

Fig. 1. Gel electrophoresis demonstrating fibrinogen patterns in riboflavin-treated and untreated plasma. The gels show riboflavin-treated plasma samples versus untreated and human plasma fibrinogen with saline as a control on 4% to 20% Tris-HCl native gel. Lane 1 = fibrinogen 3 µg with saline; Lane 2 = fibrinogen 3 µg with riboflavin; Lane 3 = fibrinogen 4 µg with saline; Lane 4 = fibrinogen 4 µg with riboflavin; Lane 5 = pretreated plasma with saline, Unit 1 (2 µL); Lane 6 = posttreated plasma with riboflavin, Unit 1 (2 µL); Lane 7 = pretreated plasma with saline, Unit 1 (4 µL); Lane 8 = posttreated plasma with riboflavin, Unit 1 (4 µL); Lane 9 = pretreated plasma with saline, Unit 2 (2 µL); Lane 10 = posttreated plasma with riboflavin, Unit 2 (2 µL); Lane 11 = pretreated plasma with saline, Unit 2 (4 µL); Lane 12 = posttreated plasma with riboflavin, Unit 2 (4 µL); Lane 13 = pretreated plasma with saline, Unit 3 (2 µL); Lane 14 = posttreated plasma with riboflavin, Unit 3 (2 µL); Lane 15 = pretreated plasma with saline, Unit 3 (4 µL); Lane 16 = posttreated plasma with riboflavin, Unit 3 (4 µL).

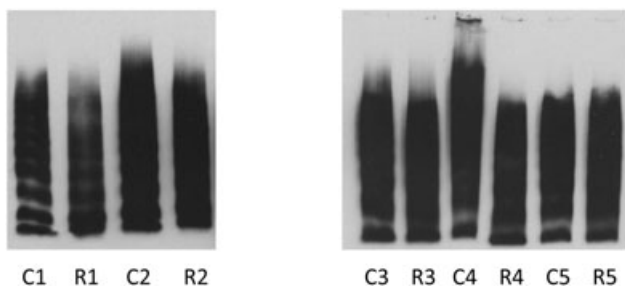


Fig. 2. VWF multimer analysis of individual samples before and after riboflavin and UV light treatment. VWF multimer patterns of untreated (C) and riboflavin-treated (R) plasma samples. Results from five paired sets are shown.

by 30%; and protein S and α_2 -antiplasmin are decreased by more than 40%.^{6,23,24}

According to various studies, riboflavin and UV treatment significantly reduces infectious levels of viruses, bacteria, and parasites in treated FFP.^{11,13,14,19,31,32} This study addresses the protein quality of apheresis-derived plasma treated with this process.

FVIIIc, being one of the most labile proteins in plasma, is usually the most affected by the various pathogen inactivation processes. Riboflavin and UV light-treated plasma also showed reduced levels of FVIIIc (75% retention). In comparison, MB-treated plasma has been reported to show 67% retention of FVIIIc after treatment.^{7,26}

Other factors were generally well preserved in riboflavin and UV light-treated plasma. The total amount of protein is fully retained; FXII, FX, FII, and F IX retained 96,

80, and 75% activity, respectively. However, FXI was sensitive to the treatment and retained only 67% activity.

These values are comparable to the data obtained by Hornsey and colleagues³³ from whole blood-derived plasma treated with riboflavin and UV light.

The fibrinogen antigen assay showed 99% retention, which was confirmed by PAGE results and quantitation of the bands. However, the fibrinogen activity was affected more by the treatment than the antigen itself. Additional studies are in progress to characterize the functional activity of the protein. In any case, the riboflavin-treated plasma showed higher retention of fibrinogen activity (77%) compared to MB, which has demonstrated retention of 64% to 69% for fibrinogen activity after treatment.²⁶⁻²⁸ Further, the pre- and posttreatment gels show similar bands indicating that there was no structural alteration of the protein as has been reported when MB is used.³⁴

ADAMTS-13, the VWF-cleaving metalloprotease cleaves the ultralarge multimers of VWF, which form PLT thrombi in patients with thrombotic thrombocytopenic purpura.^{35,36} It is considered that the replacement plasma used to treat thrombotic thrombocytopenic purpura should contain adequate levels of ADAMTS-13 activity to ensure multimer breakdown. Plasma treated with either MB or S/D retains normal levels of ADAMTS-13 activity.³⁶ Similar results were obtained after riboflavin and UV light treatment (Table 1). It is noted that, in this study, the plasma samples were all tested after a relatively short period of storage; however, the data from Bihm and colleagues³⁷ examined riboflavin-treated plasma stored for 1 and 2 years and showed similar results.

There is a tight association between ABO blood groups and the level of VWF. VWF levels are much lower in O group individuals due to the higher rate of proteolysis and in a rank of order for other groups: O < B < A < AB.³⁸ Results from this study also show the dependence of the amount of VWF on ABO groups. There were seven individuals of a total of 20 in the study who were group O, and five of these seven had fewer HMW VWF multimers. All nine individuals with group A and two individuals with group B had normal patterns of VWF multimers. However, among the two individuals with group AB, one had normal VWF multimers, as expected, and the other one had a reduction of the HMW forms before treatment. After the riboflavin and UV treatment, 13 of the 20 plasma samples had lower amounts of the HMW multimers in comparison with controls. The reason for the variable loss of some HMW multimers after treatment is not known but may be due to different susceptibilities based on glycosylation of the VWF. The complete loss of HMW forms has been reported for the Vitex solvent detergent plasma (Raritan, NJ).³⁹

Inhibitors of coagulation and plasminogen and markers of activation of fibrinolytic proteins and thrombin activation were well preserved in riboflavin and UV light-treated apheresis plasma. Antithrombin, α_2 -antiplasmin, and protein S retained 91% or more activity. These proteins have been reported to be significantly reduced in S/D-treated plasma.²⁴ Protein C was more sensitive to the treatment and retained 81% activity.

In conclusion, the percentage of protein retention of the proteins in apheresis-derived plasma after riboflavin and UV light treatment compares favorably to those reported with other pathogen inactivation methods for plasma presently in clinical use.⁴⁰⁻⁴²

ACKNOWLEDGMENTS

The authors thank Jennie Klein, MT(ASCP), and Hossein Sowdagar, HT(ASCP), OBI Laboratory, for technical assistance.

REFERENCES

- Grant IH, Gold JW, Wittner M, Tanowitz HB, Nathan C, Mayer K, Reich L, Wollner N, Steinherz L, Ghavimi F, O'Reilly RJ, Armstrong D. Transfusion-associated acute Chagas disease acquired in the United States. *Ann Intern Med* 1989;111:849-51.
- Leiby DA, Lenes BA, Tibbals MA, Tames-Olmedo MT. Prospective evaluation of a patient with *Trypanosoma cruzi* infection transmitted by transfusion. *New Engl J Med* 1999; 341:1237-9.
- Wu CG, Mason B, Jong J Erdman D, McKernan L, Oakley M, Soucie M, Evatt B, Yu MY. Parvovirus B19 transmission by a high-purity Factor VIII concentrate. *Transfusion* 2005; 45:1003-10.
- Cangelosi JJ, Sarria JC, Herwaldt BL, Indrikovs AJ. Transmission of *Babesia microti* by blood transfusion in Texas. *Vox Sang* 2008;95:331-4.
- Marques A, Torres S, Davis JM. The current infectious risks of transfusions. *Surg Infect* 2005;6:S23-31.
- Hellstern P. Solvent/detergent-treated plasma: composition, efficacy, and safety. *Curr Opin Hem* 2004;11:346-50.
- Williamson LM, Cardigan R, Prowse CV. Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? *Transfusion* 2003;43:1322-39.
- Corash L, Lin L. Novel processes for inactivation of leukocytes to prevent transfusion-associated graft-versus-host disease. *Bone Marrow Transplant* 2004;33:1-7.
- Lin L, Dikeman R, Molini B, Lukehart SA, Lane R, Dupuis K, Metzel P, Corash L. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion* 2004;44:1496-504.
- Lin L, Hanson CV, Alter HJ, Jauvin V, Bernard KA, Murthy KK, Metzel P, Corash L. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. *Transfusion* 2005;45: 580-90.
- Cardo LJ, Rentas FJ, Ketchum L, Salata J, Harman R, Melvin W, Weina PJ, Mendez J, Reddy H, Goodrich R. Pathogen inactivation of *Leishmania donovani* infantum in plasma and platelet concentrates using riboflavin and ultraviolet light. *Vox Sang* 2006;90:85-91.
- Fast LD, DiLeone G, Li J, Goodrich R. Functional inactivation of white blood cells by Mirasol treatment. *Transfusion* 2006;46:642-8.
- Rentas F, Harman R, Gomez C, Salata J, Childs J, Silva T, Lippert L, Montgomery J, Richards A, Chan C, Jiang J, Reddy H, Li J, Goodrich R. Inactivation of *Orientia tsutsugamushi* in red blood cells, plasma and platelets with riboflavin and light, as demonstrated in an animal model. *Transfusion* 2007;47:240-7.
- Ruane PH, Edrich R, Gampp D, Keil SD, Leonard L, Goodrich RP. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. *Transfusion* 2004;44:877-85.
- Zempleni J, Galloway JR, McCormick DB. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* 1996;63:54-66.
- Unna K, Greslin JG. Studies on the toxicity and pharmacology of riboflavin. *J Pharmacol* 1942;76:75-80.
- Leclerc J. Influence of thiamine, riboflavin and vitamin B6 content in food on tissue content of these vitamins in the female lactating rat and young rats. *Ann Nutr Aliment* 1974;28:11-20.
- Kumar V, Lockerbie O, Keil SD, Ruane P, Platz M, Martin C. Riboflavin and UV-light based pathogen reduction: extent and consequence of DNA damage at the molecular level. *Photochem Photobiol* 2004;80:15-21.
- Goodrich RP, Edrich RA, Li J, Seghatchian J. The Mirasol

- PRT system for pathogen reduction of platelets and plasma: an overview of current status and future trends. *Transfus Apher Sci* 2006;35:5-17.
20. Ruggeri ZM, Zimmerman TS. The complex multimeric structure composition of factor VIII/von Willebrand factor. *Blood* 1981;57:1140-3.
 21. Snider CE, Moore JC, Warkentin TE, Finch CN, Hayward CP, Kelton JG. Dissociation between the level of von Willebrand factor-cleaving protease activity and disease in a patient with congenital thrombotic thrombocytopenic purpura. *Am J Hematol* 2004;77:387-90.
 22. Tsai H, Chandler W, Sarode R, Hoffman R, Jelacic S, Habeeb RL, Watkins SL, Wong CS, Williams GD, Tarr PI. Von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in escherichia coli 0157:H7-associated hemolytic uremic syndrome. *Pediatr Res* 2001;49:5.
 23. Wieding JU, Hellstern P, Köhler M. Inactivation of viruses in fresh-frozen plasma. *Ann Hematol* 1993;67:259-66.
 24. Doyle S, O'Brien P, Murphy K, Fleming C, O'Donnell J. Coagulation factor content of solvent/detergent plasma compared with fresh frozen plasma. *Blood Coagul Fibrinolysis* 2003;14:283-7.
 25. Cardigan R, Philpot K, Cookson P, Luddington R. Thrombin generation and clot formation in methylene blue-treated plasma and cryoprecipitate. *Transfusion* 2009;49:696-703.
 26. Zeiler T, Riess H, Wittmann G, Zimmermann R, Müller C, Heuft HG, Huhn D. The effect of methylene blue phototreatment on plasma proteins and in vitro coagulation capability of single-donor fresh-frozen plasma. *Transfusion* 1994;34:685-9.
 27. Depasse F, Sensebe L, Seghatchian J, Andreu G, Samama MM. The influence of methylene blue light treatment and methylene blue removal filter on fibrinogen activity states and fibrin polymerization indices. *Trans Apher Sci* 2005;33:63-9.
 28. Suotaka AM, Blomback M, Chapman J. Changes in functional activities of plasma fibrinogen after treatment with methylene blue and red light. *Transfusion* 2003;43:568-75.
 29. De Valensart N, Rapaille A, Goossenaerts E, Irsch J, Lin L, Deneys V. Analysis of the impact of INTERCEPT treatment on fresh plasma: preservation of coagulation factors and inhibitors functional activity. *Vox Sang* 2008;95 Suppl 1:31.
 30. Alvarez-Larrán A, Del Rio J, Ramirez C, Albo C, Peña F, Campos A, Cid J, Muncunill J, Sastre JL, Sanz C, Pereira A. Methylene blue-photoinactivated plasma vs. fresh-frozen plasma as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. *Vox Sang* 2004;86:246-51.
 31. Goodrich L, Ghielli M, Hansen E, Woolum M, Gampp D, Goodrich R, Keil S. Riboflavin and UV light inactivate enveloped and non-enveloped viruses in platelet concentrates under conditions which maintain cell quality. *Vox Sang* 2002;83:111.
 32. Cardo LJ, Salata J, Mendez J, Reddy H, Goodrich R. Pathogen inactivation of Trypanosome cruzi in plasma and platelet concentrates using riboflavin and ultraviolet light. *Transfus Apher Sci* 2007;37:131-7.
 33. Hornsey VS, Drummond O, Morrison A, McMillan L, MacGregor IR, Prowse CV. Pathogen reduction of fresh plasma using riboflavin and ultraviolet light: effects on plasma coagulant proteins. *Transfusion*. 2009;49:2167-72.
 34. Crettaz D, L Sendebe L, Vu DH, Schneider P, Depasse F, Bienvenut WV, Quadroni M, Tissot JD. Proteomics of methylene blue photo-treated plasma before and after removal of the dye by an absorbent filter. *Proteomics* 2004;4:881-91.
 35. Levy GG, Motto DG, Ginsburg D. ADAMTS13 turns 3. *Blood* 2005;106:11-7.
 36. Yarranton H, Lawrie AS, Purdy G, Mackie JJ, Machin SJ. Comparison of von Willebrand factor antigen, von Willebrand factor-cleaving protease and protein S in blood components used for treatment of thrombotic thrombocytopenic purpura. *Trans Med* 2004;14:39-44.
 37. Bihm DJ, Ettinger A, Buyaert-Hoefen KA, Hendrix BK, Maldonado-Codina G, Rock G, Giclas PC, Goodrich RP. Characterization of plasma protein activity in riboflavin and UV light-treated fresh frozen plasma during two years of storage at -30°C. *Vox Sang In press* 2009.
 38. Francini M, Capra F, Targher G, Montagnana M, Lippi G. Relationship between ABO group and von Willebrand factor levels: from biology to clinical implications. *Thromb J* 2007;5:14-8.
 39. Moake J, Chintagumpala M, Turner N, McPherson P, Nolasco L, Steuber C, Santiago-Borrero P, Horowitz M, Pehta J. Solvent/detergent-treated plasma suppresses shear-induced platelet aggregation and prevents episodes of thrombotic thrombocytopenic purpura. *Blood* 1994;84:490-7.
 40. Aznar JA, Bonanad S, Montoro JM, Hurtado C, Cid AR, Soler MA, De Miguel A. Influence of Methylene blue photoinactivation treatment on coagulation factors from fresh frozen plasma, cryoprecipitate and cryosupernatants. *Vox Sang* 2000;7:156-60.
 41. Mintz PD, Neff A, MacKenzie M, Goodnough LT, Hillyer C, Kessler C, McCrae K, Menitove JE, Skikne BS, Damon L, Lopez-Plaza I, Rouault C, Crookston KP, Benjamin RJ, George J, Lin JS, Corash L, Conlan MG. A randomized, controlled Phase III trial of therapeutic plasma exchange with fresh frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. *Transfusion* 2006;46:1693-704.
 42. Evans G, Llewelyn C, Luddington R, Baglin TP, Williamson LM. Solvent/detergent fresh frozen plasma as primary treatment of acute thrombotic thrombocytopenic purpura. *Clin Lab Haem* 1999;21:119-23. ▀

Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function

Yasmin Singh, Lynette S. Sawyer, Linda S. Pinkoski, Kent W. Dupuis, Jocelyn C. Hsu, Lily Lin, and Laurence Corash

BACKGROUND: The INTERCEPT Blood System, a photochemical treatment (PCT) process, has been developed to inactivate pathogens in platelet concentrates. These studies evaluated the efficacy of PCT to inactivate pathogens in plasma and the effect of PCT on plasma function.

STUDY DESIGN AND METHODS: Jumbo (600 mL) plasma units were inoculated with high titers of test pathogens and treated with 150 μmol per L amotosalen and 3 J per cm^2 long-wavelength ultraviolet light. The viability of each pathogen before and after treatment was measured with biological assays. Plasma function was evaluated through measurement of coagulation factors and antithrombotic protein activities.

RESULTS: The levels of inactivation expressed as log-reduction were as follows: cell-free human immunodeficiency virus-1 (HIV-1), greater than 6.8; cell-associated HIV-1, greater than 6.4; human T-lymphotropic virus-I (HTLV-I), 4.5; HTLV-II, greater than 5.7; hepatitis B virus (HBV) and hepatitis C virus, greater than 4.5; duck HBV, 4.4 to 4.5; bovine viral diarrhea virus, 6.0; severe acute respiratory syndrome coronavirus, 5.5; West Nile virus, 6.8; bluetongue virus, 5.1; human adenovirus 5, 6.8; *Klebsiella pneumoniae*, greater than 7.4; *Staphylococcus epidermidis* and *Yersinia enterocolitica*, greater than 7.3; *Treponema pallidum*, greater than 5.9; *Borrelia burgdorferi*, greater than 10.6; *Plasmodium falciparum*, 6.9; *Trypanosoma cruzi*, greater than 5.0; and *Babesia microti*, greater than 5.3. Retention of coagulation factor activity after PCT was expressed as the proportion of pretreatment (baseline) activity. Retention was 72 to 73 percent of baseline fibrinogen and Factor (F)VIII activity and 78 to 98 percent for FII, FV, FVII, F IX, FX, FXI, FXIII, protein C, protein S, antithrombin, and α_2 -antiplasmin.

CONCLUSION: PCT of plasma inactivated high levels of a wide range of pathogens while maintaining adequate coagulation function. PCT has the potential to reduce the risk of transfusion-transmitted diseases in patients requiring plasma transfusion support.

Approximately 3.3 million units of fresh-frozen plasma (FFP) and 816,000 units of cryoprecipitate are transfused annually in the United States.¹ FFP is indicated for treatment of congenital and acquired coagulation factor deficiencies, coagulopathy resulting from liver disease, massive blood loss, and thrombotic thrombocytopenia purpura. In addition, FFP may be used to prepare cryoprecipitate for fibrinogen replacement and treatment of von Willebrand's disease. Typical therapeutic use of FFP for correction of coagulopathy requires transfusion of approximately 10 to 20 mL per kg FFP per transfusion episode, necessitating exposure to multiple donors.² Plasma exchange therapy for thrombotic thrombocytopenia purpura patients may require repeated large volume FFP transfusions with even greater donor exposures.

ABBREVIATIONS: APTT = activated partial thromboplastin time; BVDV = bovine viral diarrhea virus; CAD = compound adsorption device; DHBV = duck hepatitis B virus; FACT = factor assay control plasma; PC = protein C; PCT = photochemical treatment; PS = protein S; PT = prothrombin time; SARS-CoV = severe acute respiratory syndrome coronavirus; UVA = long-wavelength ultraviolet (light); VWF:RCo = von Willebrand factor:ristocetin cofactor; WNV = West Nile virus.

From Cerus Corp., Concord, California.

Address reprint requests to: Yasmin Singh, PhD, Cerus Corp., 2411 Stanwell Drive, Concord, CA 94520; e-mail: yasmin_singh@cerus.com.

These studies were supported in part by Cerus Corp., Baxter Healthcare Corp., and Grants DAMD17-01-20007, DAMD17-02-20042, and DAMD17-03-20039.

The U.S. Army Medical Research Acquisition Activity (Fort Detrick, MD) is the awarding and administering acquisition office for Grants DAMD17-01-20007, DAMD17-02-20042, and DAMD17-03-20039. The contents of this report do not necessarily reflect the position or policy of the government and no official endorsement is implied by this award.

Received for publication September 2, 2005; revision received November 7, 2005, and accepted November 13, 2005.

doi: 10.1111/j.1537-2995.2006.00867.x

TRANSFUSION 2006;46:1168-1177.

Donor screening and postdonation testing have greatly reduced the risk of transfusion-transmitted diseases in patients requiring transfusion of blood products. Residual risk persists, however, because only a limited number of pathogens are routinely screened,³ new blood-borne organisms continue to emerge,⁴ and in many cases, the diagnostic tests available are insufficiently sensitive to detect low-level contaminants and infections in the "window period."^{5,6} Even though quarantine plasma could potentially eliminate the window period by repeated donor testing within 4 to 6 months, the safety of this approach still depends on test sensitivity. Furthermore, testing remains a reactive approach to blood safety. The contaminating organisms must be identified before screening tests can be developed.

In contrast, pathogen inactivation is a proactive approach to blood safety. Since the 1990s, the transfusion community has become more receptive to this new approach provided that the method is efficacious, safe, easy to implement, and cost-effective. For plasma, two inactivation technologies, methylene blue treatment^{7,8} and solvent/detergent (S/D) treatment,⁹ have received regulatory approval and are currently in clinical use in several countries in Europe. Neither of these methods, however, has a broad range of effectiveness against pathogens, because S/D treatment only inactivates lipid-enveloped viruses,¹⁰ and methylene blue is ineffective against intracellular viruses.¹¹ Furthermore, S/D treatment has been shown to compromise some of the *in vitro* coagulation function of plasma and is contraindicated by the FDA for patients undergoing liver transplant.¹²

The INTERCEPT Blood System for platelets (PLTs) is the only pathogen inactivation technology for blood cellular components that is CE Marked and in clinical use in several countries in Europe.¹³ The INTERCEPT Blood System is a photochemical treatment (PCT) process that utilizes amotosalen (also known as S-59) and long-wavelength ultraviolet(UVA; 320-400 nm) light to permanently cross-link helical regions of DNA and RNA.¹⁴ PCT has been shown to inactivate a broad range of viruses,¹⁵⁻¹⁷ bacteria,¹⁸ and protozoa,^{19,20} as well as white blood cells (WBCs)²¹ in PLT concentrates. Because plasma has similar optical properties to PLT concentrate, it is expected that the PCT process developed for use with PLTs is applicable to plasma; thus supporting the synergy of use of the same PCT technology for two blood components in a blood center. These studies evaluated the efficacy of PCT of plasma for the preparation of pathogen inactivated fresh-frozen plasma (FFP).

MATERIALS AND METHODS

Plasma collection

Apheresis plasma (approx. 600 ± 25 mL) was collected from volunteer donors by plasmapheresis on an auto-

mated plasmapheresis machine (Haemonetics PCS 2, Haemonetics Corp., Braintree, MA; or Autopheresis-C, Baxter Healthcare Corp., Deerfield, IL) with sodium citrate anticoagulant. For the pathogen inactivation studies, apheresis FFP units were stored frozen (not greater than -18°C) before use. For the plasma function studies, apheresis plasma units were transported at room temperature and processed within 8 hours of collection.

PCT disposable sets and UVA illumination device

The PCT disposable set for treatment of plasma (Baxter Healthcare Corp.) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen hydrochloride solution in saline packaged inside a PL 2411 plastic container and protected from UVA light; a 1.3-L PL 2410 plastic container for illumination of plasma; a compound adsorption device (CAD) to reduce the concentration of amotosalen and its photoproducts, which consisted of an adsorbent disk composed of a copolymer of polystyrene and divinylbenzene particles fused with an ultrahigh-molecular-weight polyethylene plastic enclosed in an acrylic housing; and three 400-mL PL 269 plastic containers for storage of the treated plasma.

Plasma was treated by passing through each component in a series of steps. In Step 1, the plasma unit was sterile connected to the amotosalen container and the plasma content passed through the amotosalen container into the illumination container. In Step 2, the plasma containing amotosalen was illuminated with UVA light. In Step 3, the illuminated plasma mixture was passed through the CAD by gravity into the storage containers (Step 4; Fig. 1). The residual amotosalen remaining in plasma after the CAD step is $1.5 \mu\text{mol per L}$ or less on average, which is consistently achieved.

Illumination of plasma was performed in a UV illumination system (Baxter Model R4R4007, Nova Biomedical, Waltham, MA). The device was capable of illuminating 2 units of plasma per processing cycle. The illuminator delivered a 3 J per cm^2 UVA treatment to each plasma unit in approximately 7 to 9 minutes. During illumination plasma units were reciprocally agitated at approximately 70 cycles per minute.

Inactivation procedures and viability assays for pathogens

Before addition of amotosalen, plasma units of approximately 585 mL (unless otherwise specified) were inoculated with the applicable pathogen to a final concentration of approximately 10^6 infectious organisms per mL whenever possible. In all cases, the inoculum volume consisted of not greater than 10 percent of the final plasma volume. Table 1 summarizes the strain and the supplier of each pathogen evaluated in these studies.

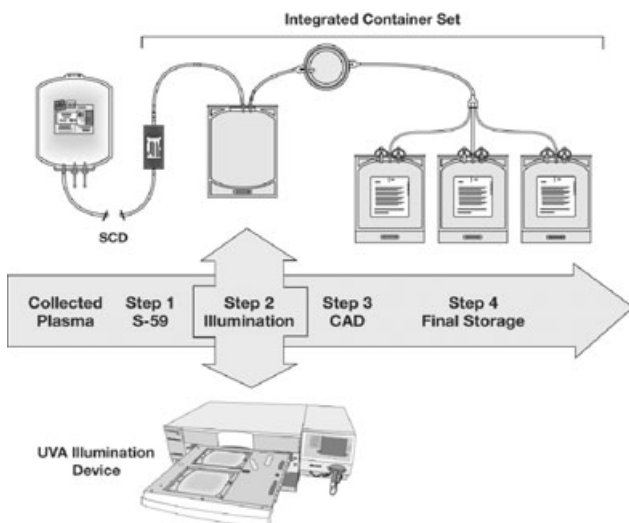


Fig. 1. The PCT system for plasma. The PCT system consists of a UVA illumination device and an integral disposable set. The device can illuminate 2 units of plasma per processing cycle. The disposable set provides a single-use, closed, integrated system for pathogen inactivation treatment of a plasma unit. The integrated disposable set is composed of the following sterile components: an amotosalen (S-59) container, a plastic illumination container, a CAD, and three plastic storage containers. The processing steps are as described under Materials and Methods section. SCD = sterile connection device.

Inoculated plasma units were then treated with 150 μmol per L amotosalen and a 3 J per cm^2 UVA treatment. Samples taken after addition of organism, but before addition of amotosalen, were used to determine the pre-PCT input titer. These samples were serially diluted in assay medium or phosphate-buffered saline before viability measurement. The post-PCT samples were taken immediately after UVA illumination. The CAD step was not performed in the pathogen inactivation studies so that measurement of log reduction was a result of illumination of plasma treated with amotosalen only and not affected by potential pathogen affinity for the CAD. The post-PCT samples were assayed for viable organisms undiluted when possible. In some cases, dilution up to 1:10 was required to prevent toxicity of plasma in the culture system. Table 1 also summarizes the biological assays used for quantifying the number of viable organisms. Detailed assay procedures are as described in the respective references. Pre- and post-PCT titers were quantified with standard assays. The Reed-Muench method was used where end-dilutions were required for quantification.²² Four replicate experiments (unless otherwise specified) for each organism were performed with four independent units (or pools) of plasma.

The level of inactivation was calculated as log-reduction with the formula

$$\text{Log-reduction} = \text{Log}(\text{pre-PCT titer}/\text{post-PCT titer}).$$

TABLE 1. The origin of the test pathogens and established biological methods used to detect and quantify viability

Organism	Strain	Origin (supplier)	Method of detection
HIV-1, cell-free	IIIB	Chronically infected H9 cells (gift from C.V. Hanson)	Microplaque in MT-2 cells ⁴²
HIV-1, cell-associated	IIIB	Chronically infected H9 cells (gift from C.V. Hanson)	Micro-plaque in MT-2 cells ⁴²
HTLV-I	2060	California Department of Health Services, Richmond, CA	β -Galactosidase production by infected pA18GBHK-21 cells ⁴³
HTLV-II	C-19	California Department of Health Services, Richmond, CA	β -Galactosidase production by infected pA18GBHK-21 cells ⁴³
HBV	MS-2	NIH repository, Bethesda, MD	Infectivity in chimpanzee ⁴⁴
HCV	Hutchinson	NIH repository, Bethesda, MD	Infectivity in chimpanzee ⁴⁵
DHBV	P-type	Congenitally infected ducks, Hepadnavirus Testing Laboratories, Menlo Park, CA	Infectivity in Legarth-Pekin ducklings ⁴⁶
BVDV	NADL	ATCC, Rockville, MD	Plaque in bovine turbinate cells ¹⁶
WNV	3356	Clone lineage I, pFL-WNV (gift from K. Bernard)	Plaque in Vero cells ⁴⁷
SARS-CoV	Urbani	CDC, Atlanta, GA	Plaque in Vero-E6 cells ¹⁵
Bluetongue virus	Station	ATCC, Rockville, MD	Plaque in embryonic bovine trachea cells ¹⁶
Human adenovirus	5	Onyx Pharmaceuticals, Inc., Richmond, CA	Plaque on lung carcinoma cells (A549) ¹⁶
<i>S. epidermidis</i>	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar ¹⁸
<i>K. pneumoniae</i>	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar ¹⁸
<i>Y. enterocolitica</i>	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar ¹⁸
<i>T. pallidum</i>	Nichols	University of Washington, Seattle, WA	Infectivity in New Zealand white rabbits ¹⁸
<i>B. burgdorferi</i>	CA4	University of California, Berkeley, CA	Dark field microscopy of cultures in BSK-H medium ¹⁸
<i>P. falciparum</i>	FcB1	Max-Planck-Institute (gift from H. Heidrich)	Infectivity in RBCs ⁴⁸
<i>T. cruzi</i>	Tulahuen	University of Washington, Seattle, WA	Infectivity in 3T3 cells ¹⁹
<i>B. microti</i>	C3H/HcN adapted	Wild strain isolated from a field mouse at State University of NY, Stony Brook, NY	Infectivity in C3H/HcN mice ⁴⁹

The pre-PCT and post-PCT titers were expressed in scientific notations. The mean level of inactivation and standard deviation (SD) were determined.

PCT of fresh plasma for plasma function studies

The total target volume in the illumination container was 600 ± 25 mL, composed of 585 ± 25 mL of plasma and 15 mL of amotosalen solution. The nominal amotosalen concentration was approximately $150 \mu\text{mol}$ per L. Each unit of plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA treatment on the R4R4007 illumination device. Plasma samples for evaluation of coagulation factor activity were taken before the addition of amotosalen (baseline, pre-PCT) and after PCT including CAD treatment (post-PCT). Samples were snap-frozen and stored at or below -65°C before testing.

Measurement of in vitro coagulation function

Soluble fibrinogen (Factor [F]I) was measured with a modified Clauss assay in which the clotting time of a diluted plasma sample, after conversion by thrombin into insoluble fibrin, is compared to a standard curve prepared with reference plasma of known fibrinogen concentration. Coagulation factors were assayed with one-stage prothrombin time (PT)-based clotting assays (FII, FV, FVII, FX) or one-stage activated partial thromboplastin time (APTT)-based clotting assays (FVIII, FIX, FXI). The clotting time of a mixture of diluted test plasma sample and plasma deficient in the factor being quantified was compared with a reference curve constructed from the clotting times of five dilutions, ranging from 1:5 to 1:320, of plasma with known activity mixed with deficient plasma. These coagulation tests, as well as the PT and APTT, were performed on an automated coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co., Lexington, MA). Reagents included brain thromboplastin (Hemoliance, Instrumentation Laboratory Co.), Platelin L (bioMérieux, Durham, NC), and congenital factor-deficient substrate. The endpoint of all tests was the formation of a clot detected photooptically and measured in seconds. The level of the factor being measured was inversely proportional to the time it takes for a clot to form. Factor assay control plasma (FACT; George King Biomedical, Inc., Overland Park, KS) was used as the reference standard for the coagulation factor assays.

FXIII was measured with the research-use-only FXIII kit (Berichrom, Dade Behring, Marburg, Germany). FXIII, activated by thrombin, releases an activation product that leads to a series of reactions resulting in a decrease in nicotinamide adenine dinucleotide (NADH), detected by monitoring absorbance at 340 nm. The assay was performed on a clot timer (BCT, Dade Behring), and standard

human plasma (Dade Behring) was used as the reference standard.

The von Willebrand factor:ristocetin cofactor (VWF:RCo) activity was measured with the BC von Willebrand reagent (Dade Behring). In the assay, lyophilized PLTs are agglutinated by the VWF in the presence of ristocetin, resulting in a decrease in turbidity measured by the change in absorbance on the Behring clot timer. FACT was used as the reference standard.

Protein C (PC) and protein S (PS) were measured with kits (Sta clot PC kit and the Sta clot PS kit, respectively, both from Diagnostica Stago, Asnieres, France). PC and PS assays were based on the prolongation of the APTT resulting from inactivation of FV and FVIII by activated PC. The activator in the PC assay is an extract of *Agkistrodon c. contortrix* snake venom; the activator in the PS assay is activated PC. The tests were performed on the Behring clot timer. A unicalibrator (Diagnostica Stago) a multi-parametric calibrator, was used as the reference standard.

Antithrombin was measured with a kit (Stachrom ATIII, Diagnostica Stago). Plasma containing antithrombin was incubated with a known excess of thrombin. A chromogenic substrate, imidolyzed by the remaining thrombin, was detected photooptically on the MLA Electra 1400C or 1600C coagulation analyzer. FACT was used as the reference standard.

α 2-Antiplasmin was quantified with reagents from Diagnostica Stago. In this chromogenic method, plasmin was added in excess to the test plasma, resulting in the formation of antiplasmin-plasmin complexes. The concentration of residual plasmin is measured by its amidolytic activity on a chromogenic substrate measured at 405 nm. α 2-Antiplasmin concentration is inversely proportional to the residual plasmin concentration and is determined by color intensity. This analysis was performed by Esoterix Laboratories (Aurora, CO) with an analyzer (STA, Diagnostica Stago).

The mean and SD were determined for each coagulation parameter quantified. The activity of each coagulation parameter remaining after PCT was expressed as percent retention of the pretreatment (baseline) activity. Comparison of the PT and APTT was based on the prolongation of the clotting time after PCT relative to baseline.

RESULTS

PCT inactivation of viruses in plasma

PCT with $150 \mu\text{mol}$ per L amotosalen and a 3 J per cm^2 UVA treatment inactivated a wide variety of viruses, including enveloped, nonenveloped, DNA, and RNA viruses in plasma (Table 2). Initial viral titers (pre-PCT) of 10^4 to 10^6 infectious viruses per mL were achieved for all viruses except for hepatitis B virus (HBV) and hepatitis C virus (HCV). The highest available titers were used in all

TABLE 2. PCT inactivates enveloped and nonenveloped viruses in plasma at high initial titers*

Viruses	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
Enveloped			
HIV-1, cell-free	10 ^{6.1±0.1} PFU/mL	<10 ^{-0.8±0.02} PFU/mL	>6.8 ± 0.1
HIV-1, cell-associated	10 ^{5.9±0.2} PFU/mL	<10 ^{-0.5±0} PFU/mL	>6.4 ± 0.2
HTLV-I	10 ^{4.0±0.2} FFU/mL	≤10 ^{-0.5±0.8} FFU/mL	≥4.5 ± 0.7
HTLV-II	10 ^{4.7±0.1} FFU/mL	<10 ^{-1.0±0} FFU/mL	>5.7 ± 0.1
HBV‡§	10 ^{4.5} CID ₅₀ /unit	None detected in 250 mL	>4.5
HCV‡§	10 ^{4.5} CID ₅₀ /unit	None detected in 250 mL	>4.5
DHBV	10 ^{5.6±0.2} ID ₅₀ /mL	10 ^{1.2-10^{1.3}} ID ₅₀ /mL	4.4-4.5
BVDV	10 ^{4.5±0.03} PFU/mL	<10 ^{-1.5±0} PFU/mL	≥6.0 ± 0.03
WNV	10 ^{6.7±0.3} PFU/mL	≤10 ^{-0.1±0.2} PFU/mL	≥6.8 ± 0.5
SARS-CoV	10 ^{4.0±0.1} PFU/mL	≤10 ^{-1.5±0} PFU/mL	≥5.5 ± 0.1
Nonenveloped			
Human adenovirus 5	10 ^{5.5±0.5} PFU/mL	≤10 ^{-1.3±0.3} PFU/mL	≥6.8 ± 0.4
Bluetongue virus	10 ^{4.0±0.2} PFU/mL	10 ^{-1.0±0.3} PFU/mL	5.1 ± 0.2

* Results are reported as mean ± SD. n = number of replicates done for each virus; PFU/mL = plaque-forming units per milliliter; FFU/mL = foci-forming units per milliliter; ID₅₀ = infectious dose necessary for infection of 50 percent of inoculated ducks; CID₅₀ = infectious dose necessary for infection of 50 percent of inoculated chimpanzees.

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

‡ n = 3.

§ Inactivation was done in 250 mL instead of the 600 mL as specified under Materials and Methods.

cases, whenever possible, to achieve the maximum dynamic range of infectivity.

PCT inactivated a mean of greater than 6.8 logs and greater than 6.4 logs of cell-free and cell-associated human immunodeficiency virus-type 1 (HIV-1), respectively. No biologically active HIV-1 were detected in any of the test samples for four replicate experiments (Table 2). Similarly, human T-cell lymphotropic virus-I (HTLV-I) and HTLV-II were sensitive to PCT and mean log-reductions of at least 4.5 and greater than 5.7, respectively, were obtained.

Inactivation of HBV and HCV by PCT was measured in nonimmune chimpanzees transfused with the entire unit of amotosalen- and UVA-treated plasma that was previously inoculated with 10^{4.5} CID₅₀ of HBV or HCV (Table 2). Inactivation of each virus was assayed in two chimpanzees. None of the animals in either the HBV or the HCV study showed evidence of viral hepatitis during a 6-month follow-up with serologic, viral nucleic acid, biochemical, or histologic examination of liver biopsies, demonstrating an inactivation of greater than 4.5 logs for both viruses. Further evidence of PCT inactivation of hepatitis A virus and flaviviridae was demonstrated with, respectively, the model systems duck hepatitis B virus (DHBV) and bovine viral diarrhea virus (BVDV; Table 2). PCT inactivated 4.4 to 4.5 logs of DHBV and at least 6.0 logs of BVDV.

In plasma, PCT inactivated high levels of recently emerging viruses. Mean log-reductions of at least 6.8 and at least 5.5 were achieved for West Nile virus (WNV) and severe acute respiratory syndrome coronavirus (SARS-CoV), respectively (Table 2). PCT inactivated a mean of 5.1 logs of nonenveloped bluetongue virus and at least 6.8 logs of nonenveloped human adenovirus 5 (Table 2).

PCT inactivation of bacteria in plasma

Gram-positive *Staphylococcus epidermidis* and gram-negative *Klebsiella pneumoniae* and *Yersinia enterocolitica* were sensitive to PCT. High levels of inactivation were achieved in plasma (Table 3). Initial pre-PCT bacterial levels of 10^{6.6} to 10^{6.7} colony-forming units (CFU) per mL were achieved with all bacterial species. PCT with 150 μmol per L amotosalen and a 3 J per cm² UVA treatment resulted in complete inactivation with no viable bacteria remaining in any of the test plasma samples in all four replicates. Mean log-reductions achieved were greater than 7.3 for *S. epidermidis* and *Y. enterocolitica* and greater than 7.4 for *K. pneumoniae*.

Spirochetes *Treponema pallidum*, which causes syphilis, and *Borrelia burgdorferi*, which causes Lyme disease, were also sensitive to PCT. No viable organisms were detected after PCT in the test plasma samples in all four replicates, demonstrating mean log reductions of >5.9 and >10.6, respectively.

PCT inactivation of protozoa in plasma

PCT inactivation studies of *Plasmodium falciparum*, the protozoan that causes malaria, *Trypanosoma cruzi*, the pathogen that causes Chagas' disease, and *Babesia microti*, an emerging protozoan causing babesiosis, exhibited mean log reductions of at least 6.9 for *P. falciparum*, greater than 5.0 for *T. cruzi*, and greater than 5.3 for *B. microti* (Table 4). In four replicate experiments, 150 μmol per L amotosalen and a 3 J per cm² UVA treatment resulted in no detectable *T. cruzi* and *B. microti* in the test plasma samples.

TABLE 3. PCT inactivates gram-positive and gram-negative bacteria and spirochetes in plasma at high initial titers*

Bacteria	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
Gram-positive			
<i>S. epidermidis</i>	10 ^{6.6±0.02} CFU/mL	<10 ^{-0.7±0} CFU/mL	>7.3 ± 0.02
Gram-negative			
<i>K. pneumoniae</i>	10 ^{6.7±0.1} CFU/mL	<10 ^{-0.7±0} CFU/mL	>7.4 ± 0.1
<i>Y. enterocolitica</i>	10 ^{6.6±0.01} CFU/mL	<10 ^{-0.7±0} CFU/mL	>7.3 ± 0.1
Spirochetes			
<i>T. pallidum</i>	10 ^{5.4±0.6} ID ₅₀ /mL	<10 ^{-0.5±0} ID ₅₀ /mL	>5.9 ± 0.6
<i>B. burgdorferi</i>	≥10 ^{9.9±2.3} ID ₅₀ /mL	<10 ^{-0.7±0} ID ₅₀ /mL	>10.6 ± 2.3

* Results are reported as mean ± SD. n = number of replicates done for each bacterium; ID₅₀/mL = infectious dose necessary for infection of 50 percent of inoculated cells or animal hosts;

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

TABLE 4. PCT inactivates protozoa in plasma at high initial titers*

Protozoa	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
<i>P. falciparum</i>	10 ^{5.9±0} iRBCs/mL	≤10 ^{-1.0±0.3} iRBCs/mL	≥6.9 ± 0.3
<i>T. cruzi</i> ‡	10 ^{6.3±0.6} TCID ₅₀ /mL	<10 ^{1.3±0} TCID ₅₀ /mL	>5.0 ± 0.6
<i>B. microti</i>	10 ^{4.9±0.4} ID ₅₀ /mL	<10 ^{0.5±0.02} ID ₅₀ /mL	>5.3 ± 0.4

* Results are reported as mean ± SD. n = number of replicates done for each protozoa; iRBCs/mL = infected red blood cells per milliliter; TCID₅₀/mL = infectious dose necessary for infection of 50 percent of inoculated cells; ID₅₀/mL = infectious dose necessary for infection of 50 percent of inoculated mice.

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

‡ Inactivation was done in 250 mL of whole-blood derived plasma instead of the 600 mL apheresis plasma as specified under Materials and Methods.

TABLE 5. Maintenance of clotting time and plasma coagulation factor activity after PCT*

Coagulation parameter	Reference range†	Pre-PCT	Post-PCT	Post/pre (% retention)
PT (n = 14)	11.1-13.5	11.2 ± 0.3 sec	11.6 ± 0.3 sec	1.0 ± 0.1 sec‡
APTT (n = 14)	23.0-35.0	26.8 ± 1.4 sec	29.1 ± 1.7 sec	4.3 ± 1.8 sec‡
Fibrinogen (n = 91)	167-379	290 ± 40 mg/dL	209 ± 36 mg/dL	72 ± 5
FII (n = 59)	71-127	96 ± 11 IU/dL	85 ± 11 IU/dL	88 ± 4
FV (n = 91)	77-153	130 ± 23 IU/dL	119 ± 19 IU/dL	92 ± 7
FVII (n = 91)	58-166	123 ± 32 IU/dL	95 ± 20 IU/dL	78 ± 6
FVIII (n = 91)	67-235	157 ± 35 IU/dL	115 ± 28 IU/dL	73 ± 7
F IX (n = 91)	63-143	108 ± 21 IU/dL	88 ± 16 IU/dL	82 ± 4
FX (n = 59)	66-134	100 ± 13 IU/dL	86 ± 11 IU/dL	86 ± 3
FXI (n = 91)	62-142	103 ± 22 IU/dL	87 ± 18 IU/dL	86 ± 5
FXIII (n = 26)	NA	110 ± 11 IU/dL	102 ± 10 IU/dL	93 ± 3
VWF:RCo (n = 12)	NA	114 ± 44 IU/dL	111 ± 41 IU/dL	97 ± 8

* Results are reported as mean ± SD. n = number of replicates (treated plasma units) done for each parameter. (A higher number of replicates was performed for a basic testing panel, with additional parameters included in a subset of studies.) IU/dL = International Units/deciliter.

† The reference range was calculated from the mean ± 2 SD of untreated, conventional plasma.

‡ For PT and PTT, the effect of PCT was calculated by subtracting the pre-PCT values from the post-PCT values.

In vitro coagulation function of plasma after PCT

Evaluation of a comprehensive panel of coagulation parameters showed that PCT conserved coagulation function within levels suitable for transfusion support.²³ On average, the PT and APPT were prolonged after PCT by 1.0 and 4.3 seconds, respectively (Table 5). Fibrinogen, FVII, and FVIII were retained, on average, 72 to 78 percent of the initial pre-PCT activity. All other coagulation parameters retained at least 82 percent of baseline activity, with FV, FXIII, and VWF:RCo retaining at least 92 percent. Antithrombotic proteins (PC, PS, antithrombin) demonstrated a high level of activity retention (Table 6), ranging between 95 and 98 percent of the initial pre-PCT level. α2-Antiplasmin was retained at 80 percent of baseline activity. Coagulation factor and antithrombotic protein activities fell within the reported reference ranges for conventional plasma.²⁴

DISCUSSION

These studies showed that PCT with amotosalen and UVA light is effective against a broad range of pathogens in plasma. This finding is not surprising based on the pathogen inactivation efficacy previously demonstrated for PLTs with the same dose of amotosalen and same dose of UVA light.¹⁵⁻²⁰ The levels of inactivation for representative viruses, bacteria, and protozoa were measured with PCT conditions developed for the commercial application of FFP.

PCT inactivated high levels of enveloped viruses including HIV-1, HBV, and HCV in plasma. Effectiveness against HBV was initially shown by inactivation of DHBV, an established infectivity model for human HBV.²⁵ A chimpanzee infectivity model was then used to confirm the level of inactivation of HBV in plasma. Effectiveness against HCV was initially shown by inactivation of BVDV, a flavivirus and model for human HCV.²⁶ Inactivation of HCV was then confirmed with the chimpanzee infectivity model as well. PCT is also effective against other flaviviruses such

TABLE 6. Maintenance of plasma antithrombotic protein activity after PCT*

Protein	Pre-PCT (IU/dL)	Post-PCT (IU/dL)	Post/pre (% retention)
PC (n = 25)	109 ± 15	102 ± 14	95 ± 9
PS (n = 25)	109 ± 12	107 ± 12	98 ± 5
Antithrombin III (n = 26)	94 ± 5	91 ± 6	96 ± 3
α 2-Antiplasmin (n = 26)	93 ± 5	75 ± 6	80 ± 4

* Results are reported as mean \pm SD. n = number of replicates done for each parameter; IU/dL = International Units/deciliter.

as WNV and, in preliminary studies, dengue virus (data not shown), examples of new pathogens that are of increasing concern to blood centers in North America.

PCT with amotosalen is very effective against cell-associated HIV-1 and against cell-associated HTLV-I and HTLV-II viruses in plasma, demonstrating that amotosalen molecules readily permeate the cell membrane. The ability of the photochemical reagent to permeate the cell membrane as well as nuclear membrane is crucial because some blood-borne viruses exist partially or largely associated with cells. Methylene blue treatment of plasma, for example, is not effective against cell-associated or intracellular viruses.¹¹ The efficacy of the methylene blue system against intracellular viruses depends on the efficacy of filtration or freeze-thaw to quantitatively disrupt WBCs.^{8,27} Recent studies, however, demonstrate that conventional WBC filters fail to eliminate cell-associated cytomegalovirus and HTLV-I from PLT products.^{28,29} Thus the methylene blue system is ineffective against these viruses.

The cumulative viral inactivation data in PLTs and plasma would predict that PCT is effective against the majority of enveloped viruses, DNA, or RNA, extracellular or intracellular. Thus PCT has the potential to inactivate new and emerging enveloped viruses and prevent them from entering the blood supply. Indeed, it was found that SARS-CoV was inactivated by PCT in both PLTs and plasma.¹⁵

Nonenveloped viruses are less common as labile blood component transfusion-transmitted pathogens. Human parvovirus B19, and in very rare cases hepatitis A virus (HAV), however, have been transmitted by component blood transfusion.^{30,31} A ubiquitous nonenveloped virus, TT virus, has been reported to be transmitted through the blood supply in Japan, but has yet to be directly linked with a specific disease state.³²

The efficacy of PCT against nonenveloped viruses in plasma continues to be evaluated and is expected to be similar to the results obtained for PLTs.¹⁶ In these studies, inactivation experiments were only performed with two nonenveloped viruses, bluetongue virus and human adenovirus 5, as examples. Inactivation results comparable to the PLT experiments were obtained. Owing to the low permeability of the capsid, some nonenveloped

viruses show little or no inactivation by PCT. The picornaviruses are known to be the most difficult to inactivate by chemical or physical means.³³ The tight capsid structure of picornaviruses such as HAV, polio virus, and encephalomyocarditis virus are thought to exclude even low-molecular-weight compounds such as psoralens from the interior of the virus and PCT is ineffective against these viruses. Human parvovirus B19

and human adenovirus 5, however, were found sensitive to PCT in PLTs.^{16,34} The level of inactivation for these non-enveloped viruses can be increased by incubating the spiked blood products with amotosalen before illumination with UVA light.³⁴

The methylene blue system also showed variable efficacy against nonenveloped viruses, ranging from ineffective to complete inactivation.⁸ Another system for treatment of plasma, the S/D system, is ineffective against nonenveloped viruses. S/D inactivates viruses by disrupting the viral membrane.¹⁰

PCT is effective for inactivation of gram-positive *S. epidermidis* and gram-negative *K. pneumoniae* and *Yersinia enterocolitica*. *Y. enterocolitica* are cryophilic bacteria that can grow in cold temperatures. High levels of inactivation were achieved for all of these bacteria in plasma. Although transfusion-transmitted bacteremia is not considered a serious problem for transfusion of FFP, it is reassuring to have a pathogen inactivation process that inactivates bacteria as well as viruses. In these studies, inactivation of two spirochetes, *T. pallidum* and *B. burgdorferi*, in plasma was also demonstrated. *T. pallidum*, which causes syphilis, is the only bacteria routinely screened for in blood banks. Under experimental conditions, *B. burgdorferi* has been reported to remain viable in frozen plasma for more than 1 month.³⁵ PCT has been shown to be effective against these agents and high levels of inactivation were obtained in plasma.

On an international level, other organisms such as *T. cruzi* (Chagas' disease), *P. falciparum* (malaria), and *B. microti* (babesiosis) may be more of a concern than bacteria for frozen plasma because, like viruses, they do not require growth during storage to be a transfusion risk. *P. falciparum* and *B. microti* are intracellular pathogens, as discussed above, and are resistant to methylene blue treatment.¹¹

Experiments with plasma are ongoing to measure the level of WBC inactivation by PCT. Based on previous studies with PLTs, it is anticipated that high levels of WBC inactivation could be demonstrated in plasma.²¹ Overall, the results presented here show that PCT offers the benefit of a broad spectrum of pathogen inactivation. Neither the methylene blue system nor the S/D system for plasma has shown this broad pathogen inactivation profile.

These studies also confirmed maintenance of adequate plasma coagulation and antithrombotic protein function after PCT. Because FFP is transfused primarily as a replacement for the liver-derived coagulation factors FII, FV, FVII, FIX, FX, and FXI, in vitro studies to evaluate plasma function after processing with PCT were conducted for a panel of coagulation factors, antithrombotic proteins, and clotting time. The proteins most affected by PCT were fibrinogen and FVIII. The changes in coagulation proteins observed in plasma treated with PCT were also associated with slight prolongation in PT and APTT. The slight changes in PT and APTT after PCT, however, were not associated with any adverse clinical observations.³⁶⁻³⁹

Levels of the anticoagulant PC and PS and antithrombin were relatively unaffected by PCT and α 2-antiplasmin was conserved by 80 percent. Previous studies on cryoprecipitate prepared from PCT plasma yielded approximately 95 and 88 percent activity retention for fibrinogen and FVIII, respectively, compared to cryoprecipitate prepared from untreated plasma.⁴⁰ Furthermore, cryosupernatant prepared from PCT plasma retained adequate levels of critical plasma proteins for plasma exchange therapy in acute thrombocytopenic purpura. The data indicate good preservation of hemostasis control proteins such as PS, α 2-antiplasmin, and VWF-cleaving protease activity.⁴¹

From a blood center perspective, the adoption of PCT would enhance safety of blood products and potentially decrease the number of donors rejected. PCT is easily integrated into the plasma preparation system by its ability to treat individual units of fresh apheresis plasma and small pools (three) of whole blood-derived plasma. For further ease of use, PCT is compatible with the existing plasma collection procedures, FFP processing, and distribution methods in use in blood centers. This PCT system allows rapid illumination and processing of plasma units and utilizes the same illumination device developed for pathogen inactivation of PLT concentrates. Thus, both plasma and PLTs can be treated with a similar processing system and the same illumination device.

In summary, photochemically treated plasma is functionally similar to untreated conventional plasma with the added benefit of pathogen inactivation.

ACKNOWLEDGMENTS


The authors thank the following investigators for conducting the pathogen inactivation studies: Bernard Guillemain, MD, HTLV; Krishna Murthy, VD, and Harvey Alter, MD, HBV and HCV; Patricia Marion, PhD, DHBV; Kristen Bernard, PhD, WNV; Sheila Lukehart, PhD, *T. pallidum*; Robert Lane, MD, *B. burgdorferi*; Phillippe Grellier, MD, *P. falciparum*; and Wesley Van Voorhis, PhD, *T. cruzi*; Jorge Benach, PhD, *B. microti*. We also appreciate the following individuals for their contributions in the development of the INTERCEPT plasma product: Jocelyn Smyers, Betsy Donnelly,

Lainie Corten, Meisa Propst, Reydan Mababangloob, Adam Sampson-Johannes, John Kinsey, Debbie Hanson, Treva Peterson, Melchor Dolor, Margaret Rheinschmidt, MT, and George Cimino, PhD.

REFERENCES

1. Sullivan MT, McCullough J, Schreiber GB, Wallace EL. Blood collection and transfusion in the United States in 1997. *Transfusion* 2002;42:1253-60.
2. Practice Guidelines for blood component therapy: a report by the American Society of Anesthesiologists Task Force on Blood Component Therapy. *Anesthesiology* 1996;84:732-47.
3. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion* 2002;42:975-9.
4. Barbara J. Why "safer than ever" may not be safe enough. *Transfus Med Hemother* 2004;31(Suppl 1):2-10.
5. Phelps R, Robbins K, Liberti T, et al. Window-period human immunodeficiency virus transmission to two recipients by an adolescent blood donor. *Transfusion* 2004;44:929-33.
6. Fiebig EW, Heldebrandt CM, Smith RI, et al. Intermittent low-level viremia in very early primary HIV-1 infection. *J Acquir Immune Defic Syndr* 2005;39:133-7.
7. Mohr H, Lambrecht B, Knuever-Hopf J. Virus inactivated single-donor fresh plasma preparations. *Infusionsther Transfusionsmed* 1992;19:79-83.
8. Williamson LM, Cardigan R, Prowse CV. Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? *Transfusion* 2003;43:1322-9.
9. Horowitz B, Bonomo R, Prince AM, et al. Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma. *Blood* 1992;79:826-31.
10. Horowitz B. Inactivation of viruses found with plasma proteins. *Biotechnology* 1991;19:417-30.
11. Wagner SJ, Robinette D, Storry J, et al. Differential sensitivities of viruses in red cell suspensions to methylene blue photosensitization. *Transfusion* 1994;34:521-6.
12. Alford BL. Important new drug warning [monograph on the Internet]. Watertown (MA): V.I. Technologies, Inc. Vitex; 2002. Available from: http://www.fda.gov/medwatch/safety/2002/plasdd_deardoc.pdf
13. Osselaer JC, Doyen C, Sonet A, et al. Routine use of platelet components prepared with photochemical treatment (INTERCEPT platelets): impact on clinical outcomes and costs. *Blood* 2004;104:987a.
14. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. *Semin Hematol* 2001;38(4 Suppl 11):4-11.
15. Pinna D, Sampson-Johannes A, Clementi M, et al. Amotosalen photochemical inactivation of severe acute respiratory syndrome coronavirus in human platelet concentrates. *Transfus Med* 2005;15:269-76.

16. Lin L, Hanson CV, Alter HJ, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. *Transfusion* 2005;45:580-90.
17. Jauvin V, Alfonso RD, Guillemain B, et al. In vitro photochemical inactivation of cell-associated human T-cell leukemia virus Type I and II in human platelet concentrates and plasma by use of amotosalen. *Transfusion* 2005;45:1151-9.
18. Lin L, Dikeman R, Molini B, et al. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion* 2004;44:1496-504.
19. Van Voorhis WC, Barrett LK, Eastman RT, et al. Trypanosoma cruzi inactivation in human platelet concentrates and plasma by a psoralen (amotosalen HCl) and long-wavelength UV. *Antimicrob Agents Chemother* 2003;47:475-9.
20. Eastman RT, Barrett LK, Dupuis K, et al. Leishmania inactivation in human pheresis platelets by a psoralen (amotosalen HCl) and long-wavelength ultraviolet irradiation. *Transfusion* 2005;45:1459-63.
21. Grass JA, Hei DJ, Metchette K, et al. Inactivation of leukocytes in platelet concentrates by photochemical treatment with psoralen plus UVA. *Blood* 1998;91:2180-8.
22. Reed LJ, Muench H. A simple method of estimating fifty-per cent endpoints. *Am J Hyg* 1938;27:493-7.
23. Heiden M, Seitz R. Quality of therapeutic plasma-requirements for marketing authorization. *Thromb Res* 2002;107 (Suppl. 1):S47-51.
24. Beeck H, Hellstern P. In vitro characterization of solvent/detergent-treated human plasma and of quarantine fresh frozen plasma. *Vox Sang* 1998;74(Suppl 1):219-23.
25. Ganem D, Varmus HE. The molecular biology of the hepatitis B viruses. *Annu Rev Biochem* 1987;56:651-93.
26. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;244:359-62.
27. Abe H, Yamada-Ohnishi Y, Hirayama J, et al. Elimination of both cell-free and cell-associated HIV infectivity in plasma by a filtration/methylene blue photoinactivation system. *Transfusion* 2000;40:1081-7.
28. Visconti MR, Pennington J, Garner SF, et al. Assessment of removal of human cytomegalovirus from blood components by leukocyte depletion filters using real-time quantitative PCR. *Blood* 2004;103:1137-9.
29. Pennington J, Taylor GP, Sutherland J, et al. Persistence of HTLV-I in blood components after leukocyte depletion. *Blood* 2002;100:677-81.
30. Siegl G, Cassinotti P. Presence and significance of parvovirus B19 in blood and blood products. *Biologicals* 1998;26:89-94.
31. Vrieling H, Reesink HW. Transfusion-transmissible infections. *Curr Opin Hematol* 1998;5:396-405.
32. Yokozaki S, Toyoda H, Nakano I, et al. Infection with TT virus, a novel transfusion-transmissible DNA virus, in haemophiliacs and in blood products. *Br J Haematol* 1999;105:1114-9.
33. Hanson CV. Photochemical inactivation of viruses with psoralens: an overview. *Blood Cells* 1992;18:7-25.
34. Sawyer L, Hanson D, Dupuis K. Inactivation of parvovirus B19 in platelets by Helinx technology. *Transfusion* 2004;44:102A.
35. McQuiston JH, Childs JE, Chamberland ME, Tabor E. Transmission of tick-borne agents of disease by blood transfusion: a review of known and potential risks in the United States. *Transfusion* 2000;40:274-84.
36. de Alarcon P, Benjamin R, Dugdale M, et al. Fresh frozen plasma prepared with amotosalen HCl (S-59) photochemical pathogen inactivation: transfusion of patients with congenital coagulation factor deficiencies. *Transfusion* 2005;45:1362-72.
37. Hambleton J, Wages D, Radu-Radulescu L, et al. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. *Transfusion* 2002;42:1302-7.
38. Mintz P, Neff A, MacKenzie M, et al. Therapeutic plasma exchange (TPE) for thrombotic thrombocytopenic purpura (TTP) using plasma prepared with photochemical treatment (INTERCEPT plasma). *Blood* 2004;104:239a.
39. Mintz P, Steadman R, Blackall D, et al. Pathogen inactivated fresh frozen plasma prepared using Helinx™ technology is efficacious and well tolerated in treatment of end-stage liver disease patients—the STEP AC trial. *Blood* 2001;98(Suppl 1):709a.
40. Mintz PD, Avery NL, Moulaison SL, et al. Preparation of cryoprecipitate from photochemically treated fresh frozen plasma. *Transfusion* 2000;40(Suppl 1):63S.
41. Yarranton H, Lawrie AS, Mackie JJ, et al. Coagulation factor levels in cryosupernatant prepared from plasma treated with amotosalen hydrochloride (S-59) and ultraviolet A light. *Transfusion* 2005;45:1453-8.
42. Hanson CV, Crawford-Miksza L, Sheppard HW. Application of a rapid microplaque assay for determination of human immunodeficiency virus neutralizing antibody titers. *J Clin Microbiol* 1990;28:2030-4.
43. Astier-Gin T, Portail JP, Lafond F, Guillemain B. Identification of HTLV-I- or HTLV-II-producing cells by cocultivation with BHK-21 cells stably transfected with a LTR-lacZ gene construct. *J Virol Meth* 1995;51:19-29.
44. Alter HJ, Creagan RP, Morel PA, et al. Photochemical decontamination of blood components containing hepatitis B and non-A, non-B virus. *Lancet* 1988;2:1446-50.
45. Feinstone SM, Alter HJ, Dienes HP, et al. Non-A, non-B hepatitis in chimpanzees and marmosets. *J Infect Dis* 1981;144:588-98.
46. Marion PL, Cullen JM, Azcarraga RR, et al. Experimental transmission of duck hepatitis B virus to Pekin ducks and to domestic geese. *Hepatology* 1987;7:724-31.

47. Shi PY, Tilgner M, Lo MK, et al. Infectious cDNA clone of the epidemic west nile virus from New York City. *J Virol* 2002;76:5847-56.
48. Grellier P, Santus R, Mouray E, et al. Photosensitized inactivation of *Plasmodium falciparum*- and *Babesia divergens*-infected erythrocytes in whole blood by lipophilic pheophorbide derivatives. *Vox Sang* 1997;72:211-20.
49. Etkind P, Piesman J, Ruebush TK 2nd, et al. Methods for detecting *Babesia microti* infection in wild rodents. *J Parasitol* 1980;66:107-10. 

Wortlaut der Gebrauchsinformation und Fachinformation im Sinne der §§ 11 und 11a AMG

Gebrauchsinformation und Fachinformation Mit Pathogeninaktivierungsverfahren behandeltes Thrombozytapheresekonzentrat

1. Identifizierung des Arzneimittels

a) Bezeichnung

Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst

b) Stoffgruppe

zelluläre Blutzubereitung

2. Anwendungsgebiete

Die Gabe von Thrombozytenkonzentraten ist indiziert zur Behandlung einer Blutungsneigung, bedingt durch eine schwere Thrombozytopenie infolge thrombozytärer Bildungsstörungen, im Notfall auch bei Umsatzstörungen, jedoch nicht bei einer niedrigen Thrombozytenzahl allein. Damit durch die Zufuhr von Plättchen eine Besserung der thrombozytär bedingten Blutungsneigung zu erwarten ist, sollte vor der Behandlung zunächst deren Ursache abgeklärt werden.

Das „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“ ist auch geeignet zur Anwendung bei gefährdeten Patienten, bei denen das Risiko einer transfusionsassoziierten Graft-Versus-Host-Reaktion vermieden werden soll, wie:

- Frühgeborene (bis zur Vollendung der 37. Schwangerschaftswoche)
- Neugeborene bei Verdacht auf Immundefizienz,
- Neugeborene bei postpartaler Austauschtransfusion*
- Patienten bei allogener Transplantation hämatopoetischer Stammzellen (aus peripherem Blut, Knochenmark oder Nabelschnurblut)
- Patienten 7 – 14 Tage vor autologer Stammzellentnahme
- Patienten bei autologer Stammzelltransplantation (bis ca. drei Monate nach Transplantation)
- Patienten mit schwerem Immundefektsyndrom oder mit AIDS
- Patienten mit M. Hodgkin (alle Stadien)
- Patienten bei Therapie mit Purin-Analoga (z.B. Fludarabin, Cladrabin, Deoxycoformycin)
- Patienten bei Therapie mit Anti-T-Lymphozyten-Antikörpern (z.B. Alemtuzumab, ATG/ALG)
- Patienten bei Hochdosis-Chemotherapie mit oder ohne Ganzkörperbestrahlung, Patienten mit Leukämien, malignen Lymphomen, soliden Tumoren*.

* nicht gesicherte Indikationen

Das „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“ ist ebenso geeignet für Patienten, bei denen eine CMV-Infektion vermieden werden muss, wie:

- Frühgeborene
- Empfänger eines allogenen Stammzellpräparates
- Empfänger mit schweren angeborenen Immundefekten (SCID)
- CMV-negative, HIV-infizierte Patienten
- CMV-negative, schwangere Frauen
- stillende Mütter.

(siehe auch „Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie)“)

3. Informationen zur Anwendung

a) Gegenanzeigen

Bekannte Überempfindlichkeit gegen Amotosalen-HCl oder Psoralene. In diesem Fall sollten

Thrombozytenkonzentrate, die mit diesem Pathogeninaktivierungsverfahren behandelt wurden, nicht verwendet werden.

Eine absolute Kontraindikation für Thrombozytentransfusionen gibt es nicht. Bei potentiellen Empfängern eines Stammzelltransplantates (Knochenmark, periphere Stammzellen, Nabelschnurblut), z.B. bei Patienten mit aplastischen Anämien, Leukämien etc., ist die Gabe von Thrombozytenkonzentraten des Transplantatspenders und seiner Blutsverwandten vor der Transplantation unbedingt zu vermeiden.

Relative Kontraindikationen sind u.a. die Anwendung bei

- bekannten Allergien des Empfängers gegen humane Plasmaproteine,
- bekannten Immunthrombozytopenien,
- posttransfusioneller Purpura,
- heparininduzierter Thrombozytopenie,
- kongenitalen Thrombozytenfunktionsstörungen, wie Thrombasthenie Glanzmann oder Bernard-Soulier-Syndrom.

b) Vorsichtsmaßnahmen für die Anwendung

Thrombozytenkonzentrate sind in der Regel AB0-kompatibel über ein Transfusionsgerät mit Standardfilter der Porengröße 170 bis 230 µm zu transfundieren.

Beim Refraktärzustand gegenüber Thrombozytentransfusionen aufgrund einer Alloimmunisierung gegen Antigene des HLA- und ggf. HPA-Systems sind nach Möglichkeit HLA-Klasse-I-kompatible und ggf. HPA-kompatible Thrombozytenkonzentrate zu transfundieren. Die Transfusionsgeschwindigkeit muss dem klinischen Zustand des Patienten angepasst werden.

Bei neonataler Transfusion sollte sorgfältig auf Anzeichen einer Zitratintoxikation geachtet und die Transfusionsgeschwindigkeit dem klinischen Zustand angepasst werden.

c) Wechselwirkungen mit anderen Arzneimitteln, soweit sie die Wirkungsweise des Arzneimittels beeinflussen können und Hauptinkompatibilitäten

Durch Medikamente, die die Thrombozytenfunktion beeinflussen, kann die Wirkung von Thrombozytenkonzentraten vermindert bzw. aufgehoben werden. Wegen der Gefahr von Gerinnselbildungen dürfen kalziumhaltige Lösungen nicht gleichzeitig in demselben Schlauchsystem gegeben werden. Die Beimischung von Medikamenten zum Thrombozytenkonzentrat ist nicht zulässig.

d) Verwendung für besondere Personengruppen

Bei Rh (D) - negativen Kindern und Frauen im gebärfähigen Alter ist wegen der praktisch in allen Thrombozytenkonzentraten vorhandenen Kontamination mit Erythrozyten die Transfusion von Thrombozytenkonzentraten Rh (D)-positiver Spender mit Ausnahme von lebensbedrohlichen Situationen unbedingt zu vermeiden. Die Transfusion von Thrombozytenkonzentraten Rh (D)-positiver Spender in Rh (D)-negative Patienten lässt sich wegen des Mangels an Rh (D)-negativem Blut nicht immer vermeiden, sollte nach Möglichkeit aber nur in Betracht gezogen werden, wenn es sich um Männer oder um Frauen im nicht gebärfähigen Alter handelt. In solchen Fällen ist stets eine serologische Nachuntersuchung 2 bis 4 Monate nach Transfusion zur Feststellung eventuell gebildeter Anti-D-Antikörper durchzuführen.

Schwangerschaft und Stillzeit: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.

Anwendung bei Kindern, Neugeborenen, Föten: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.

Anwendung bei Neugeborenen unter Lichttherapie: Neugeborene, die während einer Lichttherapie zur Behandlung von Hyperbilirubinämie Thrombozytentransfusionen benötigen, sollten nur mit Lichttherapiegeräten behandelt werden, die Licht mit einer Wellenlänge größer als 425nm aussenden. Anderenfalls kann es zu einer Wechselwirkung zwischen dem UV-A-Licht und dem Restgehalt des zur Herstellung des „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“ verwendeten Psoralens (Amotosalen) und damit zu einem Erythem kommen. Bei Lichttherapie mit einer Wellenlänge kleiner als 425nm dürfen nur unbehandelte Thrombozytenkonzentrate transfundiert werden.

Stark immunsupprimierten Patienten: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.

Auswirkung auf Kraftfahrer und die Bedienung von Maschinen: Nach der Transfusion von Thrombozytenkonzentraten sollte eine Ruhepause von mindestens 1/2 Stunde eingehalten

werden.

e) Warnhinweise

sind nicht angeordnet.

4. Hinweise zur ordnungsgemäßen Anwendung

a) Dosierung

Die Dosierung der Thrombozyten ist abhängig vom klinischen Zustand und der Thrombozytenzahl des Patienten.

Der Thrombozytenbedarf für die initiale Behandlung eines normalgewichtigen Erwachsenen ohne weitere Komplikationen beträgt mindestens $2,5 \times 10^{11}$ Thrombozyten, entsprechend einer Packungseinheit „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“. Eine Überwachung der Therapie, z.B. durch Bestimmung der Thrombozytenzahl oder der Blutungszeit beim Patienten, ist unerlässlich.

b) Art der Anwendung

zur i.v. Infusion

c) Häufigkeit der Verabreichung

nach Indikationsstellung

d) Dauer der Behandlung

nach Indikationsstellung

e) Überdosierung

Eine Gefahr der Überdosierung besteht bei Erwachsenen nicht.

f) Notfallmaßnahmen

Treten Unverträglichkeiten auf, so ist die Transfusion unverzüglich abzubrechen, der Venenzugang jedoch offen zu halten und eine Behandlung, der Schwere der Symptome gemäß, nach den aktuellen Regeln der Notfalltherapie einzuleiten.

5. Nebenwirkungen

- Unverträglichkeitsreaktionen (z.B. urtikarielle Hautreaktionen, posttransfusionelle Purpura und andere anaphylaktoide Reaktionen)
- Unverträglichkeitsreaktionen gegenüber Amotosalen und seinen Photoabbauprodukten (z.B. allergische und andere anaphylaktoide Reaktionen). Immunologische Reaktionen durch Bildung von Neoantigenen sind bisher nicht bekannt.
- Transfusionsassoziierte akute Lungeninsuffizienz (TRALI)
- anaphylaktische Reaktionen bei Empfängern mit angeborenem IgA-Mangel
- Mikrozirkulationsstörungen durch aggregierende Thrombozyten bei massiver Transfusion
- Immunisierung des Empfängers gegen thrombozytäre und nicht-thrombozytäre Antigene
- Obwohl HLA-bedingte Unverträglichkeiten stark verringert sind, können febrile Transfusionsreaktionen in unmittelbarem zeitlichem Zusammenhang mit der Transfusion auftreten.
- Bei Neugeborenen sind bei schneller Transfusion Herz-Kreislaufreaktionen infolge von Citratintoxikationen möglich.
- Durch das zusätzlich angewandte Pathogeninaktivierungsverfahren mit dem „INTERCEPT Blood System“ (Amotosalen/UVA) verlieren Viren, Bakterien, Parasiten und Restleukozyten weitgehend ihre Teilungsfähigkeit. Das Risiko einer bakteriellen, viralen oder parasitären Kontamination oder einer transfusionsassoziierten Graft-versus-Host-Erkrankung ist nicht mit allerletzter Sicherheit auszuschließen. Jedoch wird insbesondere die Inaktivierung von umhüllten Viren (HIV, HBV, HCV) und Leukozyten als sicher erachtet. Eine effiziente Inaktivierung von nicht-umhüllten Viren (z.B. HAV und Parvovirus B19) ist nicht gewährleistet. Das Pathogeninaktivierungsverfahren ist gegenüber einem breiten Spektrum von gram-positiven und gram-negativen Bakterien sehr effektiv; jedoch gegenüber einzelnen Bakterien-Spezies (z.B. *Pseudomonas aeruginosa*) nur eingeschränkt effektiv. Bakterielle Sporen (z.B. von *Clostridium perfringens* oder *Bacillus cereus*) lassen sich mit dem hier angewandten Pathogeninaktivierungsverfahren nicht abtöten. Die Belastung mit Pyrogenen wird durch das Pathogeninaktivierungsverfahren nicht unterbunden, daher sind pyrogene Reaktionen (auch schwerwiegende) des Empfängers nicht auszuschließen.

- Bei der Anwendung von aus menschlichem Blut hergestellten Arzneimitteln ist die Übertragung von Infektionskrankheiten durch Übertragung von Erregern - auch bislang unbekannter Natur - nicht völlig auszuschließen. Dies gilt z.B. für Hepatitiden, seltener für das erworbene Immundefektsyndrom (AIDS).
- Im Vereinigten Königreich Großbritannien und Nordirland wurde über Einzelfälle berichtet, in denen bei Empfängern von Transfusionen, deren Spender später an der varianten Creutzfeldt-Jakob Krankheit (vCJK) erkrankten, ebenfalls der „Erreger“ (so genannte Prionen) nachgewiesen wurde. Bei der vCJK handelt es sich um eine in Deutschland bislang nicht beobachtete Erkrankung, die durch den Verzehr von bestimmten Nahrungsmitteln aus BSE-kranken Rindern erworben werden kann.

6. Pharmakologische Eigenschaften

Die wirksamen Bestandteile von Thrombozytenkonzentraten sind morphologisch und funktionell intakte Thrombozyten, welche die zellulären Bestandteile des Hämostasesystems darstellen. Die Hämostaseaktivität der funktionell intakten Thrombozyten ist sofort nach der Transfusion gegeben. Die Funktionsfähigkeit und mittlere Überlebenszeit der Thrombozyten nimmt mit der Lagerungsdauer ab. Durch die Leukozytendepletion auf $< 1 \times 10^6$ Leukozyten pro Packungseinheit wird das Risiko einer Immunisierung gegen humane leukozytäre Alloantigene (HLA) und durch das Pathogeninaktivierungsverfahren die Übertragung mitosefähiger immunkompetenter Lymphozyten stark vermindert, somit die Gefahr einer transfusionsassoziierten Graft-versus-Host-Reaktion vermieden.

Das Pathogeninaktivierungsverfahren basiert auf der Zugabe von Amotosalen und nachfolgender UVA-Bestrahlung. Trotz der effizienten Entfernung von Amotosalen durch ein Adsorptionsverfahren sind sehr geringe Mengen von Amotosalen ($< 2\mu\text{M}$) und dessen Photoproducten D und E (Dimere von Amotosalen) im Thrombozytenkonzentrat nachweisbar. Tierversuche mit einmaliger und wiederholter Verabreichung von Amotosalen, in Dosierungen, die mehr als 100-fach über der klinisch zu erwartenden Exposition von Amotosalen lagen, ergaben keine Hinweise auf ein erhöhtes toxikologisches Risiko für die Anwendung von „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“. Es gibt keine Anzeichen auf Phototoxizität nach intravenöser Anwendung der 40-fachen üblichen klinischen Dosis bei Ratten.

7. Weitere Hinweise

a) Angaben zur Aufbewahrung und Haltbarkeit

Angaben zur Haltbarkeit, besondere Lager- und Aufbewahrungshinweise

- Das Thrombozytenkonzentrat „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“ ist 5 Tage bei 22 ± 2 °C unter gleichmäßiger Agitation haltbar. Nach Ablauf des Verfalldatums darf das Thrombozytenkonzentrat nicht mehr verwendet werden.
- Nach Unterbrechung der o.g. Lagerbedingungen ist das Thrombozytenkonzentrat unverzüglich zu transfundieren.
- Eine durch das Transfusionsbesteck geöffnete Konserve muss unverzüglich verbraucht werden.

b) Optische Prüfung

Unmittelbar vor der Transfusion muss jedes Thrombozytenkonzentrat einer optischen Qualitätsprüfung unterzogen werden, auffällige Thrombozytenkonzentrate (z.B. fehlendes "Swirling-Phänomen", erkennbare Aggregatbildung) dürfen nicht verwendet werden.

c) Zusammensetzung des Fertigarzneimittels

Wirkstoffe (qualitativ und quantitativ)

Human-Thrombozyten aus Apherese

$2,5 \times 10^{11}$ bis $6,0 \times 10^{11}$ Thrombozyten/Packungseinheit

sonstige Bestandteile:

1 ml „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst“ enthält:

InterSol	0,53 - 0,68 ml
Plasma der angegebenen Blutgruppe	0,38 - 0,28 ml
Stabilisator ACD-A	0,09 - 0,04 ml

1000 ml Thrombozytenadditivlösung InterSol enthalten:

Natriumcitrat-Dihydrat	3,18 g
Dinatriumphosphat-Anhydrat	3,05 g
Natriumdihydrogenphosphat-Dihydrat	1,05 g
Natriumacetat-Trihydrat	4,42 g
Natriumchlorid	4,52 g
Wasser für Injektionszwecke	ad 1000 ml

1000 ml des Stabilisators ACD-A enthalten:

Citronensäure-Monohydrat	8,0 g
Natriumcitrat-Dihydrat	22,0 g
Glucose-Monohydrat	25,0 g
Wasser für Injektionszwecke	ad 1000 ml

Restgehalt pro Liter:

Amotosalen < 2µM

Restzellzahlen pro Packungseinheit:

Leukozyten < 1 x 10⁶

Restzellzahlen pro ml:

Erythrozyten < 4 x 10⁶

d) Darreichungsform und Inhalt, Behältnis

210 bis 310 ml Suspension im Kunststoffbeutel mit CE-Zertifikat.

Angaben zur Zulassung

e) Zulassungsnummer

PEI.H.03610.01.1

f) Datum der Erteilung der Zulassung

23.02.2009

g) Arzneimittelstatus

Verschreibungspflichtig

8. Sonstige Hinweise

Maßnahmen zur Reduktion des Übertragungsrisikos von Infektionserregern:

Da bei der Anwendung aus menschlichem Blut hergestellten Arzneimitteln die Übertragung von Infektionskrankheiten nicht völlig auszuschließen ist, werden Maßnahmen getroffen, um das Risiko einer Übertragung von infektiösem Material zu minimieren: Für die Herstellung von „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogen-inaktivierung, DRK-Blutspendedienst“ werden ausschließlich Spenden gesunder Spender verwendet, die mit negativem Ergebnis getestet wurden auf Humanes Immundefizienz Virus (Anti-HIV-1/2-Ak, HIV-1 Genom), Hepatitis-B Virus (HBsAg, Anti-HBc-Ak), Hepatitis-C Virus (Anti-HCV-Ak, HCV-Genom) und Treponema pallidum (Anti-Treponema pallidum-Ak). Im Hinblick auf eine mögliche Hepatitis-B-Infektion werden bei Erstspendern nur Blutspenden verwendet, die Anti-HBc negativ getestet sind. Bei Blutspendern, die vor dem 01.02.2006 als Mehrfachspender Blut gespendet haben, werden nur Blutspenden verwendet, die Anti-HBc negativ sind, oder bei positivem Anti-HBc Status einen Anti-HBs Wert ≥ 100 IU/ml aufweisen. Darüber hinaus kann durch die Leukozytendepletion das Risiko einer Übertragung von leukozytenassoziierten Viren (HTLV-I/II, CMV, EBV u.a.) und Bakterien (Yersinia enterocolitica) entscheidend vermindert werden. Durch die zusätzliche Pathogeninaktivierung mittels „INTERCEPT Blood System“ (Amotosalen/UVA) kann von einer weiteren Reduktion des Übertragungsrisikos von Infektionserregern (Viren, Bakterien und Parasiten) ausgegangen werden.

Qualitätssicherung:

Für die Transfusion von Thrombozytenkonzentraten sind von den Einrichtungen der Krankenversorgung Maßnahmen im Rahmen der Qualitätssicherung nach § 15 des Transfusionsgesetzes zu ergreifen. Dazu gehören u.a. detaillierte Anweisungen sowohl für die Indikationsstellung und Dosierung abhängig von Thrombozytenanzahl und -abfall bei unterschiedlichen Grunderkrankungen, Vorsorgemaßnahmen zur Erhaltung der Unversehrtheit der Konserve vor der Transfusion und Anweisungen zur Nachuntersuchung der Patienten für die Feststellung des Transfusionserfolges, eventuell gebildeter Antikörper und zu ergreifender Prophylaxe. Die Entscheidungskriterien für die Transfusion von Thrombozyten bei primären und

sekundären Knochenmarkinsuffizienzen, aplastischer Anämie oder Myelodysplasie, disseminierter intravasaler Gerinnung, Patienten mit angeborenen Thrombozytopathien/-penien, Autoimmunthrombozytopenien, fötaler bzw. Alloimmunthrombozytopenie, für die Auswahl der Präparate und deren Dosierung sowie die Überwachung der Anwendung sind im Rahmen einer patientenbezogenen Qualitätssicherung durch die transfusionsverantwortlichen Personen festzulegen.

Besondere Vorsichtsmaßnahmen für die Beseitigung:

Die ordnungsgemäße Entsorgung von angebrochenen bzw. nicht mehr verwendbaren Präparaten ist entsprechend den Vorgaben der Einrichtung der Krankenversorgung sicherzustellen.

Die jeweils aktuellen "Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie)" sowie gegebenenfalls ergänzende Veröffentlichungen der Bundesärztekammer und des Paul-Ehrlich-Instituts sind zu berücksichtigen.

9. Datum der letzten Überarbeitung
27.02.2009

Pharmazeutischer Unternehmer / Inhaber der Zulassung:

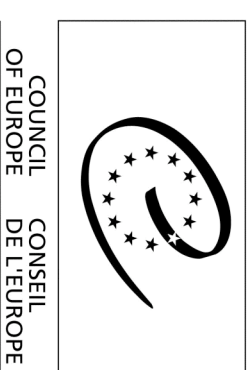
DRK-Blutspendedienst Baden-Württemberg - Hessen gemeinnützige GmbH
Friedrich-Ebert-Str. 107, 68167 Mannheim, Telefon (0621) 37060

Hersteller, der das Fertigarzneimittel für das Inverkehrbringen freigegeben hat:

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Mannheim, Friedrich-Ebert-Straße 107, 68167 Mannheim

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Frankfurt, Sandhofstraße 1, 60528 Frankfurt / Main

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Ulm, Helmholtzstraße 10, 89081 Ulm



Department of Biological Standardisation OMCL Network & Healthcare (DBO)

Pathogen Reduction Technologies for Blood Components for Transfusion: Updated Table March 2008

COUNCIL OF EUROPE
European Committee (Partial Agreement) on blood transfusion (CD-P-TS)

**Study coordinators: Miguel LOZANO¹, Jean-Pierre CAZENAVE², Marie-Emmanuelle BEHR-GROSS³
Translation to French: Michèle BACCQUE⁴**

1. M. Lozano. Hospital Clinic Provincial, Villarroel 170, 08036 Barcelona, Spain
2. J.-P. Cazenave. Etablissement Français du Sang (EFS-Alsace), 10 rue Spielmann, BP 36, 67065 Strasbourg, France
3. M-E. Behr-Gross. (Corresponding author: e-mail: marie-emmanuelle.behr-gross@edqm.eu), European Directorate for the Quality of Medicines & HealthCare, Council of Europe, 67081 Strasbourg, France
4. M. Baccqué, European Directorate for the Quality of Medicines & HealthCare, Council of Europe, 67081 Strasbourg, France

Pathogen Reduction Technologies for Blood Components for Transfusion: Updated Table March 2008

Country	FFP				Platelets		Remarks
	Quarantine	Solvent Detergent	Methylen Blue	Intercept	Intercept		
Australia	np	np	np	np	np	Pathogen reduction technologies remain under review	
Austria	23,100 units	50,100 units	0%	np	np		
Belgium	np	small percentage	96% minus a small percentage	4% Routine use in 2 centers. On-going evaluation in 1 center	17% Routine use or on-going evaluation at all centers		
Bosnia & Herzegovina	np	np	np	np	np	Pathogen reduction technologies remain under review	
Bulgaria	np	np	np	np	np		
Canada	some	some	np	np	np	Pathogen reduction technologies remain under review	
Croatia	Only plasma for fractionation	np	np	np	np		
Czech Republic	100% (about 51,000 liters)	np.	np	np	Only one very small clinical trial		
Finland	np	100% (about 60,000 units per year)	np	np	np		
France	161,597 units	130,504 units	Quarantine plasma to be replaced by methylene blue as of September 2008	5% (about 20,000 units). Use in routine in 1 regional center)	Routine use (100%) in 4 regional centers. On-going evaluation in 3 regional centers	Mirasol clinical trial phase III for platelets completed, under evaluation	
Georgia	np	np	np	np	np		
Germany	Around 90 %	Around 10%	Licensed for 2 establishments*	np	Licensed for 1 center*	* % not yet available	
Hungary	70%	np	np	np	np		
Iceland	np	np	np	np	np		
Ireland	np	About 100%	np	np	On-going evaluation in 1 center		
Italy	About 15%	About 20%	About 10%	Routine use in 2 centers. Evaluation in 1	Routine use in 8 centers. Evaluation in 1 center	Mirasol evaluation in 3 centers	
Malta	85%	np	np	np	np		
Moldova	np	np	np	np	np		

Country	FFP					Platelets		Remarks
	Quarantine	Solvent Detergent	Methylen Blue	Intercept	Intercept	Intercept		
Montenegro	np	np	np	np	np	np		
Netherlands	100%*	np	np	np	np	On-going evaluation by Sanguin	*6 months quarantine	
New Zealand	np	np	np	np	np	np		
Norway	np	100%	np	np	np	Routine use in 3 centers		
Poland	80% -100%*	np	np	np	np	np	* 4 months quarantine	
Portugal	30%	70 % About 65,000 units	np	np	np	np		
Romania	Some	np	np	np	np	np		
Serbia	np	np	np	np	np	np		
Slovak Republic	16,702 units (23% of plasma for transfusion)	np	np	np	np	np		
Slovenia	80%	np	np	np	np	70% of PC as of April 2008		
Spain	36%	np	64%	np	np	Routine use in 2 centers. Validation on-going in 2 centers		
Sweden	np	Few units	np	np	np	Routine use in 2 centers. Evaluation in 1 center		
Switzerland	80%	20%	Np	np	np	On-going evaluation in 1 center	-	
United Kingdom	np	All TTP patients	4% (imported plasma for patients below 16 years*)	np	np	np	* as vCJD precaution	
USA	Selected only (FFP WNV source season plasma)	np	np	np	np	Phase III clinical trials completed		

- FFP=fresh frozen plasma, np=not performed, PC=Platelet concentrate, TTP= Thrombotic Thrombocytopenic Purpura, WNV=West Nile Virus

Techniques de réduction des pathogènes dans les composants sanguins destinés à la transfusion - mise à jour mars 2008

Pays	PRC				Plaquettes		Observations
	Quarantaine	Solvant-détergent	Bleu de méthylène	Intercept	Intercept		
Allemagne	Env. 90 %	Env. 10%	Enregistré dans 2 établissements*	nr	Enregistré dans 1 centre*		*% pas encore connu
Australie	nr	nr	nr	nr	nr		Techniques de réduction des pathogènes en cours d'étude
Autriche	23 100	50 100	0%	nr	nr		
Belgique	nr	Faible pourcentage	96% moins faible pourcentage	4 % Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre	17 % Utilisation en routine ou évaluation en cours dans tous les centres		Techniques de réduction des pathogènes en cours d'étude
Bosnie-Herzégovine	nr	nr	nr	nr	nr		
Bulgarie	nr	nr	nr	nr	nr		
Canada	Certaines unités	Certaines unités	nr	nr	nr		Techniques de réduction des pathogènes en cours d'étude
Croatie	Plasma pour fractionnement uniquement	nr	nr	nr	nr		
Espagne	36%	nr	64%	nr	Utilisation en routine dans 2 centres. Validation en cours dans 2 centres		
Finlande	nr	100% (env. 60 000 unités par an)	nr	nr	nr		
France	161 597 unités	130 504 unités	Remplacement quarantaine par bleu de méthylène à partir de septembre 2008	5% soit env. 20 000 unités. Utilisation en routine dans 1 centre régional	Utilisation en routine (100%) dans 4 centres régionaux. Evaluation en cours dans 3 centres régionaux		Phase III d'essai clinique Mirasol pour les plaquettes terminée et en cours d'évaluation
Géorgie	nr	nr	nr	nr	nr		
Hongrie	70%	nr	nr	nr	nr		
Irlande	nr	Env. 100%	nr	nr	Evaluation en cours dans 1 centre		
Islande	nr	nr	nr	nr	nr		
Italie	Env. 15%	Env. 20%	Env. 10%	Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre	Utilisation en routine dans 8 centres. Evaluation en cours dans 1 centre		Mirasol en cours d'évaluation dans 3 centres
Malte	85%	nr	nr	nr	nr		
Moldova	nr	nr	nr	nr	nr		

Pays	PFC				Plaquettes		Observations
	Quarantaine	Solvant-détergent	Bleu de méthylène	Intercept	Intercept		
Monténégro	nr	nr	nr	nr	nr		
Norvège	nr	100%	nr	nr	Utilisation en routine dans 3 centres		
Nouvelle-Zélande	nr	nr	nr	nr	nr		
Pays-Bas	100%*	nr	nr	nr	Evaluation en cours par Sanguin		*Quarantaine de 6 mois
Pologne	80% - 100%*	nr	nr	nr	nr		*Quarantaine de 4 mois
Portugal	30%	70 % (env. 65 000 unités)	nr	nr	nr		
République slovaque	16 702 unités (23% du plasma pour transfusion)	nr	nr	nr	nr		
République tchèque	100% (env. 51 000 litres)	nr	nr	nr	Une étude clinique à très petite échelle uniquement		
Roumanie	Certaines unités	nr	nr	nr	nr		
Royaume-Uni	nr	Tous les patients PTT	4% (plasma importé pour les patients de moins de 16 ans*)	nr	nr		* à titre de précaution contre le vMCI
Serbie	nr	nr	nr	nr	nr		
Slovenie	80%	nr	nr	nr	Traitement de 70% des CP à partir d'avril 2008		
Suède	nr	petit nombre d'unités	nr	nr	Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre		
Suisse	80%	20%	nr	nr	Évaluation en cours dans 1 centre		
USA	Sur sélection uniquement (PFC à risque VNO saisonnier)	nr	nr	nr	Essais de phase III en cours		

- CP = concentrés plaquettaires – Env. = environ - nr = non réalisé - PFC = Plasma frais congelé – PTT= Purpura thrombotique thrombocytopénique, VNO = virus du Nil occidental – vMCI = variant de la maladie de Creutzfeldt-Jakob

February, 2009

Dear Valued Customer,

We have recently been informed of the early conclusion of INTERCEPT data collection for the HOVON 82 "TriPlate" study. We would like to take this opportunity to share with you the information currently provided to us by HOVON and Sanquin, to review information regarding the safety and efficacy of INTERCEPT Platelets, and to outline the steps we will take to clarify the results of this trial and ensure that you receive updated information as it becomes available.

The HOVON 82 study is a three-arm clinical trial comparing (a) platelets collected in 100% plasma, (b) platelets collected in reduced plasma (35%) with Intersol platelet additive solution, and (c) platelets collected in reduced plasma with Intersol and treated with the INTERCEPT Blood System. Platelet components are stored for up to 7 days and all are treated with gamma irradiation when ordered by treating physicians. The study was designed to include approximately 300 patients, resulting in approximately 100 patients for each study arm. The primary endpoint is 1-hour CCI, and secondary endpoints include 24-hour CCI and measures of hemostatic efficacy and safety. The study has been conducted by the HOVON foundation of Dutch oncology centers, in the role of principal investigator, with Sanquin supplying blood products. The study was initiated in 2007.

Cerus was not involved in the design, conduct or data analysis for the HOVON 82 study. Periodically, we have received informal reports that the study was ongoing and that the study was projected to finish in late 2008 or early 2009. Recently, we were informed that enrollment had been halted in the INTERCEPT test arm due to observation of lower CCI data. Cerus has not received data or analyses related to INTERCEPT or the other treatment conditions, and we understand from the study organizers that their analysis of the data is ongoing.

Apart from the HOVON 82 study, the efficacy and safety of INTERCEPT Platelets have been evaluated in eight Phase III/IV clinical studies and an ongoing haemovigilance program documenting over 30,000 transfusions to more than 5000 patients. In comparison to untreated platelets, some studies have observed reduced CI and/or CCI values for INTERCEPT Platelets. However, the studies have not shown clinically significant differences in hemostatic efficacy, and the data supported our 2002 CE mark approval confirming that INTERCEPT Platelets are not clinically different from conventional platelets. Furthermore, the INTERCEPT Platelet clinical dossier has been reviewed and approved by both Afssaps and the Paul Ehrlich Institute.

Our hemovigilance data have documented INTERCEPT Platelet safety in a broad patient population, and longitudinal analyses by long-term users in Belgium and France before and after INTERCEPT implementation have not revealed increased use of platelet components to patients receiving pathogen inactivated platelets.

We are confident that any results emerging from the HOVON data analysis will not change the safety and efficacy conclusions from the existing body of INTERCEPT studies. A summary of these studies is attached for your convenience.

We take the safety and efficacy of our products very seriously. We are in contact with the HOVON study investigators, and are attempting to gain access to the trial data. As soon as results are available to us, and in cooperation with HOVON, we will provide you with updated information.

Thank you for choosing INTERCEPT as your platelet pathogen inactivation treatment. Please feel free to contact us at any time with your questions on this or any other matter.

With kind regards,

Cerus Europe BV



Platelet Clinical Trials

Buffy-Coat Platelets (euroSPRITE Phase III Clinical Trial)	page 3
Apheresis Platelets (SPRINT)	page 4
Pediatric Study (Gent, Belgium)	page 5
Open-Label Transfusion Study - Germany	page 6
Open-Label Transfusion Study - Switzerland	page 7

INTERCEPT Haemovigilance Program

Summary to Date - Platelets	page 8
-----------------------------	--------

Buffy-Coat Platelets (EuroSprite Phase III Clinical Trial)

Trial Size:	103 patients (52 INTERCEPT + 51 control)
Primary Endpoint:	1-hour CI, 1-hour CCI
Study Sites:	Rotterdam, Netherlands Stockholm, Sweden Bristol, UK Strasbourg, France

This was a randomized, controlled, double-blinded clinical trial with 103 patients to evaluate the efficacy and safety of pooled buffy-coat platelets treated with the INTERCEPT Blood System. The majority (90 out of 103) of patients had a primary diagnosis of hematological diseases. Each patient received either untreated reference or INTERCEPT treated platelets for up to an 8-week period of transfusion support followed by a 4 week surveillance period. Efficacy data on platelet CI/CCI and safety data using assessments of clinical hemostasis based on WHO bleeding scores are reviewed in the following summary. Full results of the clinical trial have been reported (D van Rhenen et al. *Blood* 2003;101(6):2426-33).

The mean 1-hour CI for the INTERCEPT group ($27 \times 10^9/L$) was significantly less than the mean 1-hour CI for the reference group ($36 \times 10^9/L$, $p=0.03$). Similarly, the mean 24-hour CI for the INTERCEPT group ($16.4 \times 10^9/L$) was also significantly less than the mean 24-hour CI for the reference group ($24.7 \times 10^9/L$, $p=0.004$). However the mean INTERCEPT platelet dose was $3.89 \pm 1.0 \times 10^{11}$ per transfusion compared to $4.32 \pm 1.2 \times 10^{11}$ per transfusion for the reference group ($p \leq 0.001$). When the 1-hour CI was corrected for body size and platelet dose using the CCI, there was no significant difference between INTERCEPT (13.1×10^3) and reference (14.9×10^3) groups. However, there was a significant difference in the 24-hour CCI between INTERCEPT (7.4×10^3) and reference (10.6×10^3) groups. When all transfusions (INTERCEPT = 390; reference = 256) were analyzed for 1- and 24-hour platelet count increments using longitudinal regression analysis with multiple covariates, INTERCEPT platelets and reference platelets demonstrated comparable 1-hour CI ($p=0.53$) and 24-hour CI ($p=0.19$) for equal platelet doses.

Despite the observed differences in the 1-hour CI, 24-hour CI, and 24-hour CCI, INTERCEPT platelets demonstrated similar hemostatic efficacy to reference platelets. Before platelet transfusion, 71% of patients in the INTERCEPT group and 63% patients in the reference group had at least one episode of bleeding. After platelet transfusion the incidence of bleeding events was lower and similar in both groups (54% INTERCEPT and 49% reference; $p=0.62$).

CONCLUSIONS: The results of this trial show that equal doses of INTERCEPT buffy coat platelets provided similar 1- and 24-hour post-transfusion platelet CI, and patients treated with INTERCEPT buffy coat platelets exhibited adverse event profiles similar to those who received reference platelets.

Apheresis Platelets (SPRINT Phase III Clinical Trial)

Trial Size:	645 patients (318 INTERCEPT + 327 control)
Primary Endpoint:	Proportion of patients with Grade 2 bleeding (WHO criteria)
Study Sites:	12 blood centers in the United States

This was a randomized, controlled, double-blinded clinical trial with 645 patients to evaluate the efficacy and safety of apheresis platelets treated with the INTERCEPT Blood System. The majority (565 out of 645) of patients had a primary diagnosis of hematological diseases and 75% of patients in each group underwent hematopoietic stem cell transplant (HSCT). Each patient received either untreated reference or INTERCEPT treated platelets for up to a 4-week period of transfusion support followed by a one week surveillance period. Efficacy data on the proportion of patients with WHO Grade 2 bleeding and platelet CI/CCI are summarized in the following sections. Full clinical trial results have been published previously (J McCullough et al. *Blood* 2004;104(5):1534-41, S Murphy et al. *Transfusion* 2006;46(1):24-33, S Murphy et al. *Transfusion* 2006;46(1):24-33).

SPRINT was a powered non-inferiority trial to detect a small difference in Grade 2 bleeding in patients. The primary endpoint of the trial was met despite significant differences in the surrogate efficacy endpoints. INTERCEPT platelets were not inferior to reference platelets in maintaining hemostasis in severely thrombocytopenic patients for up to 28 days. The proportion of patients with Grade 2 bleeding was 58.5% for INTERCEPT group compared to 57.5% for reference group. The trial was not highly powered to demonstrate small differences in more severe bleeding of WHO Grades 3 or 4. These grades of bleeding were comparable between treatment groups (4.1% INTERCEPT group compared to 6.1% Reference group).

The mean 1-hour and 24-hour CI for the INTERCEPT group ($21.4 \times 10^9/L$ and $13.2 \times 10^9/L$) was significantly less ($p < 0.001$) than the mean 1-hour and 24-hour CI for the Reference group ($34.1 \times 10^9/L$ and $21.5 \times 10^9/L$). Similarly, the mean 1-hour and 24-hour CCI for the INTERCEPT group (11.1×10^3 and 6.7×10^3) was significantly less ($p < 0.001$) than the mean 1-hour and 24-hour CCI for the Reference group (16.0×10^3 and 10.1×10^3). Similar to the buffy-coat clinical trial, this finding primarily reflected the lower mean platelet dose of 3.7×10^{11} per transfusion in the INTERCEPT group compared to 4.0×10^{11} per transfusion in the Reference group ($p < 0.001$).

The time to onset of Grade 2 bleeding after beginning the study was not significantly different between INTERCEPT patients and reference patients ($p = 0.78$). However, Grade 2 bleeding occurred on a mean of 3.2 days in the INTERCEPT group as compared with 2.5 days in the reference group ($p = 0.02$). This finding again reflected the differences in the mean platelet dose per transfusion in the two groups. More INTERCEPT patients received platelet doses containing less than 3.0×10^{11} ($n = 190$) than reference patients ($n = 118$, $p < 0.01$). Comparison of patients receiving comparable platelet doses showed no significant differences between INTERCEPT and reference groups for bleeding or number of platelet or RBC transfusions; despite observation that the CI in response to INTERCEPT platelets (and transfusion intervals) were statistically significantly greater for the reference group. The lower CI values for INTERCEPT platelets suggested that some platelet injury may occur during the INTERCEPT process. This injury does not appear to result in a detectable increase in bleeding, however (S Murphy et al. *Transfusion* 2006;46(1):24-33).

CONCLUSIONS: These data support the conclusion that INTERCEPT platelets may be used according to standard transfusion guidelines whenever platelet transfusions are required. INTERCEPT platelets appear to be safe and effective in management of thrombocytopenic patients.

Pediatric Study

Trial Size:	83 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute transfusion reactions
Study Site:	Gent, Belgium

Following the CE mark registration, an investigator study of INTERCEPT Platelets in the pediatric population in routine clinical setting has been conducted in one center in Belgium (I Van Haute et al. *Vox Sang* 2006;91(s3):177).

The investigators transfused 500 INTERCEPT platelet components prepared by the buffy coat method to 83 pediatric patients with predominately hematology-oncology diagnoses. The platelet concentrates were prepared by pooling 5 whole blood-derived buffy coats. The pooled platelets were leukoreduced by filtration followed by INTERCEPT treatment for pathogen and leukocyte inactivation. The INTERCEPT Platelet concentrates were not gamma irradiated or tested for CMV. Platelet transfusions were ordered according to hospital guidelines and patients managed according to hospital clinical practice. Eligible pediatric patients were thrombocytopenic, expected to develop thrombocytopenia, diagnosed with a condition associated with thrombocytopenia or receiving therapy that will result in severe thrombocytopenia. Patients were mainly in oncology. Safety and efficacy, assessed by registration of adverse reactions < 24h after transfusion and calculation of corrected count increment [CCI] < 1.5hr post transfusion, were monitored.

Of the 500 transfusions, seven acute transfusion reactions in 6 patients have been noted. No transfusion reactions were of clinical severity greater than grade 1, including fever, urticaria, skin rash, nausea, vomiting, and abdominal pain. Bacterial cultures on INTERCEPT Platelet concentrates were negative. Efficacy was assessed for 493 of the 500 transfused INTERCEPT platelet concentrates. Transfusion episodes per patient was a mean of 6 (range 1 to 49). The number of platelets transfused per unit was a mean of 3.1×10^{11} . Mean platelet 1-hour CCI per transfusion episode was 12,300 (SD 9,450).

CONCLUSIONS: Transfusion of pediatric patients with INTERCEPT platelets provided therapeutic count increments. No unexpected transfusion reactions were attributed specifically to the use of INTERCEPT Platelets.

Open-Label Transfusion Study - Germany

Trial Size:	52 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute platelet transfusion reactions
Study Site:	University Lünebeck, Germany

This was an observational, single arm, open label study (P Schlenke et al. 2007 *Vox Sang* 2007;93:(s1):171). INTERCEPT platelets were transfused into thrombocytopenic patients according to standard local practices. The primary endpoint was the frequency of overall acute platelet transfusion reactions. INTERCEPT Blood System for platelets was used in place of gamma irradiation for prevention of TA-GVHD. Fifty-two patients were enrolled (54% male/ 46% female) with a median age of 57.5 yrs (range 22 to 78 yrs). All patients had hematological malignancy as primary disease with the largest proportion 40.4% (21/52) having a diagnosis of Acute Myelogenous Leukemia (AML). Most patients had received chemotherapy without stem cell transplant in 65.4% (34/52) and while 32.7% (17/52) patients had received autologous stem cell transplant with chemotherapy and/or radiotherapy.

A total of 560 INTERCEPT platelet components were administered with the mean number of transfusions per patient being 11.0 (median = 6, range 1-71). For 553 reported transfusions, 10 acute transfusion reactions were associated with 9 transfusions (ATR, 1.6%). All ATR were of low Grade 1 severity. Mean 24- hour CI and CCI were $10.9 \times 10^9/L$ and 6.6×10^3 , respectively.

CONCLUSIONS: No bleeding complications were attributable to the INTERCEPT platelets. This study confirmed the safety and efficacy of INTERCEPT platelets for support of thrombocytopenia.

Open-Label Transfusion Study - Switzerland

Trial Size:	46 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute platelet transfusion reactions
Study Site:	University Hospital Basel, Switzerland

This was an observational, single arm, open label study (L Infanti et al. *Vox Sang* 2008; 95(s1):289). INTERCEPT platelets were transfused into thrombocytopenic patients according to standard local practices. The primary endpoint was the frequency of overall acute transfusion reactions (ATR). INTERCEPT Blood System for platelets was used in place of gamma irradiation for prevention of TA-GVHD.

A total of 551 INTERCEPT platelet components were administered to 46 patients (61% male/39% female), median age 52.8 yrs (range 22 to 80 yrs). The majority (38 patients, 82.6%) of the patients had hematological malignancy as primary disease receiving chemotherapy without stem cell transplant (22 patients) or with stem cell transplant (12 allogeneic, 3 autologous). Preliminary analysis showed that the mean number of transfusion per patient was 12.0 ± 12.52 (range 1-58) and the mean 1-hour CCI was $10.12 \pm 8.06 \times 10^3$.

The rate of adverse events reported in this study was low; 97.8% of INTERCEPT platelet transfusions were without reported ATR.

CONCLUSIONS: This observational study found that transfusions with INTERCEPT platelets were well tolerated, in routine use and exhibited a safety profile consistent with that generally observed with conventional platelet components.

Post-Marketing Surveillance – Summary to Date

Transfusions Monitored: 31,225
 Study Sites: 21 sites / 11 countries

An active haemovigilance (HV) program was implemented following CE marking of INTERCEPT Blood System for platelets. This is an open label observational surveillance program to characterize and extend the safety profile of transfusing platelet components treated with the INTERCEPT process in a routine use setting and to document any unexpected adverse events that were not reported in early clinical trials and in patient populations that were not studied before. Please refer to haemovigilance publications for detailed analysis (Osselaer et al. *Vox Sang* 2008;94;(4):315-323, Osselaer et al. *Transfusion* 2008;48(6):1061-71, Osselaer et al. *Vox Sang* 2008; 95(s1):284, Osselaer et al. *Transfusion* 2009:in press, Cazenave et al. *Vox Sang* 2008;95(s1):302-6).

INTERCEPT Haemovigilance Program

Study	Transfusions	Countries	Sites	Locations
HV1	5,106	4	5	Mont Godinne, Belgium Pescara, Italy Bergen, Norway Trondheim, Norway Madrid, Spain
HV2	7,437	3	7	Brugge, Belgium Erasme, Belgium Mont Godinne, Belgium Rennes, France Strasbourg, France St Etienne, France Valladolid, Spain
HV3 (open)	~4,800*	8	11	Luebeck, Germany La Reunion, France Rennes, France St Etienne, France Reykjavik, Iceland Bergen, Norway Lisbon, Portugal Barcelona, Spain Madrid, Spain Uppsala, Sweden Basel, Switzerland
EFS Alsace	13,241	1	1	Strasbourg, France
Pediatric	641	2	2	Vienna, Austria Gent, Belgium

* Including 1,950 transfusions in La Reunion during CHKV epidemic and 489 transfusions in pediatric patients

To date approximately 200,000 doses of INTERCEPT platelets have been administered in patients and over 30,000 transfusions have been documented in the HV program. Data are summarized in three large interim analyses:

INTERIM ANALYSIS #1

One of the interim analyses was a meta-analysis of 16,631 transfusions of INTERCEPT platelet components to 3,274 patients (1,973 (60.3%) males/ 1297 (39.7%) females) with a mean age of 57 years (range <1 to 96 years) (Osselaer et al. *Vox Sang* 2008;94;(4):315-323, Osselaer et al. *Transfusion* 2008;48(6):1061-71, Rasongles et al. *Vox Sang* 2008;95(s1):15). Half of the recipients were hematology/oncology patients (1,643, or 50.2%) many of whom received hematopoietic stem cell transplants (n=307).

Transfusions associated with “related” (possibly related, probably related, or related) adverse events following INTERCEPT platelet transfusion were infrequent (110/16,631 n=0.66%). Eighty-two pts (2.5%) experienced at least one related adverse event following one or more INTERCEPT transfusion. Most reactions were of grade 1 severity and were representative of the events expected with conventional platelet transfusion. The most frequently reported signs/symptoms were chills, fever, and urticaria. Eleven SAE’s were reported, with one having causal relationship (hypotension possibly related) to INTERCEPT platelet transfusion. No cases of Transfusion Related Acute Lung Injury (TRALI), TA-GVHD, transfusion-related sepsis, or death due to an INTERCEPT transfusion were reported.

CONCLUSIONS: In summary, 99.34% of INTERCEPT platelet administrations were without a related transfusion reaction. Adverse events following INTERCEPT platelet transfusions classified as related to transfusions were infrequent, mild in severity, and representative of the events expected with platelet transfusion.

INTERIM ANALYSIS #2

The second analysis was based on the routine use experience from EFS-Alsace (Cazenave et al. *Vox Sang* 2008;95(s1):302-6). More than 99% of the patients hospitalized in Alsace that were transfused, according to conventional medical indications, in period 1 (1/1/2003-1/2/2004) with conventional leukoreduced platelet components (100% plasma) or in period 2 (1/9/2006-1/8/2007) with leukoreduced inactivated platelet components (INTERCEPT). Platelet components were prepared either from apheresis or buffy-coats (40/60). The average dose for all platelet components was $4.2 \pm 0.8 \times 10^{11}$. The demography of patients for both periods 1 versus 2 were, respectively, similar in terms of number of patients, age, and gender, and clinical indications: oncohematology (56%/58%), cardiovascular surgery (7%/6%), general medicine and surgery (37%/36%).

The number of platelet components transfused in period 1 was 10,629 (to 2,050 patients); and during period 2, 13,241 (to 2,069 patients) INTERCEPT platelet components were transfused. Although processing conditions changed during the two periods, the mean total dose of platelets per patient required in each period remained the same (26.0×10^{11} vs. 27.0×10^{11}). Thus no increase in platelet utilization was observed. During periods 1 and 2, 83.7%/85.2% of patients receiving platelet components were transfused with red blood cell concentrates (RBCC). Mean RBCC transfusions were similar in both periods (14.4 vs. 13.5 units/patient). Responses to platelet transfusion within 48 hours after transfusions were reported for $\geq 99.4\%$ platelet components transfused. The incidence of transfusion reactions per transfusion and per patient decreased with INTERCEPT platelet components (0.53% vs. 0.14% per transfusion, or 2.9% vs. 1.7% per patient). During the two periods, severity and imputation of transfusion reactions was similar and no bacterial sepsis was reported.

CONCLUSIONS: In summary, transfusion of platelet components treated with INTERCEPT to a broad patient population for a spectrum of indications was well tolerated in routine practice. The incidence of adverse events was less than untreated platelet components suspended in plasma. INTERCEPT offers the potential to improve the safety and availability of platelet components for transfusion. Importantly, an increase in the total platelet dose and RBCC transfused to patients in this study was not observed.

INTERIM ANALYSIS #3

The third analysis was based on the routine use experience in the Blood Transfusion Center of Cliniques Universitaires Mont Godinne (Osselaer et al. *Vox Sang* 2008;95(s1):284. Universal routine use of INTERCEPT platelets were initiated in 2003 for transfusion support of patients with thrombocytopenia. The blood component usage and clinical outcome observed for 3 years after adoption of INTERCEPT was compared to those observed during 3 years before INTERCEPT adoption.

The number of patients supported with platelets increased in the period after adoption of INTERCEPT, and approximately 91% required RBCC transfusions in both periods. The distributions (%) of indications for platelet transfusions (Hematology/Cardiovascular Surgery/Medical/Oncology), respectively, were approximately similar in the two periods (Pre: 39/32/22/7 vs. Post: 34/35/22/9). To compensate for loss of platelets due to INTERCEPT processing, approximately 10% more platelets were harvested for INTERCEPT components, resulting in larger average platelet collections (6.3 vs 6.8×10^{11}).

Days of platelet transfusion support (14.2 vs. 13.1), number of platelet transfusions per patient (9.9 vs. 10.1), and total platelet dose per patient (41.5×10^{11} vs. 42.0×10^{11}) did not increase significantly with universal implementation of INTERCEPT. INTERCEPT platelets had no impact on RBCC use.

CONCLUSIONS: In summary, the adoption of INTERCEPT Blood System into routine practice did not affect platelet or RBCC component usage over a 3 year observation period.

OVERALL POST-MARKETING SAFETY EXPERIENCE CONCLUSION: Periodic review of post-marketing surveillance data by Cerus indicates that INTERCEPT Platelet components, transfused in routine practice to a broad patient population, are safe and well tolerated. No unexpected safety issues related to the INTERCEPT Platelet product have been identified.

EUROSPRITE TRIAL

Cazenave JP, Davis K, Corash L. Design of clinical trials to evaluate the efficacy of platelet transfusion: the euroSPRITE trial for components treated with Helinx™ technology. *Seminars in Hematology* 2001;38,4 (S11):46-54.

van Rhenen D, Gulliksson H, Cazenave JP, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. *Blood* 2003;101(6):2426-33.

SPRINT TRIAL

McCullough J, Vesole DH, Benjamin RJ et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. *Blood* 2004;104(5):1534-41.

Murphy S, Snyder E, Cable R et al. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. *Transfusion* 2006;46(1):24-33.

Pineda A, McCullough J, Benjamin RJ et al. Pathogen inactivation of platelets with a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT trial. *Transfusion* 2006;46(4):562-71.

Snyder E, McCullough J, Slichter SJ et al. Clinical safety of platelets photochemically treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. *Transfusion* 2005;45(12):1864-75.

HAEMOVIGILANCE PROGRAM

Osselaer JC, Cazenave JP, Waller C et al. An active hemovigilance program characterizing the safety profile of 7,437 platelet transfusions prepared with photochemical treatment. *Vox Sang* 2008; 94; (4):315-323.

Osselaer JC, Doyen C, Defoin L et al. Universal adoption of photochemical treatment of platelet components: impact on platelet and red cell component use. *Transfusion* 2009. In press.

Osselaer JC, Messe N, Hervig T et al. A prospective observational cohort safety study of 5,106 platelet transfusions using components prepared with photochemical treatment. *Transfusion* 2008;48(6):1061-71.

Rasongles P, Angelini-Tibert MF, Simon P et al. Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a chikungunya virus epidemic in Ile de la Reunion. *Transfusion* 2009. In press.

ADDITIONAL CLINICAL PUBLICATIONS

Corash L. Confounding variables and co-interventions in the design of clinical trials: real life experience. *Vox Sang* 2002;83 (S1):261-266.

Janetzko K, Cazenave JP, Kluter H et al. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. *Transfusion* 2005;45(9):1443-52.

Simonsen AC, Johansson PI, Conlan MG et al. Transfusion of 7-day-old amotosalen photochemically treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. *Transfusion* 2006;46(3):424-33.

Slichter SJ, Raife TJ, Davis K et al. Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. *Transfusion* 2006;46(5):731-40.

Snyder E, Raife T, Lin L et al. Recovery and life span of ¹¹¹indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet A light. *Transfusion* 2004;44(12):1732-40.

van Rhenen DJ, Gulliksson H, Cazenave JP, et al. Therapeutic efficacy of pooled buffy-coat platelet components prepared and stored with a platelet additive solution. *Transfus Med* 2004;14(4):289-95.