly higher in Control RBC; whereas MCHC and hemolysis were lower (**Table 2**).

In Study 2, the RT PI hold was carried out for either 4h (Test 4 h) or 18 h (Test 18 h). RBC characteristics were comparable amongst Test units, suggesting that the differences between Test and Control are not due to the 18 h RT hold (**Table 3**). Comparison between Test and Control showed that K^+, total protein, and lactate were statistically significant higher in Control; whereas hemolysis was statistically significantly lower (**Table 3**).

Since the Hct is affected by suspension volume of the RBC units, the MCHC measurement provides a more precise indication of RBC hydration status. MCHC provides a direct measure of intracellular water. MCHC of the Test units were comparable to Control in both studies (**Tables 2 and 3**).

The S-303 PI process results in reduced pH (37°C) in Test RBC units, throughout storage; 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**).

The removal step is also responsible for the lower extracellular K^+ observed in Test units for both studies (**Tables 2 and 3**); K^+ accumulated during the RT PI hold is removed and the rate of K^+ accumulation during storage is comparable between Test and Control (**Figure 3A**).

Although glucose consumption is similar during storage (**Tables 2 and 3**), the rate of lactate production is slower in Test units throughout storage (**Figure 3B**). Lactate is lower at the beginning of storage due to the removal step and remains low due to the reduced pH during storage.

At the beginning of storage, mean ATP values are highest for the Test 18 h units, due to increased glycolysis during the RT PI hold, but ATP levels are comparable between Test and Control units after 42 days of storage (**Figure 3C**).

In both studies hemolysis met the EU guideline for post-storage hemolysis (<0.8% hemolysis) for all units after 42 days of storage (**Tables 2 and 3; Figure 3D**).

Conclusions

- **All treated units met the EU guidelines for hematocrit, hemoglobin and hemolysis for leukocyte-depleted RBCs.**
- **ATP levels were greater than 3 µmol/g Hb throughout storage.**
- **The S-303 PI process is compatible with standard EU blood bank practices for RBC production and can be easily implemented within blood center workflow.**
- **The *in vitro* characteristics of S-303 RBC are suitable for transfusion or further clinical development of the S-303 PI process for RBC in Europe.**

Figure 3: A-D. S-303 Treated RBC (Test 4 h and Test 18 h) vs. Control Over 42 Days Storage

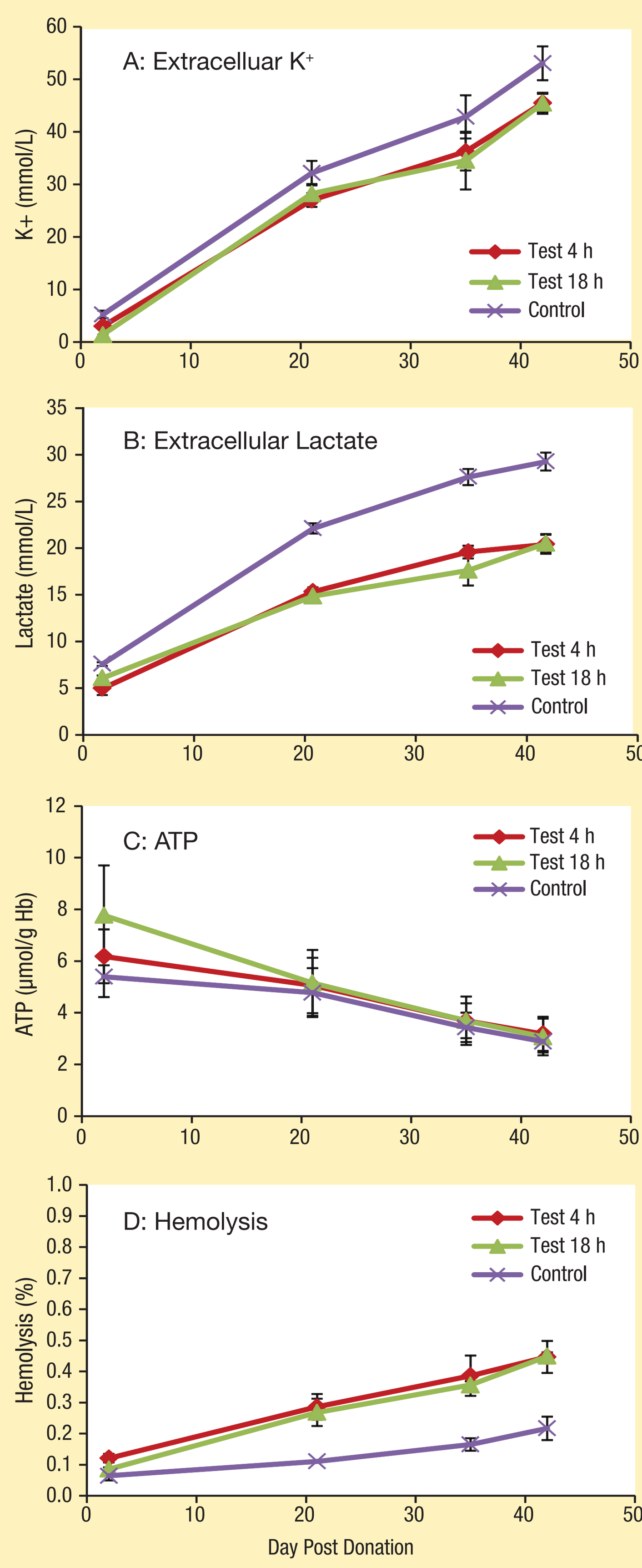


Table 2: RBC Characteristics After 42 Days of Storage (mean±SD)

	Study #1 (n=3)		
	Test Manual	Test Automated	Control
MCV (fL)	88.8±3.4	88.7±3.5	90.6±3.8
MCHC (g/dL)	33.2±0.4	33.3±0.4	32.4±0.2*
Hct (%)	59.1±0.9	58.5±0.5	61.5±1.3*
Hemolysis (%)	0.71±0.90	0.72±0.17	0.37±0.12*
ATP (µmol/gHb)	3.4±0.8	3.2±0.7	2.9±0.3
pH (37°C)	6.32±0.03	6.31±0.02	6.40±0.02*
K^+(mM)	48.5±2.0	46.6±1.7	55.3±0.8*
Glucose (mM)	18.9±1.4	18.7±0.5	17.4±0.6
Lactate (mM)	19.9±0.9	19.7±0.2	29.7±0.4*

*Repeated measures ANOVA between combined Test and Control: p < 0.05
aValue is from S-303 PI process, D2 post collection

Table 3: RBC Characteristics After 42 Days of Storage (mean±SD)

	Study #2 (n=3)		
	Test 4h	Test 18h	Control
MCV (fL)	91.0±2.5	90.7±2.6	91.7±2.9
MCHC (g/dL)	32.2±0.4	32.1±0.3	31.1±1.6
Hct (%)	59.6±0.9	59.6±1.5	61.2±1.6
Hemolysis (%)	0.45±0.05	0.45±0.01	0.22±0.04*
ATP (µmol/gHb)	3.2±0.7	3.1±0.7	2.9±0.4
pH (37°C)	6.34±0.05	6.33±0.05	6.41±0.04
K^+(mM)	45.1±1.8	45.0±2.0	52.6±3.2*
Glucose (mM)	17.3±0.7	17.4±0.5	17.8±0.4
Lactate (mM)	20.4±1.0	20.5±1.0	29.3±1.0*

*Repeated measures ANOVA between combined Test and Control: p < 0.05
aValue is from S-303 PI process, D2 post collection