

Inactivation of Emergent Blood-borne Pathogens in Plasma and Platelets

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Introduction & Background

Pathogens continuously emerge and reemerge around the world, significantly impacting blood transfusion safety. Current measures to assure safety are reactive and only detect known pathogens for which tests have been implemented. In addition, even with the introduction of nucleic acid testing for a limited number of viruses, the window period persists. A pathogen inactivation method for platelets, plasma, and red blood cells would help protect the blood supply against emerging pathogens, logically following inactivation safeguards in place for plasma-derived proteins. The INTERCEPT Blood System™ for platelet concentrates and plasma

has been developed by Cerus Corporation. This system incorporates Cerus' technology, which utilizes amotosalen (S-59) and UVA light (Figure 1) to inactivate a broad spectrum of pathogens, providing a prospective defense against transfusion-transmitted diseases.

Inactivation efficacy of the INTERCEPT Blood System for platelets has been demonstrated in apheresis and pooled buffy coat platelet preparations using components and conditions representative of those currently in commercial use in Europe (Figure 2). Similar studies undertaken to evaluate the inactivation efficacy of this system for plasma

primarily evaluated inactivation of organisms in jumbo apheresis plasma using treatment components and conditions representative of those intended for commercial use (Figure 3). The results of these inactivation studies in platelet concentrates and plasma appear in Table 1, and most have been previously published (Lin 2004, Lin 2005, Singh 2006). Inactivation studies attempt to focus on organisms of concern in the blood product under study. Since bacteria are of minimal concern in fresh frozen plasma, inactivation of only a few representative bacteria was evaluated in this product.

Table 1: Inactivation of a Variety of Pathogens in Blood Products by 150 µM Amotosalen and 3.0 J/cm² UVA Treatment, using the INTERCEPT Blood System

Organism	Method of Detection	Mean Log Reduction ^a in Platelet Concentrates	Mean Log Reduction ^a In Plasma
HIV, cell-associated	Micro-plaque in MT-2 cells	>6.1	>6.4
HIV, cell-free	Micro-plaque in MT-2 cells	>6.2	>6.8
HCV ^b	Infectivity in chimpanzee	>4.5	>4.5
BVDV (HCV model)	Plaque in bovine turbinate cells	>6.0	≥6.0
HBV ^b	Infectivity in chimpanzee	>5.5	>4.5
DHBV (HBV model)	Infectivity in ducklings	>6.2	4.6
HTLV-I	Beta-galactosidase production by infected pA18GBHK-21 cells	4.7 ^c	≥4.5
HTLV-II	Beta-galactosidase production by infected pA18GBHK-21 cells	5.1 ^c	>5.7
CMV, cell associated	Plaque in fibroblastoid cells	>5.9	- ^d
Bluetongue (model non-enveloped virus)	Plaque in embryonic bovine trachea cells	6.1 to 6.4	5.1
Human Adenovirus 5	Plaque in A549 cells	>5.9 ^e	≥6.9
<i>Escherichia coli</i>	Colony formation on Luria Bertani agar	>6.4	-
<i>Serratia marcescens</i>	Colony formation on Luria Bertani agar	>6.7	-
<i>Pseudomonas aeruginosa</i>	Colony formation on Luria Bertani agar	4.5	-
<i>Klebsiella pneumoniae</i>	Colony formation on Luria Bertani agar	>5.6	≥7.4
<i>Salmonella choleraesuis</i>	Colony formation on Luria Bertani agar	>6.2	-
<i>Enterobacter cloacae</i>	Colony formation on Luria Bertani agar	5.9	-
<i>Yersinia enterocolitica</i>	Colony formation on Luria Bertani agar	>5.9	>7.3
<i>Staphylococcus epidermidis</i>	Colony formation on Luria Bertani agar	>6.6	>7.3
<i>Staphylococcus aureus</i>	Colony formation on Luria Bertani agar	6.6	-
<i>Streptococcus pyogenes</i>	Colony formation on trypticase soy agar with 5% sheep blood	>6.8	-
<i>Listeria monocytogenes</i>	Colony formation on trypticase soy agar with 5% sheep blood	>6.3	-
<i>Corynebacterium minutissimum</i>	Colony formation on trypticase soy agar with 5% sheep blood	>6.3	-
<i>Treponema pallidum</i> (syphilis)	Infectivity in New Zealand white rabbits	≥6.8 to ≤7.0	>5.9

a. Log reduction is calculated as log (pre-treatment titer ÷ post-treatment titer), where titer is expressed as 10⁶ organisms/mL.
 b. Studies were performed using standard size (approximately 300 mL) plasma units.
 c. Inherent low-level background in non-infected cells indicator cells precludes ">" for HTLV in platelets.
 d. "-" Indicates inactivation data not available. Since bacteria are of minimal concern in fresh frozen plasma, inactivation of only a few representative organisms was evaluated in this product.
 e. Results of feasibility study.

Figure 1: Mechanism of Action

The mechanism of action for inactivation of pathogens in platelets and plasma consists of amotosalen HCl, a psoralen molecule, and illumination with 3.0 J/cm² ultraviolet A (UVA) light treatment. The amotosalen compound penetrates cellular and nuclear membranes and intercalates into the helical regions of DNA and RNA. Covalent crosslinks to the nucleic acid base pairs form upon exposure to UVA light, blocking DNA and RNA replication. This process inactivates white blood cells and pathogens, rendering them unable to cause disease, while retaining the function of plasma and platelets, which do not require nucleic acid replication for therapeutic efficacy.

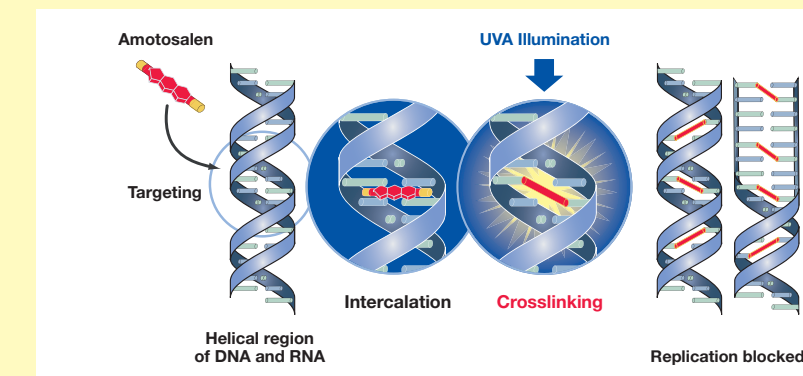


Figure 2: INTERCEPT Blood System for Platelets

The collected platelets are sterile docked to the container set (1-4) for processing. After addition of amotosalen HCl (1) by gravity flow, and removal of the platelet and amotosalen HCl containers, the platelets are illuminated with UVA light (2). Residual amotosalen and its photoproducts are reduced to low levels using a compound adsorption device (CAD) (3), before transfer to the storage container (4).

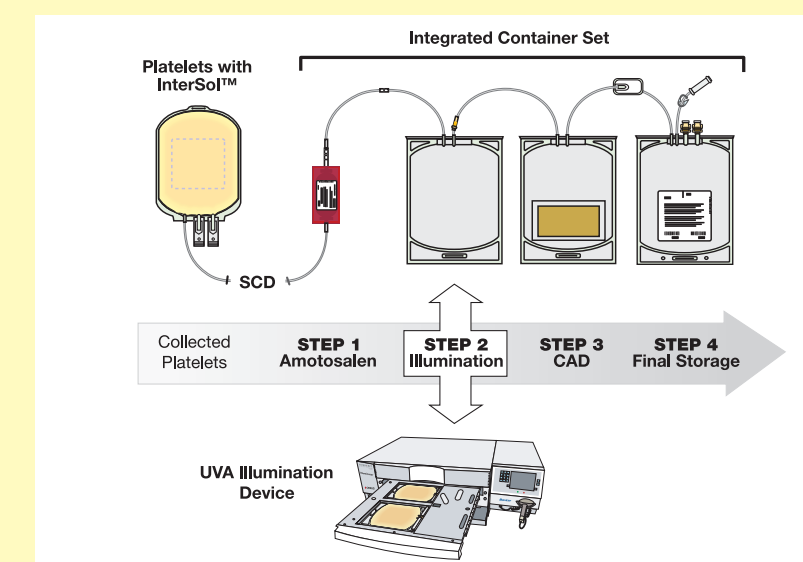
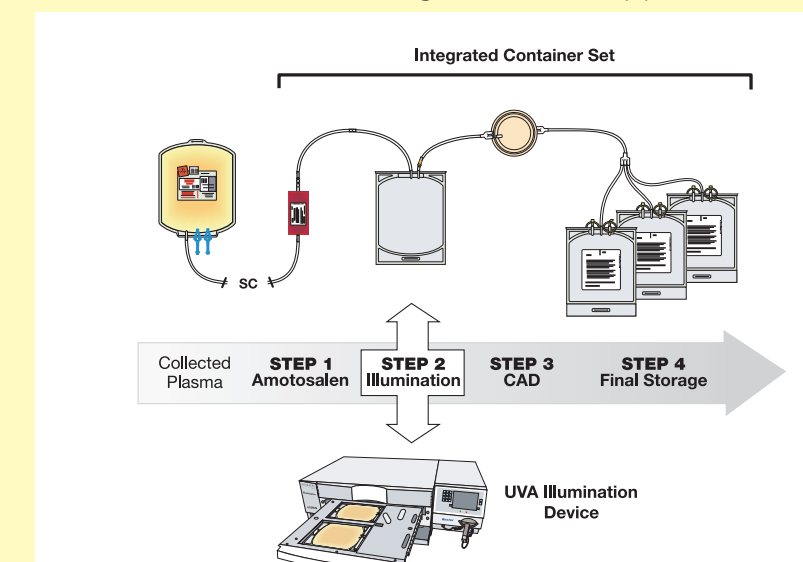


Figure 3: INTERCEPT Blood System for Plasma

The collected plasma is sterile docked to the container set (1-4) for processing. After addition of amotosalen (1) by gravity flow, and removal of the plasma and amotosalen containers, the plasma is illuminated with UVA light (2). Residual amotosalen and its photoproducts are reduced to low levels using a compound adsorption device (CAD) (3), before transfer to the storage containers (4).



Methods

Plasma units or platelet concentrates suspended in 35% plasma and 65% InterSol™ (platelet additive solution) were inoculated with the applicable organism to a final concentration of approximately 10⁶ infectious organisms/mL whenever possible. In all cases, the inoculum was 10% or less of the final blood product volume. Pathogens were inoculated as cell free organisms. For most studies presented here jumbo apheresis plasma units were pooled as necessary (pooled units were ABO-matched) to create units of approximately 585 mL. For the *T. cruzi* study ABO-matched, pooled, whole blood-derived plasma was used. Full-sized apheresis platelet units were used for most inactivation studies using platelet concentrates.

In a few studies limited infectious inoculum necessitated the use of one-tenth volume, mini-units of platelet concentrates and the chikungunya study utilized 3 mL volumes of platelets. Inoculated blood products were treated with 150 µM amotosalen and 3.0 J/cm² UVA treatment. Samples taken after addition of organism, but prior to treatment, were used to determine input titer. These samples were serially diluted in assay medium or PBS prior to inoculation of the assay system for viability detection (Table 2). Samples taken after treatment were tested undilute when possible; in some cases dilution up to 1:10 was required to prevent plasma or platelet toxicity in the detection system. Inactivation was expressed as log reduction.

Results

The studies reported in Table 2 evaluated the efficacy of the INTERCEPT Blood System for inactivation of a number of pathogens of emerging concern in the blood supply, including viruses, parasites and a spirochete bacterium.

- pfu/mL = plaque forming units per mL
ID₅₀/mL = 50% infectious dose per mL (in animals or culture)
iRBC/mL = infected red blood cells per mL
- When no residual organism was detected in any replicate or when input titers didn't vary, or when fewer than 3 replicates were performed no standard deviation (SD) was calculated.
- Log reduction is calculated as log (pre-treatment titer ÷ post-treatment titer), where titer is expressed as 10⁶ organisms/mL.
- Pinna, et al., 2005
- Results of feasibility study.
- Van Voorhis, et al., 2003
- Eastman, et al., 2005

References

Eastman R, Barrett L, Dupuis K, Buckner R, Van Voorhis W. 2005. Leishmania inactivation in human pheresis platelets by a psoralen (amotosalen HCl) and long-wavelength ultraviolet irradiation. *Transfusion*. 45:1459-1463.
 Lin L, Dikeman R, Molini B, Lukehart S, Lane R, Dupuis K, Metzger P, Corash L. 2004. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion*. 44:1496-1504.
 Lin L, Hanson C, Alter H, Jauvin V, Bernard K, Murthy K, Metzger P, Corash L. 2005. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. *Transfusion*. 45:580-590.
 Pinna D, Sampson-Johannes A, Clementi M, Poli G, Rossini S, Lin L, Vicenzi E. 2005. Amotosalen photochemical inactivation of severe acute respiratory syndrome coronavirus in human platelet concentrates. *Transfusion Medicine*. 15:269-276.
 Singh Y, Sawyer L, Pinkoski L, Dupuis K, Hsu J, Lin L, Corash L. 2006. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. *Transfusion*. 46:1198-1217.
 Van Voorhis W, Barrett L, Eastman R, Alfonso R, Dupuis K. 2003. Trypanosoma cruzi inactivation in human platelet concentrates and plasma by a psoralen (amotosalen HCl) and long-wavelength UV. *Antimicrobial Agents and Chemotherapy*. 47:475-479.

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Conclusions

- High titers of a variety of viruses, bacteria and parasites of emerging concern in the blood supply were inactivated in platelets and plasma by photochemical treatment with 150 µM amotosalen and 3.0 J/cm² UVA light.
- The ability to inactivate high titers of these emerging and reemerging pathogens, along with the clinical efficacy of treated platelets and plasma as demonstrated in commercial use and clinical studies, make the implementation of the INTERCEPT Blood System an effective defense strategy against future pathogens in the blood supply, even before their presence may be recognized.

Table 2: Inactivation of Pathogens of Emerging Concern in the Blood Supply by 150 µM Amotosalen and 3.0 J/cm² UVA Treatment

Organism	Method of Detection	Blood Product	N	Log Input Titer ^a (Mean ±SD) ^b	Log Post-Treatment Titer (Mean ±SD)	Log Reduction ^c (Mean ±SD)
West Nile Virus	Plaque in vero cells	Platelets	4	5.7 ±0.4 pfu/mL	<-0.3 pfu/mL	>6.0 ±0.4
		Plasma	4	6.7 ±0.3 pfu/mL	≤-0.1 ±0.4 pfu/mL	≥6.8 ±0.5
SARS-CoV	Plaque in vero E6 cells	Platelets	7	4.9 ±0.5 pfu/mL	<-1.2 ±0.6 pfu/mL	>6.2 ±0.7 ^d
		Plasma	4	4.0 ±0.1 pfu/mL	≤-1.5 pfu/mL	≥5.5 ±0.1
Vaccinia Virus (smallpox model)	Plaque in vero 76 cells	Platelets	3	5.4 ±0.2 pfu/mL	<0.2 pfu/mL	>5.2 ±0.2
Chikungunya virus	Plaque in BHK cells	Platelets	1	7.1 pfu/mL	<1.8 pfu/mL	>5.3 ^e
Lymphocytic choriomeningitis virus (LCMV)	Plaque in vero cells	Plasma	2	4.3 pfu/mL	<-1.3 pfu/mL	>5.6 ^e
<i>Borrelia burgdorferi</i> (Lyme disease)	Growth in BSK-H medium	Platelets	4	≥7.1 ±0.2 ID ₅₀ /mL	<0.3 ID ₅₀ /mL	>6.8 ±0.2
		Plasma	4	≥9.9 ±2.3 ID ₅₀ /mL	<-0.7 ID ₅₀ /mL	>10.6 ±2.3
<i>Plasmodium falciparum</i> (malaria)	Infectivity in RBC	Platelets	4	5.6 ±0.05 iRBC/mL	≤-0.5 ±0.06 iRBC/mL	≥6.0 ±0.2
		Plasma	4	5.9 ±0 iRBC/mL	≤-1.0 ±0.3 iRBC/mL	≥6.9 ±0.3
<i>Trypanozoma cruzi</i> (Chagas' disease)	Growth on 3T3 cells	Platelets	4	6.2 ±0.1 ID ₅₀ /mL	≤0.8 ±0.2 ID ₅₀ /mL	≥5.4 ±0.1 ^f
		Plasma	4	6.3 ±0.6 ID ₅₀ /mL	<1.3 ±0.5 ID ₅₀ /mL	>5.0 ±0.6 ^f
<i>Babesia microti</i> (babesiosis)	Mouse infectivity	Platelets	2	4.9 ID ₅₀ /mL	<-0.5 ID ₅₀ /mL	>5.3
		Plasma	4	4.9 ±0.4 ID ₅₀ /mL	<-0.5 ±0.02 ID ₅₀ /mL	>5.3 ±0.4
<i>Leishmania mexicana</i> (Leishmaniasis)	Mouse infectivity	Platelets	4	6.3 ±0.7 ID ₅₀ /mL	<1.3 ID ₅₀ /mL	>5.0 ±0.5 ^g