Whole blood pathogen inactivation: preliminary results of a treatment combining S-303 and different glutathione concentrations on red blood cell parameters in treated *vs*. untreated whole blood

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BACKGROUND Blood transfusion is used to treat several life threatening diseases including acute anemia. Every day in many low resources countries, women are frequently dying during childbirth due to the lack of blood availability in transfusion centers. Moreover, these countries can experience significant problems with the transmission of infectious diseases through the use of blood infected by bacteria, viruses and parasites.

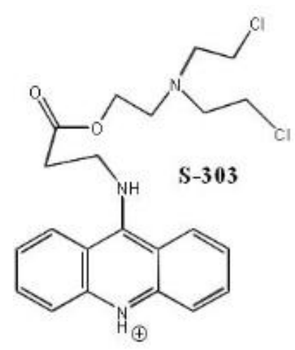
Our project, supported by the Humanitarian Foundation of the Swiss Red Cross, aims at finding a solution to improve the safety of transfusion practice in Africa. It entails the adaptation of Cerus' INTERCEPT Blood System for red blood cells (RBCs) for pathogen inactivation (PI) of whole blood (WB) and is specifically being developed for sub-Saharan African countries where infrastructures and resources are lacking.

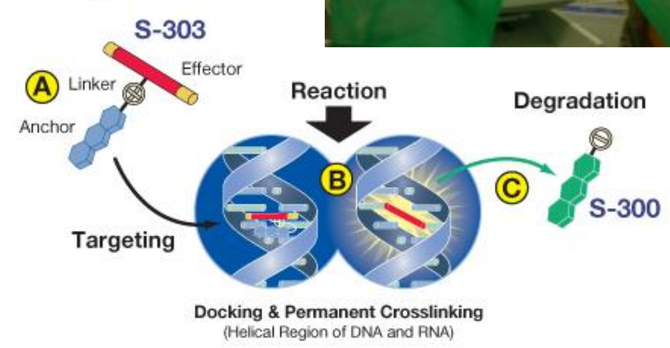
AIM **The first step of this project was to determine the effects of pathogen inactivation on different RBC parameters in treated whole blood.**

MATERIALS AND METHODS WB units were treated with S-303, an alkylating agent used to crosslink nucleic acids and prevent replication of contaminating pathogens, and glutathione (GSH) used to quench non-specific reactions with proteins . The concentrations used for the treatment were 0.2 mM of S-303 and 2, 5, 10 and 20mM of GSH. The blood units were stored at room temperature during the experiment and were tested at different time points after treatment (24h, 48h, 72h and 7 days) for the following parameters: hematocrit, osmotic fragility, ATP, pH, lactate, K+ and the percentage of hemolysis.

- **Hematocrit (HTC)** is the relative percentage of the volume of cells circulating in blood reported to the total volume of blood. It gives an indication of the volume occupied by the RBCs. Hematocrit was obtained using a Sysmex KX-21.
- **Osmotic fragility** was determined by the measure of hemoglobin released from RBCs, when placed in an environment containing serial dilutions of Phosphate Buffered Saline (PBS).
- The percentage of hemolysis was calculated using the mathematical formula:

$$\% \text{ Hemolysis} = \frac{\text{free Hb} \times (100-\text{HTC})}{\text{Hb Sysmex}}$$





S-303: Chemical structure and mode of action

RESULTS

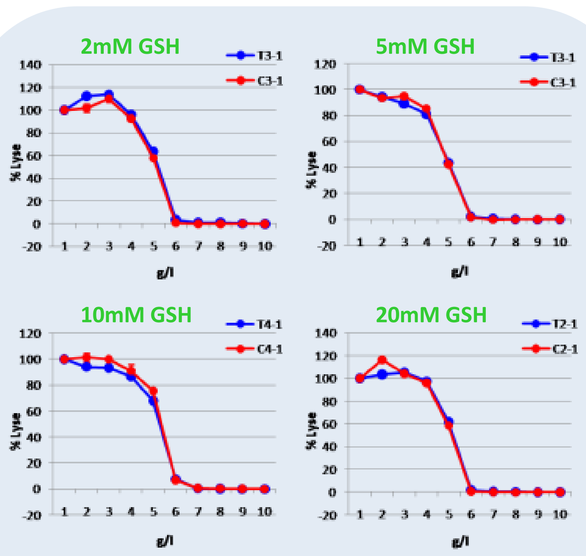


FIGURE 1: OSMOTIC FRAGILITY

- The more RBCs are stressed, the more the curves are left shifted (Blasi et al., Transfusion Medicine, 2014)
- **Perfect overlaping of C and T curves**
- **No effect of treatment on osmotic fragility**

2mM GSH

		Free Hb (umol/L)	HCT (%)	HGB (umol/L)	HGB (g/L)	% hemolysis
T1	J1	16.7	34	6696	108	0.16
	J2	23.2	34.5	6696	108	0.23
	J3	23.4	34.5	6696	108	0.23
	J7	15.9	36.1	6758	109	0.15
C1	J1	5.2	35.3	7130	115	0.05
	J2	10.5	35.4	6944	112	0.1
	J3	10.8	35.3	6944	112	0.1
	J7	12.7	36.6	7006	113	0.11
T2	J1	30.8	36.7	7502	121	0.26
	J2	35.9	37.9	7502	121	0.3
	J3	37.1	37.3	7502	121	0.31
	J7	24.1	39	7502	121	0.2
C2	J1	7.2	39.1	7874	127	0.06
	J2	17.5	39.3	7874	127	0.13
	J3	31.2	38.8	7874	127	0.24
	J7	19.2	39.8	7874	127	0.15
T3	J1	19.4	37.7	7564	122	0.16
	J2	20	37.9	7564	122	0.16
	J3	17.6	37.7	7502	121	0.15
	J7	15.7	39.3	7564	122	0.13
C3	J1	6.5	39.4	7998	129	0.05
	J2	6.8	39.4	7936	128	0.05
	J3	8.5	39.6	7936	128	0.07
	J7	13.2	40.1	7874	127	0.1

20mM GSH

		Free Hb (umol/L)	HCT (%)	HGB (umol/L)	HGB (g/L)	% hemolysis
T1	J1	29.3	33.6	6572	106	0.3
	J2	23.1	34.4	6572	106	0.23
	J3	14	34.1	6510	105	0.14
	J7	18	34.8	6510	105	0.18
C1	J1	1.2	35.6	7006	113	0.01
	J2	1.8	35.7	7068	114	0.02
	J3	2.7	36.4	7006	113	0.02
	J7	6	37.2	7068	114	0.05
T2	J1	4.9	32.2	6138	99	0.05
	J2	3.5	32.4	6200	100	0.04
	J3	4.6	32.2	6200	100	0.05
	J7	4.6	33.3	6262	101	0.05
C2	J1	4.2	34.6	6634	107	0.04
	J2	3.6	34.6	6634	107	0.04
	J3	5.2	34.7	6696	108	0.05
	J7	5.1	35	6634	107	0.05
T3	J1	12.2	31.9	5704	92	0.15
	J2	7.2	33.6	6448	104	0.07
	J3	8	33.6	6510	105	0.08
	J7	8	34.7	6448	104	0.08
C3	J1	2.5	35.7	7006	113	0.02
	J2	4	35.9	7006	113	0.04
	J3	4.8	35.9	7006	113	0.04
	J7	5.7	37.3	7006	113	0.05

FIGURE 2: HEMATOCRIT AND % OF HEMOLYSIS

- A rise in free hemoglobin indicates hemolysis.

In blood RBC units, hemolysis has to be < 0.8% → our results are in the accepted range, according to the EDQM blood transfusion guides.

- HTC % and total hemoglobin concentration are stable with time, and slightly higher in control samples than in treated samples.

		2mM GSH	5mM GSH	10mM GSH	20mM GSH
T1	J1	4	4.6	4.4	4.3
	J2	5.3	6.6	6.1	6.1
	J3	6.7	8.7	7.6	7.6
C1	J1	3.9	4.4	4.2	4.2
	J2	5	6.4	5.8	5.6
	J3	6.3	8.3	7.3	7
T2	J1	3.9	4.5	4.4	3.7
	J2	5.7	6	6.1	5
	J3	7.4	7.8	7.8	6.2
C2	J1	4.2	4.2	4.2	3.5
	J2	5.8	5.8	5.7	4.6
	J3	7.6	7.4	7.3	5.6
T3	J1	5	4.9	3.9	3.6
	J2	7	6.8	5.5	5.2
	J3	8.7	8.6	6.8	6.5
C3	J1	4.6	5	3.8	3.7
	J2	6.6	7	5.2	4.7
	J3	8.5	8.7	6.5	5.8

FIGURE 3: K^+

- Stable and homogenous effect of time on K^+ concentration: it increases in T and C samples with time.
- Consistent with literature

		2mM GSH	5mM GSH	10mM GSH	20mM GSH
T1	J1	4.7	4.1	3.8	3.2
	J2	4.1	3.7	3.6	2.6
	J3	3.7	3	3.1	2.5
C1	J1	4.3	4.6	4	3.6
	J2	4.7	3.9	3.7	2.9
	J3	4	3.5	3.5	2.6
T2	J1	4.2	4.2	4.5	4.1
	J2	3.6	3.5	3.9	4.2
	J3	3	3.2	3.3	3
C2	J1	4.1	4.7	4.3	4.5
	J2	3.5	3.9	4.2	3.9
	J3	3.8	3.5	3.9	3.4
T3	J1	4	3.8	3.8	4.3
	J2	3.9	3.9	2.7	3.4
	J3	3.3	3.1	2.6	3.4
C3	J1	4.1	4	3.6	4.3
	J2	3.8	3.6	3.5	4.3
	J3	3.4	3.3	2.9	3.2

FIGURE 4: ATP

- Effect of time is observed with ATP decreasing over time
- All conditions result in >2.5umol/g Hb consistent with ATP level observed by Cerus in RBCs stored at 4°C during 42 days

		2mM GSH	5mM GSH	10mM GSH	20mM GSH
T1	J1	6.764	6.759	6.727	6.723
	J2	6.661	6.651	6.62	6.594
	J3	6.577	6.575	6.528	6.503
C1	J1	6.831	6.824	6.835	6.823
	J2	6.724	6.726	6.741	6.716
	J3	6.648	6.664	6.675	6.653
T2	J1	6.788	6.723	6.733	6.749
	J2	6.663	6.61	6.615	6.636
	J3	6.572	6.524	6.521	6.557
C2	J1	6.856	6.778	6.86	6.858
	J2	6.743	6.675	6.728	6.76
	J3	6.675	6.602	6.675	6.697
T3	J1	6.779	6.756	6.722	6.733
	J2	6.665	6.647	6.625	6.605
	J3	6.579	6.576	6.533	6.522
C3	J1	6.826	6.804	6.812	6.865
	J2	6.715	6.71	6.723	6.732
	J3	6.649	6.652	6.664	6.651

FIGURE 5: pH

- The pH decreased over time likely due to the increase of lactate with time.
- pH slightly lower in T compared to C; may be due to acidic S-303 molecule

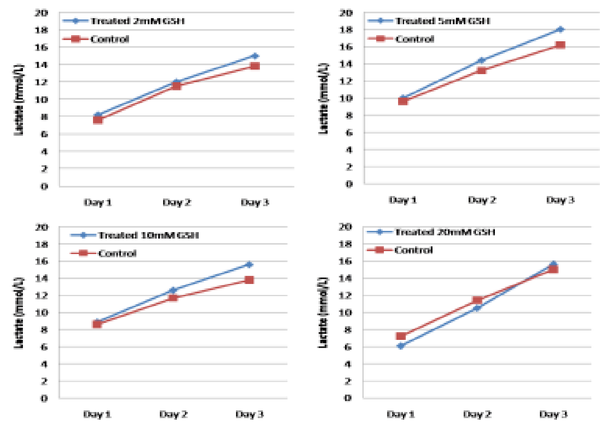


FIGURE 6: LACTATE

- Lactate production increases with time.
- Lactate levels in T samples are slightly higher than in C samples

CONCLUSIONS **Although the sample size was small, interestingly the experiments showed acceptable results in treated and control WB units for most of the tested parameters, indicating that RBC viability and function are preserved after treatment.**

These preliminary results for S-303-treated whole blood are very encouraging, suggesting that pathogen inactivated whole blood could represent a safe and innovative blood product for transfusion. The next step will consist of in-depth studies of the treatment's effects, in particular on coagulation factors and platelet functions.