

# Methicillin Resistance Does Not Impact the Sensitivity of *Staphylococcus aureus* to Inactivation by Amotosalen and UVA Illumination



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## Background

A photochemical treatment process utilizing 150 µM amotosalen HCl (S-59) and 3 J/cm<sup>2</sup> long wavelength ultraviolet light (UVA), the INTERCEPT Blood System™, has been developed for inactivation of viruses, bacteria, parasites and leukocytes that can contaminate platelet and plasma components intended for transfusion. This process has been demonstrated to inactivate high titers of a large number of Gram positive and Gram negative bacteria, both cell-free and cell-associated, enveloped and non-enveloped viruses, and protozoan parasites (Table 1). It is currently in use in more than 15 countries and under development in the U.S.

Multiply drug resistant bacteria are an increasing problem, particularly in the health care setting, and methicillin resistant *Staphylococcus aureus* is one of the most significant

of these pathogens. The severity of the problem has caused concern and occasional questions about the sensitivity of drug-resistant bacteria to pathogen inactivation. The mechanism of action of the INTERCEPT Blood System, which relies on intercalation of amotosalen into nucleic acids and adduct formation upon UVA illumination, is not sequence specific but can occur at any pyrimidine base pair. This lack of sequence specificity means that inactivation does not put evolutionary pressure on the bacteria to develop resistance mutations. Thus, mutations and plasmids that confer antibiotic resistance should have no impact on the efficacy of pathogen inactivation by the INTERCEPT™ process.

## Aims

This study evaluated the efficacy of the INTERCEPT Blood System for plasma to inactivate multiply drug resistant bacteria by directly comparing the inactivation of methicillin resistant *Staphylococcus aureus* (MRSA) with methicillin sensitive *Staphylococcus aureus* (MSSA).

## Methods

Isolates of the bacteria used in this study, MRSA (ATCC 25923) and MSSA (ATCC 43300) were obtained from ATCC, and are commonly used in Quality Control for methods distinguishing MRSA and MSSA. For each of 4 replicates, previously frozen apheresis plasma units were pooled as necessary and divided into two units of approximately 585 mL each. Paired units were inoculated with an approximate 1:100 dilution of an overnight culture of either MRSA or MSSA to a final concentration of approximately

10<sup>6</sup> organisms/mL. After inoculation with bacteria, the plasma units were treated with 150 µM amotosalen and UVA light using the INTERCEPT Blood System for plasma. The UVA dose response was evaluated with 1, and cumulative 2 and 3 J/cm<sup>2</sup> UVA treatment. Control samples were taken before illumination to determine input titer and Test samples were taken after illumination to detect residual viable pathogens. Titer was determined by colony formation on LB agar.

## Results

Tables 2 and 3 show the inactivation of each organism achieved in the four individual replicate experiments. In all cases no residual viable bacteria were detected following as little as 1 J/cm<sup>2</sup> UVA. Table 4 directly compares inactivation of MRSA to that of MSSA under the commercial treatment conditions, illustrating that multiple drug resistance does not impact susceptibility to inactivation by amotosalen and UVA light.

**Table 2: Inactivation of MRSA Using the INTERCEPT Blood System for Plasma**

Replicate	Input titer (Logs per mL)	Log Inactivation Following the Indicated UVA Treatment		
		1 J/cm <sup>2</sup>	2 J/cm <sup>2</sup>	3 J/cm <sup>2</sup>
1	6.2	>6.9	>6.9	>6.9
2	6.5	>7.2	>7.2	>7.2
3	5.9	>6.5	>6.5	>6.5
4	5.8	>6.5	>6.4	>6.5

**Table 3: Inactivation of MSSA Using the INTERCEPT Blood System for Plasma**

Replicate	Input titer (Logs per mL)	Log Inactivation Following the Indicated UVA Treatment		
		1 J/cm <sup>2</sup>	2 J/cm <sup>2</sup>	3 J/cm <sup>2</sup>
1	6.7	>7.4	>7.4	>7.4
2	6.6	>7.3	5.1	>7.3
3	6.0	>6.8	>6.8	>5.5
4	5.7	>6.4	>6.4	>6.4

**Table 4: Comparison of MRSA and MSSA Inactivation by the INTERCEPT Blood System for Plasma**

Organism	Log Inactivation Following 3 J/cm <sup>2</sup> UVA Treatment (Mean±SD)
MRSA	>6.8±0.3
MSSA	>6.7±0.9

## Conclusions

- *Staphylococcus aureus* is inactivated below the limit of detection with amotosalen and as little as one third of the standard UVA treatment.
- Resistance to multiple antibiotics does not impact the sensitivity of *Staphylococcus aureus* to inactivation by the INTERCEPT Blood System.

## Footnotes

- Log reduction is calculated as log (pre-treatment titer ÷ post-treatment titer), where titer is expressed as 10x organisms/mL.
- 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.
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**Table 1: Pathogen Inactivation by the INTERCEPT Blood System**

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

Presented at the American Association of Blood Banks Annual Conference & CCTXPO (AABB)

San Diego, California • October 22nd - 25th, 2011