

Pathogen Inactivation Achieved with Amotosalen and UVA in Platelets Suspended in Plasma/SSP+ is Comparable to that Achieved in Platelets Suspended in Plasma/InterSol

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Background

The INTERCEPT Blood System™ for platelets was developed to prevent transfusion-transmitted infections by inactivating pathogens in platelet components for transfusion. This system utilizes amotosalen HCl and UVA illumination, which has been demonstrated to inactivate leukocytes (Grass 1998) and blood-borne pathogens in platelet and plasma components, including a broad spectrum of both cell-free and cell-associated, enveloped and non-enveloped viruses, gram negative and gram positive bacteria, and protozoan parasites (Table 1).

The INTERCEPT Blood System for platelets was originally CE Marked for treatment of platelet concentrates obtained by either apheresis collection or by pooling of buffy coats and suspended in approximately 35% plasma and 65% InterSol® platelet additive solution. Recently, the device label claim was expanded to include platelets suspended in 35% plasma and 65% SSP+.* SSP+ is a recently introduced platelet additive

solution that differs from InterSol primarily in the addition of magnesium and potassium. To determine whether SSP+ is compatible with INTERCEPT™, both platelet function and pathogen inactivation have been evaluated. A study previously presented at AABB evaluated platelet in vitro functional parameters during storage following treatment of platelets in InterSol and in SSP+ (Stebler 2004). This study found platelet in vitro functional parameters following INTERCEPT treatment to be comparable in the two additive solutions. Platelets treated and stored in SSP+ showed somewhat better in vitro storage parameters through day 6, but they were indistinguishable from platelets treated and stored in InterSol by day 8. This is consistent with more recent data showing that untreated platelets stored in SSP+ has slightly better in vitro characteristics than those stored in InterSol, but this could not be verified by in vivo recovery and survival (Diedrich 2008).

Table 1: Inactivation of a Variety of Pathogens in Platelets and in Plasma by 150 µM Amotosalen and 3 J/cm² UVA Treatment, Using the INTERCEPT Blood System (Platelets in 65% InterSol).

Organism	Mean Log Reduction ^a	
	Platelets	Plasma
HIV, cell-associated	>6.1 ^b	>6.4 ^c
HIV, cell-free	>6.2 ^b	>6.8 ^c
HCV	>4.5 ^b	>4.5 ^c
BVDV (HCV model)	>6.0 ^b	≥6.0 ^c
HBV	>5.5 ^b	>6.5 ^c
DHBV (HBV model)	>6.2 ^b	4.6 ^c
HTLV-I	4.7 ^{b,d}	≥4.5 ^c
HTLV-II	5.1 ^{b,d}	>5.7 ^c
CMV, cell-associated	>5.9 ^b	- ^e
Bluetongue (model non-enveloped virus)	6.1 to 6.4 ^b	5.1 ^c
Human Adenovirus 5	>5.9	≥6.9 ^c
Parvovirus B-19	2.0 to >6.0 ^f	1.8 to 2.8 ^g
West Nile virus	>6.0 ^b	≥6.8 ^c
SARS-CoV	>6.2 ^b	≥5.5 ^c
Vaccinia virus	>5.2	-
Chikungunya virus	>6.4	≥7.6
Influenza A virus	>5.9 ^b	>5.7 ^h
Lymphocytic choriomeningitis virus (LCMV)	-	>5.6 ^h
<i>Treponema pallidum</i>	≥6.8 to ≤7.0 ⁱ	>5.9 ^c

a Log reduction is calculated as log (pre-treatment titer ÷ post-treatment titer), where titer is expressed as 10⁶ organisms/mL.
b Lin 2005

c Singh 2006
d Inherent low-level background in non-infected indicator cells precludes ">" for HTLV in platelets.

e "-" indicates inactivation data not available
f Sawyer 2007
g Pinna 2005
h Sawyer 2008

i Lin 2004
j Rentas 2004
k Grellier 2009
l Van Voorhis 2003
m Eastman 2005

*The INTERCEPT Blood System, InterSol and SSP+ are not approved for sale in the US.

Aims

The in vitro efficacy of pathogen inactivation was evaluated in platelets in SSP+ as compared to platelets in InterSol.

Methods

The organisms evaluated in this study were selected to represent a variety of viruses and bacteria, including enveloped (HIV) and non-enveloped (bluetongue virus (BV) and human adenovirus 5 (Ad5)) viruses, RNA (HIV and BV) and DNA (Ad5) viruses, and viruses with a single stranded genome (HIV) and those with a double stranded genome (BV and Ad5). Bacteria evaluated include both Gram positive (*Staphylococcus epidermidis*) and Gram negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) organisms. In addition to representing classes of organisms, as described above, *K. pneumoniae* and BV were

selected because of their relative resistance to inactivation and *Ps. aeruginosa* was included specifically because it is one of the most difficult organisms to inactivate and therefore is a sensitive indicator of changes in inactivation efficacy.

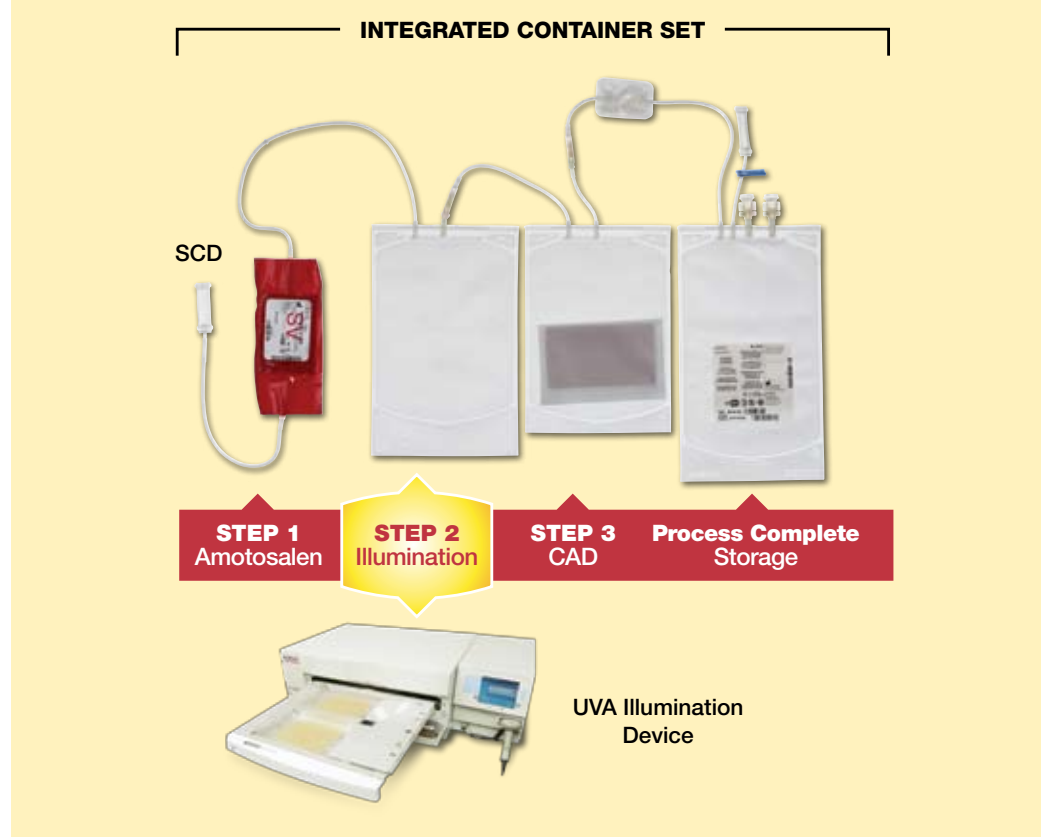
Four replicate experiments were performed for each of the studies reported here. Each replicate utilized a single-dose platelet unit, collected by apheresis to contain 2.5 – 6.0 x10¹¹ platelets in approximately 300 mL of plasma. Each unit was processed to remove plasma then the platelets were resuspended into approximately 285 mL of 35% plasma/65% SSP+. For five of the six organisms evaluated, historical data obtained in prior studies of inactivation in platelets suspended in 35% plasma/65% InterSol was used for comparison to the data obtained in this study. No comparable prior data was available for comparison of adenovirus inactivation, so the adenovirus study utilized double-dose platelet units that were split, with one of the resulting two units resuspended in 35% plasma/65% InterSol. The historical *Ps. aeruginosa* inactivation data included data from multiple input titers of *Ps. aeruginosa*. Only one bacterial input titer was used for the SSP+ study and only the historical InterSol data from the same input titer is

shown in Table 1 to provide a more direct comparison. Units were inoculated with virus or bacteria at approximately 10⁶ organisms/mL when possible and the inoculated platelet units were treated with 150 µM amotosalen and 3.0 J/cm² UVA treatment using the INTERCEPT Blood System for platelets (Figure 1). CAD and storage containers remained attached during the illumination step, but were not used in these studies.

Samples were taken from each unit prior to illumination to determine viable input titer, and following illumination to determine the titer of any residual viable organisms. Samples taken before illumination were serially diluted prior to plating on the appropriate medium for viability detection, while samples taken after illumination were plated undilute or following 1:10 and 1:100 dilutions. Bacteria samples were plated to Luria Bertoni agar and viability was detected by colony formation. Viable virus titers were determined by plaque formation in appropriate cell culture: HIV in MT-2 cells, Ad5 in A-549 cells and BV in bovine turbinate (BT) cells.

Figure 3: 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

Similar inactivation was achieved whether platelets were suspended in plasma/SSP+ or in plasma/InterSol (see Table 2). In both cases, HIV, human adenovirus 5, and *S. epidermidis* were completely inactivated to below the limit of detection in the volumes assayed. Inactivation of *P. aeruginosa* was comparable in the two platelet additive solutions and inactivation of *K. pneumoniae* and bluetongue virus in plasma/SSP+ was consistent with that obtained in plasma/InterSol.

Table 2: INTERCEPT Blood System Inactivation of Pathogens in Platelets Suspended in Either Plasma/SSP+ or Plasma/InterSol

Organism	Additive Solution	Log Inactivation				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean ±SD ^a
HIV	SSP+	>6.5	>5.6	>5.8	>6.0	>6.0 ± 0.4
	InterSol	>6.0	>6.2	>6.5	>5.8	>6.1 ± 0.3
Human Adenovirus 5	SSP+	>5.8	>5.8	>6.4	>5.5	>5.9 ± 0.4
	InterSol	>5.7	>5.8	>6.4	>5.7	>5.9 ± 0.3
Bluetongue virus	SSP+	>4.9	>4.8	>5.2	>5.1	>5.0 ± 0.2
	InterSol	6.4	>6.1	6.4	>6.4	≥6.1 to 6.4
<i>Klebsiella pneumoniae</i>	SSP+	>7.2	>7.2	>7.1	>7.1	>7.1 ± 0.04
	InterSol	>5.4	>5.4	>5.6	>5.9	>5.6 ± 0.2
<i>Staphylococcus epidermidis</i>	SSP+	>6.7	>6.9	>6.7	>6.1	>6.7 ± 0.1
	InterSol	>6.6	>6.6	>6.5	>6.6	>6.6 ± 0.1
<i>Pseudomonas aeruginosa</i>	SSP+	>5.0	>4.9	>4.9	5.1	≥5.0 ± 0.1
	InterSol	4.9	4.3	>4.7	>5.0	≥4.8 ± 0.1

^a Means are calculated from unrounded data

Conclusions

The results of this study indicate that the inactivation efficacy of the INTERCEPT Blood System for platelets is maintained when platelets are suspended in plasma and SSP+, suggesting that SSP+ could be an alternative to InterSol for use with the INTERCEPT Blood System.

References

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