

# **Robustness of Bacteria and Virus Inactivation with S-303 Treatment in RBC Concentrates at Variable Hematocrit**

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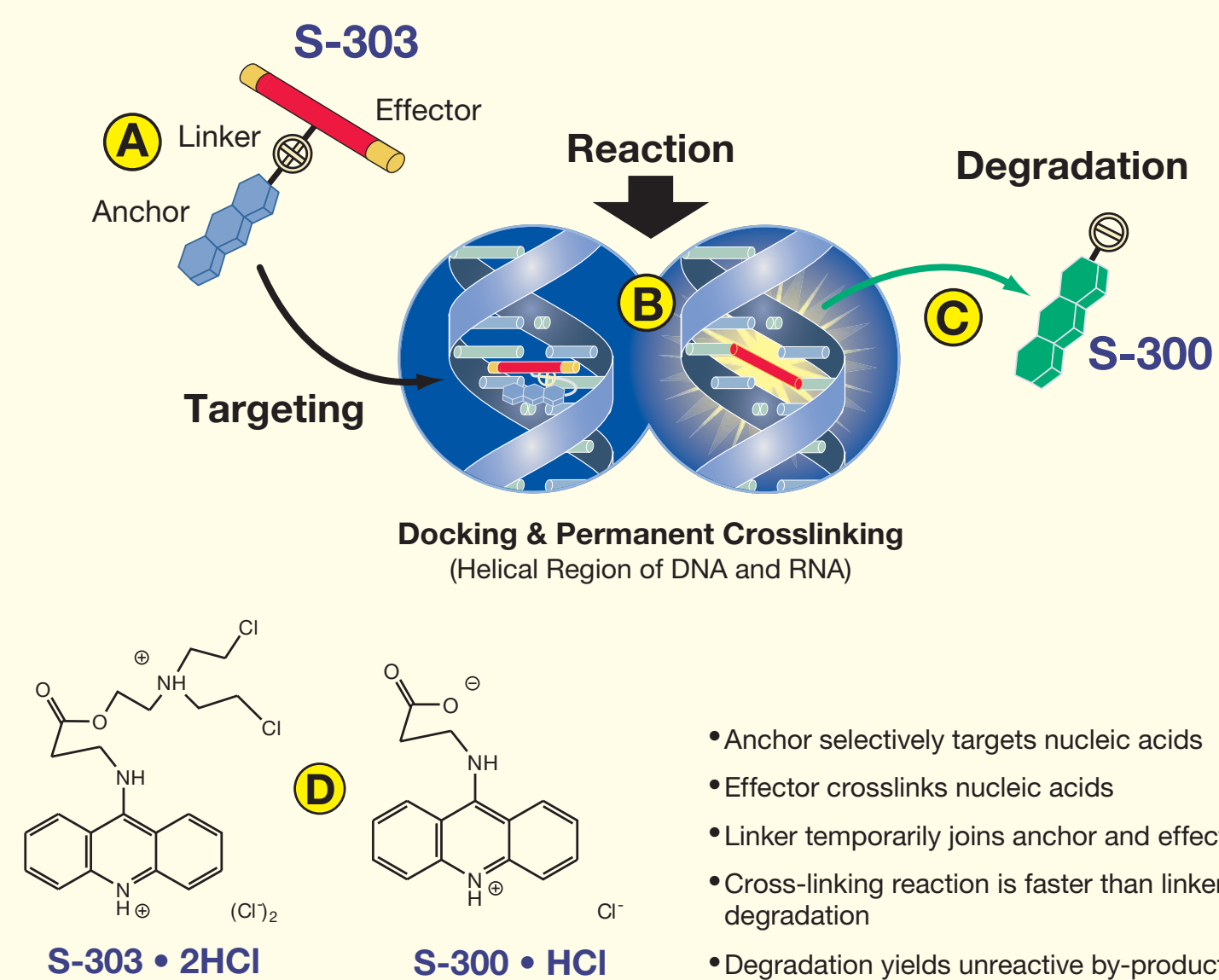
## Background

Cerus is developing a pathogen inactivation process for red blood cells (RBC) utilizing the small molecule S-303, a member of a class of compounds known as FRALEs (frangible anchor-linker-effector). S-303 rapidly crosses membranes and forms covalent crosslinks with nucleic acids (Figure 1). These covalent DNA and RNA crosslinks prevent replication of contaminating pathogens and leukocytes while retaining the function of RBC, which do not require nucleic acid replication for therapeutic efficacy. S-303 is a modular compound (Figure 1A-1C) designed to decompose by hydrolysis to the nonreactive compound S-300. Quenching of the unreacted

S-303 is achieved by including pH-adjusted glutathione (GSH), a natural anti-oxidant, in the treatment process.

Unlike photochemical pathogen inactivation processes for RBC, the S-303 RBC treatment system is not dependent on low hematocrit to allow light penetration, and therefore has the potential to be used with RBC at varying hematocrit values. The studies reported here evaluated the effectiveness of this treatment process for RBC suspended at hematocrits of 40%, 60% and 80% using representative bacteria and vesicular stomatitis virus (VSV) as a model for virus inactivation.

Figure 1: S-303 Treatment Process Mechanism of Action



## Aims

This study was designed to evaluate the efficacy of S-303 pathogen inactivation over a range of hematocrits in RBC.

## Methods

### Preparation of RBC units

RBC units were prepared from 450 to 500 mL whole blood collections (the whole blood had been leukofiltered unless otherwise indicated in Table 1). The RBC were prepared without additive solution (80% spun hematocrit), in a custom additive solution (60% spun hematocrit), or in a commercial additive solution with 150 mL of a custom diluent solution added immediately before treatment (40% spun hematocrit) (Figure 2).

### Bacterial and Viral Inocula

The bacterial species *Staphylococcus aureus*, *Yersinia enterocolitica*, *Serratia marcescens*, and *Escherichia coli* were chosen for these studies because of their prevalence and safety concerns in RBC transfusions: *S. aureus* because it commonly contaminates units during venipuncture and is a representative Gram positive bacteria, and *Y. enterocolitica* and *S. marcescens* due to their ability to grow at the low temperatures at which RBC are stored. In addition, *Y. enterocolitica*,

*S. marcescens*, and *E. coli* also serve as representative Gram negative bacteria. Vesicular stomatitis virus (VSV) was chosen as a model enveloped, RNA virus.

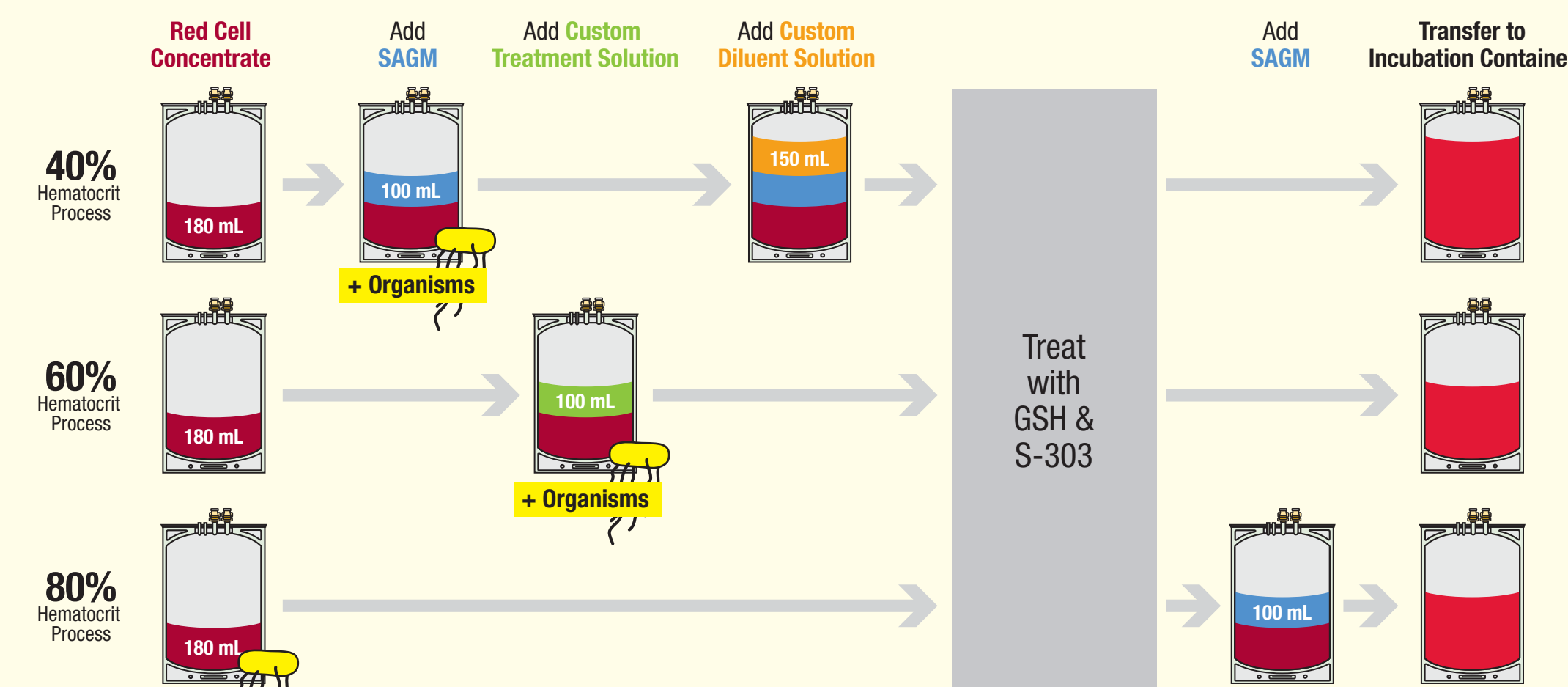
### S-303 Treatment Process and Sample Analysis

For the high level input studies, RBC units were inoculated with approximately  $10^6$  organisms/mL and a Control sample of 28 mL was removed from each inoculated unit prior to S-303 treatment. For the low level bacterial input study, individual Test and Control units were prepared by pooling and splitting RBC units to generate multiple full size Test units and one full size Control unit. Test units were inoculated with approximately  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$  or  $10^5$  bacteria per unit and the Control unit was inoculated with approximately  $10^1$  organisms per unit.

Test units with 80% and 60% spun hematocrit were treated with 0.2 mM S-303 and 20 mM GSH, pH 4. Test units with 40% spun

hematocrit were treated with 0.13 mM S-303 and 13 mM GSH, pH 4. Following treatment all test units were transferred to an incubation container. The Control samples or units were treated with the same 20 mM GSH or 13 mM GSH, pH 4, as were the corresponding Test units. For studies with high level bacterial or viral input, Control samples were assayed for viable organisms at the time the Test unit was treated. After 3 hours of incubation at room temperature both the Test units and Control samples were assayed for viable organisms, which were quantified by growth on rich agar plates (bacteria) or by plaque assay on vero cells (VSV). For units with low level input, the Control and Test units were incubated at room temperature for 20 hours and then at 37°C for ~20 hours. The units were then visually evaluated for evidence of bacterial growth and samples were plated on a rich agar medium to detect bacterial growth.

Figure 2: Pathogen Inactivation Process



## Results

The results in Table 1 show effective pathogen inactivation for a range of bacterial species that may contaminate blood components, and the model virus, VSV in each hematocrit tested. Organism input titers varied from  $10^5$  to  $10^7$  organisms/mL. The inactivation of VSV was greater than 5 logs while inactivation of the bacteria *S. marcescens*, *Y. enterocolitica* and *E. coli*, exceeded 3 logs, and greater than 5 log reduction was achieved for *S. aureus*. Complete inactivation of greater than 2.5 logs of *P. aeruginosa* was obtained. The bacterial inactivation demonstrated is in excess of the bacterial contamination expected in freshly donated blood, which is generally less than 10 cfu/mL at time of collection and processing (Wagner et al. 1994; Hillyer et al. 2003).

Table 1: S-303 Inactivation of Pathogens in RBC at Different Hematocrit Levels

Organism	80% Hematocrit	60% Hematocrit	40% Hematocrit
<b>High Level Input<sup>a</sup></b>			
<b>Mean Log<sub>10</sub> Reduction (N=2)</b>			
<i>Yersinia enterocolitica</i>	3.4 <sup>b</sup>	4.9	6.1
<i>Escherichia coli</i>	3.8 <sup>b</sup>	6.1 <sup>c</sup>	6.6
<i>Serratia marcescens</i>	4.4 <sup>b</sup>	4.5	3.1
<i>Staphylococcus aureus</i>	≥5.8 <sup>b</sup>	6.7	>7
Vesicular stomatitis virus (VSV)	>6.2 <sup>b</sup>	>5.9	5.9
<b>Low Level Input<sup>a</sup></b>			
<b>Full Unit Inactivation<sup>d</sup> (log<sub>10</sub>, N=1)</b>			
<i>Pseudomonas aeruginosa</i>	>2.7	>2.5	>2.5

a. Log reduction is calculated as Log (Untreated titer/Post-treatment titer), with titer expressed as 10<sup>7</sup>/mL

b. Pathogen reduction without leukofiltration  
c. n=3

d. In all cases, control units at the lowest input level were positive for bacterial growth, indicating that inactivation was not due to the blood itself.

e. Log reduction calculated as highest number of inoculated organisms that showed no growth.

## Conclusion

The S-303 pathogen inactivation system for red blood cells (RBC) provides effective pathogen inactivation when used with RBC suspended at hematocrits of 80%, 60%, and 40% for a range of bacterial species and the model virus VSV.

## References

- Hillyer C, et al., 2003, Hematology Am Soc Hematol Educ Program. 575-89.
- Wagner S, et al., 1994, Transfusion. 34(6):521-6.