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INTERCEPT Blood System Inactivates *Enterococcus faecalis*, Multiple Species of *Streptococcus* and *Serratia liquefaciens* in Platelet Components in Platelet Additive Solution and in Plasma

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Background

A photochemical treatment process utilizing 150 µM amotosalen (S-59) and 3 J/cm^2 long wavelength ultraviolet light (UVA), the INTERCEPT™ Blood System, has been developed to inactivate high titers of a large number of Gram positive and Gram negative bacteria, both cell-free and cell-associated, enveloped and nonenveloped viruses, and protozoan parasites (**Table 1**). It is currently in use in more than 100 sites in 20 countries and has recently been approved for use in the US.

Previous studies have shown inactivation of *Streptococcus pyogenes* and *Serratia marcescens* by the INTERCEPT Blood System for platelets. Additional species of *Serratia* and *Streptococcus* have recently emerged as organisms of concern to blood transfusion in the UK. The INTERCEPT mechanism of action is not species specific and evaluation of these additional species offers the opportunity to demonstrate this breadth of efficacy.

Figure 1: INTERCEPT Mechanism of Action Targeting DNA and RNA to Prevent Pathogen Proliferation

Small molecules (amotosalen) penetrate cellular and nuclear membranes and intercalate into helical regions of DNA or RNA present.

Amotosalen forms covalent crosslinks to nucleic acid base pairs upon exposure to UVA light.

DNA and RNA replication is blocked. Pathogens and leukocytes cannot reproduce and are 'inactivated'.

Aims

The aim of these studies was to demonstrate that the inactivation of *Serratia marcescens* and *Streptococcus pyogenes* is representative of the two genera by showing that inactivation of *Serratia liquefaciens*, *Streptococcus agalactiae*, *Streptococcus mitis*, *Streptococcus pneumoniae* and *Enterococcus faecalis* by INTERCEPT treatment is comparable to that previously demonstrated for *Serratia marcescens* and *Streptococcus pyogenes*.

Methods

Apheresis platelet components containing ~2.5–7.0 x 10^{11} platelets in ~330-400 mL of 100% plasma or 35% plasma/65% Platelet Additive Solution (PAS) were inoculated to a titer of ~10^6 organisms per mL with one of the five organisms under investigation, then treated with the INTERCEPT Blood System for platelets using large volume processing sets (**Figure 2**). Samples were taken before illumination to determine input titer and after illumination to detect and quantify any residual viable bacteria. Bacterial viability was determined by colony formation on agar incubated at 37°C. The species of bacteria, the type of agar, and the incubation atmosphere are shown in **Table 2**.

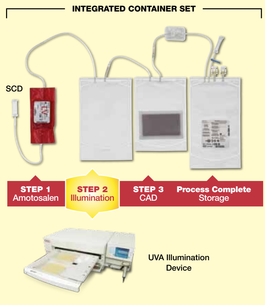
Historic data on inactivation of *Streptococcus pyogenes* and *Serratia marcescens* were obtained using platelet concentrates (PC) of approximately 300 mL and small volume INTERCEPT platelet processing sets.

Table 2: Culture Conditions

Organism	Agar	Atmosphere
Serratia liquefaciens	Rich	Ambient
Streptococcus agalactiae	Rich	Ambient
Streptococcus mitis	Blood	5% CO_2
Streptococcus pneumoniae	Blood	5% CO_2
Enterococcus faecalis	Rich	Ambient

Figure 2: The INTERCEPT Blood System for Platelets

Using a sterile connecting device (SCD), the platelet container is sterilely connected to the INTERCEPT kit. Amotosalen (1) is added by gravity flow and the platelet mixture is illuminated with UVA light (2). Residual amotosalen and its photoproducts in the platelet mixture are reduced to low levels using a compound adsorption device (CAD) (3) before the platelets are transferred to the storage container.



Results

Historical data for inactivation of *S. marcescens* and *S. pyogenes* in PC in PAS are shown in **Table 3**, along with the data obtained in the current studies. In the current study, inactivation of at least 6.5 $\log_{10}$ of one additional species of *Serratia* and four additional species of *Streptococcus*/*Enterococcus* was demonstrated in PC suspended in 100% plasma or 35% plasma/65% PAS (**Table 3**).

Table 3: $\log_{10}$ Inactivation of Multiple Species of *Serratia* and *Streptococcus* in Platelet Components in 65% PAS or 100% Plasma by the INTERCEPT Blood System (Mean ± SD, n=4)

Pathogen	65% PAS	100% Plasma
Serratia marcescens	>6.7±0.1*	Not Done
Serratia liquefaciens	>6.3±0.3	>7.2±0.6
Streptococcus pyogenes	>6.8±0.1*	Not Done
Streptococcus agalactiae	≥7.1±0.1	≥6.6±0.0
Streptococcus mitis	>6.8±0.3	>6.8±0.4
Streptococcus pneumoniae	>7.3±0.1	>7.3±0.2
Enterococcus faecalis (n=3)	>7.0±0.2	>6.8±0.2

*Historical data

Table 1: Pathogen Inactivation by the INTERCEPT Blood System

Pathogen	Mean $\log_{10}$ Reduction[a]	
	Platelets in ~65% Additive Solution/ 35% Plasma	Plasma and Platelets in 100% Plasma[b]
VIRUSES		
Enveloped Viruses		
HIV-1, cell-associated	>6.1[c]	>6.7[d]
HIV-1, cell-free	>6.2[c]	>6.8[c] (≥4.7)[e]
HIV-1 (clinical isolate)	>3.4[c]	-[f]
HIV-2 (clinical isolate)	>2.5[c]	-
HCV	>4.5[c]	>4.5[d]
BVDV (model for HCV)	>6.0[c]	≥6.0[d] (≥5.4)[e]
HBV	>5.5[c]	>4.5[d]
DHBV (model for HBV)	>6.2[c]	4.4 – 4.5[d]
HTLV-I	4.7[c]	≥4.5[d]
HTLV-II	5.1[c]	>5.7[d]
XMRV	>4.0[g]	-
CMV, cell-associated	>5.9[c]	-
PRV (model for CMV)		(≥4.7)[e]
WNV	>6.0[c]	≥6.8[d]
Dengue virus	>4.0[h]	-
SARS corona virus (SARS-CoV)	>6.2[i]	≥5.5[d]
Vaccinia virus	>5.2[c]	-
Chikungunya virus	>6.4[j]	≥7.6[j]
LCMV	-	>5.6[k]
Influenza A H5N1	>5.9[l]	>5.7[l]
Non-enveloped Viruses		
Bluetongue virus	>5.0[c]	5.1[d]
Human Adenovirus 5	>5.9[i]	≥6.9[d]
Calicivirus	1.7 to 2.4[c]	-
Parvovirus B-19	2.0 to >6.0[m]	1.8 to 2.8[j]
BACTERIA		
Rickettsiales		
Orientia Tsutusgamushi	>5.0[n]	>5.5[o]
Anaplasma phagocytophilum	-	>4.2[o]
Spirochetes		
Treponema pallidum	≥6.8 to ≤7.0[q]	>5.9[d]
Borrelia burgdorferi	>6.8[q]	>10.6[d]
Gram Negative		
Escherichia coli	>6.4[q]	(≥7.3)[e]
Serratia marcescens	>6.7[q]	-
Pseudomonas aeruginosa	4.5[q]	-
Klebsiella pneumonia	>5.6[q]	≥7.4[d] (≥6.7)[e]
Salmonella choleraesuis	>6.2[q]	-
Enterobacter cloacae	5.9[q]	-
Yersinia enterocolitica	>5.9[q]	>7.3[d]
Gram Positive		
Staphylococcus epidermidis	>6.6[q]	>7.3[d] (>7.4)[e]
Staphylococcus aureus	6.6[q]	(>7.6)[e]
Listeria monocytogenes	>6.3[q]	-
Corynebacterium minitissimum	>6.3[q]	-
Streptococcus pyogenes	>6.8[q]	-
Bacillus cereus (vegetative)	>6.0[q]	-
Anaerobic Gram Positive		
Bifidobacterium adolescentis	>6.5[q]	-
Propionibacterium acnes	>6.7[q]	-
Clostridium perfringens (vegetative)	>7.0[q]	-
Lactobacillus species	>6.9[q]	-
PROTOZOAN PARASITES		
Plasmodium falciparum	≥6.0[r]	≥6.9[r]
Trypanosoma cruzi	≥5.4[s]	>5.0[s]
Babesia microti	>5.3[r]	>5.3[r]
Leishmania species	>5.0[t]	-

a) Log reduction is calculated as log (pre-treatment titer ÷ post-treatment titer), where titer is expressed as 10^x organisms/mL.

b) Where data for inactivation in platelets in 100% plasma differ from that in plasma, the inactivation data for platelets in 100% plasma is shown in parentheses.

c) Lin L, et al. 2005. Transfusion. 45:580

d) Singh Y, et al. 2006. Transfusion. 46:1168

e) Brussel A, et al. 2008. Vox Sang. 95(Suppl. 1):301

f) "-" indicates inactivation studies not performed.

g) Mikovitz JA, et al. 2010 Abstract presented at the 1st Annual XMRV Workshop (NIH)

h) Lam S, et al. 2007. Transfusion. 47:134A.

i) Pinna D, et al. 2005. Transfusion Medicine. 15:269.

j) Stramer S, et al. 2009 Transfusion. 49(2S):1S

k) Sawyer L, et al. 2006. Transfusion. 46:115A.

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p) Sawyer L, et al. 2009. Vox Sang. 96(Suppl. 1):233

q) Lin L, et al. 2004. Transfusion. 44:1496

r) Grellier P, et al. 2008. Transfusion. 48:1676

s) Van Voorhis W, et al. 2003. Antimicrobial Agents and Chemotherapy. 47:475

t) Eastman R, et al. 2005. Transfusion. 45:1459

Conclusions

- **INTERCEPT treatment inactivates high titers of multiple species of *Serratia* and *Streptococcus* in platelet components suspended in PAS and in plasma.**
- **These results support the applicability of inactivation data obtained using one species as a model for efficacy across a genus.**

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