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# INACTIVATION OF PATHOGENS IN RED BLOOD CELLS BY A MODIFIED TREATMENT PROCESS UTILIZING S-303 AND GLUTATHIONE

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**Background:** Treatment of red blood cells (RBC) with 0.2 mM S-303 (an acridine compound) and 2.0 mM glutathione (GSH) has previously been shown to inactivate a variety of pathogens, including viruses, bacteria, protozoan parasites and contaminating leukocytes. During Phase III clinical trials antibodies to S-303-treated RBC were detected in a few trial participants. These antibodies were later determined to be specific to residual acridine bound to the RBC surface. As a result of these antibodies, a modification of the S-303 treatment process has been developed to reduce the potential immunogenicity of S-303-treated RBC. This modified process utilizes 0.2 mM S-303 and 20 mM GSH. In this abstract we report the first data from an on-going series of studies designed to evaluate the efficacy of bacterial and viral inactivation in RBC using the modified S-303 treatment process. **Aims:** The aim of this study was to evaluate the efficacy of pathogen inactivation in RBC using the modified S-303 treatment process.
**Methods:** Leukoreduced RBC units with a hematocrit of approximately 60% were prepared in AS-3 storage medium. Most studies utilized full-size units of approximately 280 mL, but some of the data presented here was obtained using 1/4 size units of approximately 70 mL. When smaller volume units were used parameters such as container size and volume of S-303 and GSH used were reduced proportionally. RBC units were inoculated with approximately 6 logs/mL of organism, and an aliquot was removed to serve as the untreated, input control. GSH in a solution of 2 equivalents NaOH was added to the inoculated units to a final concentration of 20 mM and mixed well. Following a 10-minute room temperature incubation, S-303 was added to a final concentration of 0.2 mM and the units were again mixed well and incubated at 20 to 25 °C for three hours. Following incubation, samples were removed and assayed to detect residual viable organisms. Control preparations were titered immediately after preparation and again after the 3-hour incubation. Bacterial titers were determined by colony formation on agar plates and VSV and HIV titers were determined by plaque formation in vero 76 and MT-2 cells, respectively. Bacteria were chosen to represent Gram positive and Gram negative organisms of relevance to transfusion. Vesicular stomatis virus (VSV) was used as a representative negative sense RNA virus, and HIV inactivation was evaluated because of its relevance to blood transfusion. Replicate experiments were performed for each organism and the results are expressed as the mean and standard deviation (SD) of the observed inactivation levels. Some studies in this abstract are currently in progress, when data reported are from fewer than three replicates no SD was calculated. Inactivation is expressed as log reduction.
**Results:** See table below. Both Gram positive and Gram negative bacteria were effectively inactivated by treatment with the modified S-303 process. This process also resulted in effective inactivation of the two virus tested so far: HIV and VSV.
**Conclusion:** The modified S-303 process effectively inactivates bacterial and viral pathogens in RBC.

| Organism | Log Reduction[a] (Mean ±SD) |
|---|---|
| Staphylococcus aureus | ≥6.9 ±0.4 |
| Staphylococcus epidermidis | >7.0 ±0.1 |
| Yersinia enterocolitica | ≥4.7 ±1.4 |
| Escherichia coli | >6.6 ±0.1 |
| Serratia marcescens | 3.8[b] |
| Vesicular stomatitis virus (VSV) | 4.5 ±0.3 |
| HIV | >5.8 ±0.2 |

[a]Additional replicates of some studies are in progress and data presented is preliminary
[b]No SD was calculated because data presented is from fewer than 3 replicates.


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