Pathogen Inactivation
The Definitive Safeguard for the Blood Supply

Barbara J. Bryant, MD; Harvey G. Klein, MD

● Context.—Pathogen inactivation provides a proactive approach to cleansing the blood supply. In the plasma fractionation and manufacturing industry, pathogen inactivation technologies have been successfully implemented resulting in no transmission of human immunodeficiency, hepatitis C, or hepatitis B viruses by US-licensed plasma derivatives since 1985. However, these technologies cannot be used to pathogen inactivate cellular blood components. Although current blood donor screening and disease testing has drastically reduced the incidence of transfusion-transmitted diseases, there still looms the threat to the blood supply of a new or reemerging pathogen. Of particular concern is the silent emergence of a new agent with a prolonged latent period in which asymptomatic infected carriers would donate and spread infection.

Objective.—To review and summarize the principles, challenges, achievements, prospective technologies, and future goals of pathogen inactivation of the blood supply.

The present large scale use of blood and plasma transfusion may lead to the occurrence of a considerable number of such cases of hepatitis. It seems highly probable that they may be occurring not infrequently but are not being recognized. If one were not aware of the fact that jaundice may follow inoculation with homologous serum or plasma after a long latent period, one would be unlikely to attach any significance to a history of transfusion three months previous to onset of a patient’s illness.

Paul B. Beeson

More than halfway through the first decade of the 21st century, the blood bank community continues to confront challenges of blood safety in an age of emerging and re-emerging pathogens that threaten the blood supply. Blood collectors have celebrated the dramatic reduction in risk of transmissible infections, even as they have agonized over past failures of 20th-century systems to prevent widespread transmission of human immunodeficiency virus (HIV) and hepatitis viruses.2–4 The specter of a new, as yet undiscovered agent with an extended latent phase raises the concern that the current system of overlapping safeguards that protect patients from infectious blood components is still disturbingly vulnerable.

Until recently, the approach to reduce pathogens in the blood supply depended on a combination of donor education; donor selection (volunteer, nonremunerated); donor screening by specific, direct questioning regarding risk behavior; testing for selected known causative agents; consulting lists of previously deferred donors; meticulous examination and preparation of the phlebotomy site; and discarding components in quarantine or inventory if evidence of donor exposure or illness was reported postdonation. This strategy, although effective, is reactive. For example, years may elapse from the discovery of an etiologic agent to implementation of a licensed test. Depending on the prevalence and infectivity of the agent in question, the delay can result in thousands of transfusion transmissions of the pathologic agent.

Pathogen inactivation of blood products represents a proactive approach to blood safety. Inactivation technologies promise an additional layer of protection both from infectious agents that are known and from those not yet recognized as threats to the blood supply. An agent with broad antimicrobial activity could eliminate emerging agents before they were recognized as transfusion-transmitted pathogens. However, because of the very nature of blood—a liquid organ containing numerous labile proteins and fragile cells—and the wide array of potentially

Data Sources.—The current published English-language literature from 1968 through 2006 and a historical landmark article from 1943 are integrated into a review of this subject.

Conclusions.—The ultimate goal of pathogen inactivation is to maximally reduce the transmission of potential pathogens without significantly compromising the therapeutic efficacy of the cellular and protein constituents of blood. This must be accomplished without introducing toxicities into the blood supply and without causing neoantigen formation and subsequent antibody production. Several promising pathogen inactivation technologies are being developed and clinically tested, and others are currently in use. Pathogen inactivation offers additional layers of protection from infectious agents that threaten the blood supply and has the potential to impact the safety of blood transfusions worldwide.

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PATHOGENS—KNOWN AND SCREENED BY TESTING

In the United States, and almost universally in the developed world, blood is screened for human immunodeficiency viruses, type 1 and type 2 (HIV-1 and HIV-2); hepatitis B virus (HBV); hepatitis C virus (HCV); human T-cell lymphotropic virus, type I and type II (HTLV-I and HTLV-II); West Nile virus (WNV); and the causative agent for syphilis, Treponema pallidum (Table 1). Serologic testing is used to detect donor exposure to HBV, HTLV-I and HTLV-II, HIV, HCV, and T. pallidum; however, direct virus detection by nucleic acid–based testing is used for HIV-1 and HIV-2, HCV, and WNV testing. Nucleic acid-based testing is more sensitive than serologic methods thus decreasing the interval between infection of the donor and appearance of a positive marker (“latency” or “window period”) and increasing the safety of the blood supply. Calculated residual risks of transfusion-transmitted viruses in repeat US blood donors are approximately 1 in 2135,000 for HIV, 1 in 1935,000 for HCV, 1 in 144,000 for HBV, and 1 in 641,000 for HTLV-I and HTLV-II.23 Risks are estimated to be even lower in Europe.4 In 2003, only 6 cases of transfusion-transmitted WNV were documented in the United States from approximately 12.6 million blood units donated and 23 million blood transfusions.7 Not a case of transfusion-transmitted syphilis has been reported in the United States since 1966.8

Bacterial contamination has surpassed viral transmission as a transfusion associated risk in Western countries. Bacteriologic testing of platelets, which are stored at room temperature and thus more susceptible to bacterial growth, has been required since March 2004.9 Culture assays detect only about half of the contaminated units; the surrogate assays of pH and glucose concentration are virtually ineffective. The prevalence of positive microbiologic testing of platelet products lies between 1 in 2000 and 1 in 4000.10,11 Clinical sepsis is expected in 10% to 40% of transfused contaminated platelet units, and the risk of death from the transfusion of bacterially contaminated platelets is estimated at 1 in 2500 to 1 in 100,000.10-14 Although red cell units are refrigerated during storage, which reduces the risk of bacterial growth, a few organisms such as the Enterobacteriaceae Yersinia enterocolitica and Serratia liquefaciens thrive at 4°C. The bacterial contamination rate of red blood cells by Yersinia, resulting in post-transfusion septicemia, has been reported in as many as 1 in 40,000 transfusions. Overall, 70% of the reported cases of transfusion-related sepsis are from gram-positive organisms associated with skin flora at the phlebotomy site; however, more than 80% of the transfusion-related fatalities are caused by gram-negative organisms that circulate transiently in the blood of healthy donors.15-15

PATHOGENS—KNOWN BUT NOT SCREENED BY TESTING

Many pathogens that have the potential to invade the blood supply are not yet screened by testing because of low prevalence of the disease in the general population, unknown transmission rate of infection through transfusion, or the lack of a readily available test for the agent (Table 1). Often the lack of a developed testing methodology is the result of the pathogenic agent not being perceived as a significant threat and/or the lack of sufficient commercial interest in developing and marketing the assay. Agents with the potential to infect the blood supply are numerous and extensive. The known viral pathogens include more than 35 arboviruses such as the flaviviruses DENV-1 through DENV-4 and St Louis encephalitis virus, the togaviruses western and eastern equine encephalitis and Chikungunya, the Coronavirus severe acute respiratory virus, the Circovirus TT and its variant SEN, and the Delavarius hepatitis D. Other blood-borne viruses include the herpesviruses such as the Epstein-Barr virus, and human herpesvirus 6, human herpesvirus 7, and human herpesvirus 8, as well as the human parvovirus B19.16,17 Blood collectors do not routinely screen for the herpesvirus cytomegalovirus; however, some institutions test cellular components intended for premature infants and other immunocompromised patients such as organ and marrow transplant recipients for whom infection can prove lethal.
Numerous animal blood-borne agents (zoonotics) have been able to cause infection across species barriers; most do not cause disease but have the potential to do so. Of particular concern are the simian viruses such as the foamy viruses and SV 40, which have been reported in animal handlers and in some vaccine recipients.18,19

Protozoa that threaten the blood supply include the 4 malarial parasites Plasmodium vivax, P falciparum, P ovale, and P malariae, probably the most important transfusion-transmitted infection on a worldwide basis. Another red cell parasite, Babesia microti, found primarily in the northeastern United States, is readily transmitted by blood, but like Toxoplasma gondii the causative agent of toxoplasmosis, causes disease primarily in immunocompromised patients. Leishmania donovani and numerous other subspecies result in a high disease burden in the developing world. Borrelia burgdorferi, the cause of tick-borne Lyme disease, has the potential for blood transmission and another tick-borne illness, human granulocytic ehrlichiosis, has been reported from transfusion transmission of the bacterial pathogen Anaplasma phagocytophilum.5,17,20

Recently, 3 instances of transfusion transmission of the infectious protein or prion PrPsc have been reported in the United Kingdom, at least 1 of which almost certainly caused the human equivalent of "mad cow disease," variant Creutzfeldt-Jakob disease.21–23 Prions have been associated with a number of other transmissible spongiform encephalopathies (Creutzfeldt-Jacob disease, Gerstmann-Straussler-Scheinker disease, fatal familial insomnia), as well as the deer- and elk-associated prions that cause chronic wasting diseases in these animals. There is no blood screening test for the prion diseases.

**EMERGING AND REEMERGING PATHOGENS**

Of particular concern to blood transfusion safety is the danger posed by emerging or reemerging pathogens, agents whose incidence of infectivity and disease burden have increased in the past 20 years or threatens to increase in the near future. Such agents include newly discovered pathogens or known pathogens that have acquired new virulence factors or drug resistance. With climate changes, destruction of wildlife reserves, urbanization of rural lands and the forests, and population increases, contacts between humans and different animal species are changing, which abets microbial colonization across the species barrier.17 In addition, pathogens continue to adapt and mutate as was likely the case when HIV "emerged" in the 1970s.

Illustrative of an emerging pathogen that posed a recent threat to the blood supply is WNV, recognized previously for mosquito-borne outbreaks in the 1950s limited to southern Europe, the Middle East, Africa, Russia, and parts of Asia. Prior to 1999, there were no reported cases of WNV in the Western Hemisphere.24 Following identification of the first mosquito-borne cases in Queens, New York in 1999, 62 cases of WNV resulting in 7 fatalities, and a seropositive prevalence rate of 2.6% was reported in a limited study of 677 persons the same year.25,26 Between 2000 and 2001, another 86 cases and 11 deaths were confirmed. By 2002, WNV has spread through 44 states and Canada resulting in 4156 and 414 cases, respectively.17,24,27,28 In 2002, WNV was recognized as transmitted by blood transfusions, and 23 confirmed cases of transfusion-transmitted WNV were reported that year.17,29,30 West Nile virus estimated transfusion-transmitted risks were as high as 1 in 5000 blood donations in New York during the 1999 epidemic escalating to as high as 10 to 15 in 10 000 units in the most severely affected areas of the United States by 2002.2 Only the fortunate preexistence of a research assay for WNV, the unprecedented speed of developing a commercial screening test, and the relatively short viremic phase of this agent prevented more widespread transmission through transfusion. It is not hard to imagine the silent emergence of another new disease with a long latent period in which asymptomatic infected carriers would donate blood and spread infection worldwide.

**DEVELOPING COUNTRIES**

More than 66% of the world does not have access to safe blood.31 Most developing countries do not screen donor units for all viral markers because the technology is sophistication, the cost prohibitive, and/or the incidence of infected individuals so high that little blood would be available if all marker-positive donors were excluded. Approximately 50% of blood donations in developing nations are from family members or paid donors.32 Each year, unsafe blood transfusions in third-world countries result in 8 to 16 million HBV infections, 2.3 to 4.7 million HCV infections, and 80 000 to 160 000 HIV infections.32 Pathogen-reduced blood could have a dramatic impact on blood safety in these circumstances.

**PATHOGEN REDUCTION VERSUS PATHOGEN INACTIVATION**

The terms pathogen reduction and pathogen inactivation are often used interchangeably. Both definitions are relative and neither guarantees sterility of a blood component. Pathogen removal is generally considered a separate process for modeling plasma fractionation processes but is considered part of the pathogen reduction process for the cellular components. Potential infectivity is determined by several measures. Genomic equivalents refers to the number of viral particles detected by molecular techniques; the particles may or may not be infectious. The TCID50 refers to the dose of viral particles in a tissue culture assay capable of infecting 50% of susceptible animals. Most candidate pathogen inactivation methods report the TCID50 log reduction in organisms, usually from specimens “spiked” with specific laboratory-derived organisms but occasionally from specimens derived from patients infected in vivo. Although the “acceptable” log-reduction number for a given organism is not standardized, experience suggests the fewer than 4 log reductions may be insufficient and greater than 6 log reductions is ordinarily sufficient.

No inactivation method can be tested against every conceivable organism. Different classes of organisms are selected to cover the range of potential pathogens. Often similar “model” agents are used in place of pathogens that cannot be cultured in the laboratory. Each pathogen inactivation technology has its own profile of the spectrum of pathogens affected, effectiveness against high viral loads, degree of reduction in infectivity in any given blood component, and adverse reactions. The definitive determination of effectiveness (and safety) comes only after years of clinical use. The challenge of pathogen inactivation is to reduce maximally the greatest number of potential pathogens in blood without significantly compromising the cellular or protein constituents or introducing some new toxicity, carcinogenicity, or teratogenicity. Each
method is subjected to rigorous protocols for toxicity detection; however, no series of laboratory studies can predict safety with absolute certainty, particularly in the most vulnerable patients such as neonates, gravid women, and critically ill immunosuppressed patients.

**PATHOGEN INACTIVATION OF PLASMA AND PLASMA DERIVATIVES—EARLY SUCCESSES**

**Cohn Fractionation and Heat Treatment**

In 1946, Edwin Cohn published what has become the most widely used commercial fractionation method for plasma proteins (Figure 1). Cohn fractionation involved multiple steps of precipitation and physical separation by centrifugation or filtration of the precipitant and effluent using changes in pH, temperature, ionic strength, and ethanol concentration gradients. Because pathogen reduction occurred after different steps of precipitation and filtration in the fractionation process, proteins isolated later in the schematic were generally safer from infectious agents. However, pathogen removal depends critically on the protein concentration and specific separation conditions. The albumin and globulin fractions proved extremely safe, particularly regarding transmission of hepatitis viruses and HIV. However, the process was not infallible and deviations from proper processing have resulted in disease transmission. Also of note is that some of the most important plasma protein derivatives are separated early in the fractionation process and do not reap the benefits of added layers of fractionation and processing (Figure 1).

In 1985, when viral inactivation of specific plasma protein fractions was introduced, more than 90% of hemophiliacs were already infected with HCV and most were HIV positive from prior exposure to plasma-derived clotting factors made from pools of tens of thousands of liters of plasma. Antihemophilic factors (factor VIII) and factor IX complexes are precipitated in the first and second Cohn fractionation steps, respectively. Since the advent of additional multistep viral inactivation processing of plasma derivatives, no new cases of HBV, HCV, or HIV transmission have occurred in the United States. Currently at least 2 different methods of pathogen reduction or removal are included in production of each plasma protein concentrate.

Figure 1. **Flow diagram of Cohn plasma fractionation process.** Major fractionation steps, intermediates, and final products are listed. The “Additional Processing Steps” heading includes manufacturer-specific operations (such as filtration, or chromatography) that vary among manufacturers. Reprinted from Burdick et al with permission from Elsevier.
Plasma protein concentrates have been made from human albumin, immunoglobulins, and Triton X-100 is removed by chromatographic adsorption. The tri-butyl phosphate is removed by oil extraction, and Triton X-100 is removed by chromatographic adsorption. The solvent and detergent combination disrupts the lipid envelope and prevents the virus from binding to cells and replicating. Incorporation of a virucidal detergent and solvent (solvent 1% tri-n-butyl phosphate and the detergent 1% Triton X-100 for 4 hours at 30°C) into the processing of plasma from pools of approximately 12,000 donors produces a product known as solvent-detergent (S-D) plasma. The tri-n-butyl phosphate is removed by oil extraction, and Triton X-100 is removed by chromatographic adsorption. Plasma protein concentrates have been made from S-D treated plasma, and fresh frozen plasma (FFP) equivalent has been licensed in the United States and Europe. As with the heat-inactivated coagulation factors, S-D technology does not inactivate the nonenveloped viruses. Solvent-detergent treatment also results in some loss of integral plasma proteins such as α2-antiplasmin and protein S. α2-Antiplasmin is crucial in maintaining hemostasis especially in patients with liver dysfunction. Decreased levels of the natural anticoagulant protein S can lead to a hypercoagulable state especially when massive S-D plasma transfusions are used as the major source of plasma replacement in massive traumas or therapeutic plasma exchanges. These concerns, along with economic factors, resulted in the removal of S-D plasma from the US market. Solvent-detergent plasma is still used widely in Europe. Recently, 2 new S-D treatment procedures have been developed for single unit or mini pools of 10 to 12 units of plasma that yield more than 90% mean recovery of coagulation factors, anticoagulants (including protein S), protease inhibitors (including α2-antiplasmin), total protein, albumin, and immunoglobulins. Single unit and mini pool S-D treatment technologies show promise and have the potential to overcome some of the drawbacks of the original industrial S-D treatment processes such as the need for plasma fractionation facilities, large-scale pooling of plasma, and reduction of protein S and α2-antiplasmin.

**Nanofiltration**

Nanofiltration, also known as viral filtration, is a robust viral removal system that uses sieving or size exclusion to remove infectious agents. Most viruses range in size from approximately 20 to 200 nm, and the typical nanofilter has a pore size of 15 to 40 nm (Table 1). Nanofiltration has proven effective in removing a wide range of viruses including the nonenveloped viruses and may even remove viruses smaller than the filter pore size. Proposed mechanisms for the removal of these small agents include viral adsorption at the charged membrane surface, aggregation of the smaller viruses, or association of the viruses with cellular components. Nanofiltration typically results in a 4 to 6-log removal of most viruses, with 90% to 95% recovery of protein activity. Prion removal from plasma has been achieved when a 35-10 pore size is used; however, the amount of prion removed depends on the degree of aggregation of the prions. Critical factors for effective nanofiltration include ratio of protein volume to filter area, temperature, pressure, and flow rate of filtration. Extensive experience with nanofiltration fails to demonstrate protein alteration or neoantigen formation. Many currently licensed plasma-derived coagulation factors and immunoglobulins that are subjected to heat, pasteurization, and/or S-D treatment are also nanofiltered. All classes of plasma protein fractions such as antithrombin, C-1 inhibitor, protein C, fibrinogen, and ceruloplasmin have been nanofiltered without apparent change in the protein characteristics. The US Food and Drug Administration recommends viral filtration during the production of monoclonal antibodies.

**Methylene Blue**

Methylene blue (MB) is a photosensitive phenothiazine dye that has been used in Europe for approximately 15 years for the pathogen inactivation of single units of plasma. Phenothiazine dyes were first used clinically by Paul Ehrlich in the 19th century and have been recognized for their virucidal properties since the 1930s. Methylene blue has an affinity for nucleic acids and the surfaces of viruses. When MB-treated plasma is exposed to ultraviolet (UV) light, most enveloped viruses are easily inactivated; however, nonenveloped viruses are more resistant to treatment. Intracellular viruses are not inactivated by MB/UV light, but freezing and thawing plasma often disrupts the cell membranes of leukocytes thus liberating the viral particles and leaving them susceptible to MB pathogen inactivation. Residual intact white blood cells containing viruses are removed by a micropore filter. Neither protozoa nor bacteria are inactivated by MB treatment. Plasma proteins are moderately affected; fibrinogen and factor VIII activity is reduced by up to 30%.

Methylene blue treatment can be used for pathogen inactivation of single units of plasma, thus eliminating the risk of large plasma pools currently used to manufacture S-D plasma. First-generation MB systems involved the addition of an MB stock solution to each thawed plasma unit for a final concentration of 1μM. Then the MB-treated plasma was subjected to a white light luminescence of 45,000 lux or more for 1 hour. A more advanced commercially manufactured MB system by MacoPharma (Mouvaux, France) uses an inline system consisting of a
membrane filter, MB dye, illumination bag, elimination filter, and storage bag (Figure 2). The 0.65-μm membrane filter removes platelets, leukocytes, and debris. The plasma then passes through tubing containing an MB pill that dissolves as the plasma flows through the tubing into the illumination bag. The MB-containing plasma is subjected to double-sided illumination by sodium high-intensity low-pressure lamps emitting yellow light at a wavelength of 590 nm for 15 to 20 minutes. Plasma is then passed through an MB elimination filter that removes greater than 95% of the residual dye and photoderivative byproducts (especially phenothiazide dyes azure A and B) that exhibit in vitro mutagenic properties.58

Millions of MB single units of plasma have been transfused in Europe without unexpected adverse outcomes.58 Methylene blue–treated plasma has replaced S-D plasma in Belgium and is the sole plasma product used in the United Kingdom for patients born after January 1, 1996. The long-term effects of exposure to even minute amounts of residual MB and its photoderivatives have not been studied in larger cohorts. To date, no adverse effects have been reported; however, long-term studies of carcinogenicity and reproductive toxicity have not been conducted.

ONGOING DEVELOPMENT OF PATHOGEN INACTIVATION—PLASMA COMPONENTS AND PLATELETS

Until recently, attempts at pathogen reduction for cellular blood components have achieved little success. Leukoreduction of blood has reportedly decreased the risks of transfusion-transmitted cell-associated viruses such as cytomegalovirus, HTLV-I and HTLV-II, and probably Epstein-Barr virus and human herpesvirus 8, because leukocytes are the principal reservoir for these infectious agents. Although graft-versus-host disease is not traditionally viewed to be a “pathogen-related” consequence of transfusions, alloimmune donor lymphocytes can become pathogenic in certain instances. Blood irradiation is currently used to eliminate the risk of transfusion-associated graft-versus-host disease in selected patient groups. Irradiation renders the potentially pathogenic T cells incapable of replication, whereas removal of lymphocytes by filtration has proved ineffective. A pathogen inactivation technology that effectively disrupts the replication of microbial nucleic acids should also prevent transfusion-associated graft-versus-host disease and eventually eliminate the need for blood irradiation.

Psoralens

Psoralens are small, planar molecules that cross cell membranes and viral capsids and intercalate between the bases of the nucleic acids (Figure 3). On illumination with UV-A (320–400 nm), the psoralens react with the DNA or RNA pyrimidine bases to form covalently bonded intranucleic and internucleic acid cross-links (Figure 4). This cross-linking prevents replication and transcription of the RNA or DNA.59,60 Psoralen treatment with UV-A light results in the reduction of a broad array of viruses, bacteria, and protozoa to a level unlikely to transmit infection (Table 2).

Aminomethyl-trimethyl psoralen, a 3-ringed synthetic psoralen known as amotosalen hydrochloride or S-59, has been extensively tested in the pathogen reduction of platelets and plasma (Figure 3). Amotosalen is advantageous for this process because lower doses of UV-A can be applied for shorter periods, thus protecting platelets from high-energy UV damage, without the loss of virucidal activity.61 Amotosalen and photochemical treatment have demonstrated an acceptable safety profile through extensive toxicologic studies for acute toxicity, repeat dose toxicity, reproductive toxicity, phototoxicity, and mutagenic and carcinogenic potential.

To pathogen inactivate with psoralens, the platelet concentrate must be volume reduced and resuspended in 30% to 45% plasma and 70% to 55% platelet additive solution containing sodium chloride, acetate, citrate, and phosphate (Figure 5).62–65 The amotosalen (150 μmol/L) is added to the platelet and incubated for 3 to 5 minutes.66 The product is then exposed to 3 J/cm2 UV-A light for 3 minutes with agitation. After UV-A treatment, approximately 90% of the psoralen has been photodegraded to byproducts. The remaining psoralen and byproducts are removed by a “compound absorption disc” to avoid potential toxicity.64 The photochemically treated (PCT) platelets are transferred to a bag containing the S-59 absorbent and incubated at room temperature with agitation for 4 to 16 hours before transfer to the final platelet storage bag (Figure 3).65,66

INTERCEPT, an S-59 amotosalen system, has been evaluated in 3 clinical trials in Europe (euroSPRITE) involving 166 thrombocytopenic patients. Two of these trials evaluated whole blood–derived buffy coat platelets, and 1 assessed single-donor apheresis platelets. These studies demonstrated that when equal platelet doses were transfused, the INTERCEPT and conventional platelet transfusions resulted in comparable posttransfusion platelet count increments without significant differences in adverse reactions.

In the United States, the SPRINT trial evaluated the hemostatic efficacy and safety in 645 thrombocytopenic oncology patients receiving INTERCEPT single-donor apheresis platelets collected on the Amicus separator.65 A total of 4719 platelet transfusions were given: 2678 INTERCEPT platelets and 2041 conventional platelets. The incidence of World Health Organization grade 2 bleeding between the
Figure 3. Chemical structure of 7 pathogen reduction agents: psoralen S-59 (Amotosalen), riboflavin, binary ethyleneimine (PEN110), S-303 (Helinx), thiopyrylium (TP), dipyridamole (DP), and TO (thiazole orange). These planar structures intercalate into the helical regions of nucleic acids forming intranucleic and internucleic acid cross-links thus preventing replication and transcription of the RNA and DNA. Although the other chemicals have fixed bonds, TP, DP, and TO have flexible single bonds capable of rotation (designated by arrows). Dipyridamole is a competitive inhibitor of TP, and the dotted ellipses in the DP structure indicate the areas that are similar to DP. Reprinted from Klein,35 Wagner et al,105 and Skripchenko et al106 with permission from Blackwell Publishing.
**Figure 4.** Intercalation of pathogen reduction agent. Planar structure permits intercalation of pathogen reduction agent into the helical regions of nucleic acids. Activation by illumination or change in surrounding pH results in cross-linking of DNA and RNA thus blocking the replication and transcription of the nucleic acids or photolytic breakage of the nucleic acids preventing replication.

### Table 2. Inactivation of Viruses and Microbial Pathogens by Treatment With Amotosalen and UV-A Light*109–113

<table>
<thead>
<tr>
<th>Viral Pathogens</th>
<th>Genome</th>
<th>Enveloped</th>
<th>Infectivity Log Reduction Amotosalen/UV-A Platelets</th>
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<tr>
<td>Human immunodeficiency 1/2</td>
<td>ss-RNA</td>
<td>+</td>
<td><img src="https://via.placeholder.com/150" alt="" /> cell free; &gt;6.1 cell associated</td>
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<tr>
<td>Human T-cell lymphotropic I/II</td>
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<td>4.7/5.1 cell associated</td>
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<td>ss-RNA</td>
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<td>Yersinia enterocolitica</td>
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<table>
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<th>Microbial Pathogens</th>
<th>Class</th>
<th>Infectivity Log Reduction Amotosalen/UV-A Platelets</th>
</tr>
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<td>Treponema pallidum</td>
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<td>Spirochete</td>
<td>&gt;6.9 ± 0.1</td>
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<td>Protozoa</td>
<td>&gt;5.3</td>
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</tr>
<tr>
<td>Leishmania mexicana</td>
<td>Protozoa</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Leishmania major</td>
<td>Protozoa</td>
<td>&gt;4.5</td>
</tr>
</tbody>
</table>

* Apheresis platelets were treated with 150 μmol/L Amotosalen and 3 J/cm² UV-A light. ss indicates single stranded; ds, double stranded; +, presence of an envelope; -, no envelope; Neg, gram negative; and Pos, gram positive.
groups was comparable (58.8% PCT vs 57.7% control), and the incidence between the groups of World Health Organization grade 3 or 4 bleeding was equivalent (4.1% PCT vs 6.1% control). Patients receiving the INTERCEPT platelets had lower posttransfusion platelet count increments (corrected count increment of $11.1 \times 10^9$ PCT vs $16.0 \times 10^9$ control), required more platelet transfusions (8.4 PCT vs 6.2 control), and had a shorter interval between transfusions (1.9 days PCT vs 2.4 days control). Transfusion reactions were fewer with PCT platelets (3.0% PCT vs 4.4% control; $P = .02$) most likely attributed to the reduced volume of plasma in the PCT units as well as increased leukocyte inactivation resulting in less cytokine production during storage.

Explanations for the differences in posttransfusion platelet count increments in the PCT platelets were partly explained by the lower mean platelet dose ($3.7 \times 10^{11}$ PCT vs $4.0 \times 10^{11}$ control; $P < .001$) and disproportionate number of transfusions containing platelet doses less that $3.0 \times 10^{11}$ (n = 190 or 20% PCT vs n = 118 or 12% control; $P < .01$). The rationale for the observed lower platelet increments between PCT platelets and control platelets.65

There was no evidence of coagulation factor activation as measured by activated protein C, factor V, VIII, IX, X, XII, protein C and protein S as well as antithrombin were maintained functional activity of approximately 87%. Protein C and protein S as well as antithrombin were maintained at more than 95% pretreatment activity levels. Factor VIII levels, although decreased to 73% to 98% of control thawed plasma. Factor VIII levels, although decreased to 73%, are still sufficient for therapeutic use. There were no significant differences in the quantity and activity of the von Willebrand factor, the pattern and distribution of the von Willebrand multimers, or the activity of the ADAM TS-13 (von Willebrand factor) metalloprotease. Fibrinogen maintained functional activity of approximately 87%. Protein C and protein S as well as antithrombin were maintained at more than 95% pretreatment activity levels. There was no evidence of coagulation factor activation as a result of treatment. The factor VII kinetics of posttransfusion PCT plasma was compared with standard FFP in a crossover study. Autologous plasma collected by apheresis was divided and one half was treated with amotosalen and UV-A light. The other half was handled as stan-

Figure 5. Amotosalen (S-59) pathogen inactivation system for platelets. Platelets are volume reduced and resuspended in 35% plasma and 65% platelet additive solution. Using an integrated closed system, the platelets are passed through a pouch containing S-59 and into an illumination bag. The platelets are illuminated and then transferred to a bag containing the compound absorption disc (CAD) that lowers the concentration of residual psoralen and byproducts to reduce toxicity. After incubation with the CAD, the platelets are then transferred to the final storage bag. Reprinted from Klein35 with permission from Blackwell Publishing.
and found to have comparable hemostatic properties when salen and UV-A treated plasma has also been evaluated plasma has not been tested in clinical trials.76 As of yet, cryoprecipitate prepared from PCT and UV-A treated plasma. Preliminary studies indicate activities were expressed as specific activity per gram of the normal reference ranges for CSP. When the factor activities were expressed as specific activity per gram of plasma protein to compensate for the dilutional factor, the differences were no longer significant.77

Riboflavin/UV Light

Riboflavin (vitamin B2), a naturally occurring essential nutrient, has been used as a pathogen-inactivating agent for platelets and plasma. Riboflavin is a 3-ringed planar structure that binds to nucleic acids and intercalates between DNA and RNA bases (Figures 3 and 4). On activation of cross-linked riboflavin with UV or visible light, guanosine bases are oxidized resulting in single-strand breaks in the nucleic acids. The mechanisms of damage to the nucleic acids in this process are 3-fold: (1) direct electron transfer reaction by the oxidation of guanosine, (2) generation of oxygen radical, and (3) production of peroxide and hydroxyl radicals.79 The damaged and disrupted nucleic acids are incapable of repair and replication. Toxicities of riboflavin and its photoderivative byproducts do not appear to cause concern because riboflavin and its breakdown products are present in many food and natural products.79 Removal of the spent riboflavin and products postillumination may not be necessary in a pathogen inactivation system using riboflavin. The US Food and Drug Administration has classified riboflavin as a ‘‘Generally Regarded As Safe’’ compound.80 Riboflavin and its metabolic products are normally found in the blood. Standard phototherapy for neonatal jaundice activates riboflavin without apparent toxicities to the newborn.

The Mirasol PRT System contains 30 mL of riboflavin (500μM) in a light-protective opaque foil pouch and a pathogen-reduction illumination/storage bag. Platelets or plasma are sterile connected to the system, and 250 mL of plasma or platelet product is transferred to the bag containing the riboflavin diluting it to a final concentration of 50μM. The riboflavin-treated product is subjected to 6.24 J/mL double-sided UV illumination in a temperature-controlled environment with agitation for 10 minutes.81 Riboflavin/UV light treatment has been evaluated in preclinical studies and found to result in reduction of infectivity by many pathogens including WNV, intracellular HIV, bacteria, and protozoa (Table 3).81–84 The Mirasol system demonstrated successful pathogen reduction of selected pathogen-spiked platelet units after treatment and storage for 5 days at 20°C to 24°C. The viral reduction of platelets was sufficient to close the window period of transmission of HIV and chronic phase of parvovirus B19, eliminate the viremic period of WNV, prevent infection due to Staphylococcus epidermidis and Escherichia coli, and result in a 5- to 6-log reduction of Leishmania donovani infantum.81,84 Additionally, Leishmania-spiked plasma units treated with riboflavin/UV light demonstrated a 5- to 7-log reduction in parasites.84 Studies have demonstrated significant differences in control and treated platelets after 5 days of storage at 20°C to 24°C in regards to accelerated changes in platelet morphology, increased platelet activation, and induced partial platelet aggregation suggesting that treatment produces increased glycolysis. However, a recent report indicates that the increased platelet glycolytic

<table>
<thead>
<tr>
<th>Viral Pathogens</th>
<th>Genome</th>
<th>Enveloped</th>
<th>Infectivity Log Reduction Riboflavin/UV-A Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immunodeficiency 1/2</td>
<td>ss-RNA</td>
<td>+</td>
<td>5.93 ± 0.20 cell associated and cell free;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.46 ± 0.39 intracellular</td>
</tr>
<tr>
<td>West Nile</td>
<td>ss-RNA</td>
<td>+</td>
<td>5.19 ± 0.50</td>
</tr>
<tr>
<td>PPV (surrogate for human erythro B19)</td>
<td>ss-DNA</td>
<td>–</td>
<td>≥5.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbial Pathogens</th>
<th>Gram</th>
<th>Aerobes Vs Anaerobes</th>
<th>Infectivity Log Reduction Riboflavin/UV-A Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Neg</td>
<td>Aerobe</td>
<td>≥4.38</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Pos</td>
<td>Aerobe</td>
<td>≥4.5</td>
</tr>
<tr>
<td>Leishmania donovani infantum</td>
<td>Protozoa</td>
<td></td>
<td>≥5.0</td>
</tr>
</tbody>
</table>

* Apheresis platelets were treated with 50μM riboflavin and 6.2 J/mL UV-A light. PPV indicates porcine parvovirus; ss, single stranded; ds, double stranded; +, presence of an envelope; −, no envelope; Neg, gram negative; and Pos, gram positive.
flux stimulated by UV-induced stress is not the direct cause of these platelet storage lesions.85 Riboflavin/UV light treated plasma has been shown to retain acceptable levels of clotting factors without evidence of increased complement activation.86 Additional clinical studies are currently underway.

**Thionine/UV Light**

Platelets are extensively damaged by the MB/UV light technology that is successfully used in the pathogen inactivation of plasma. However, thionine, another phenothiazine dye, can be safely used in place of MB with the same type low-pressure sodium UV light leading to a comparable spectrum of pathogen inactivation.58 Measurements of platelet function such as change in morphology, aggregation, and activation as well as biochemical parameters are just slightly affected by thionine/UV light treatment. There is no change in storage duration after treatment. However, thionine with UV light treatment alone does not inactivate bacteria and leukocytes. This can be accomplished with an additional step consisting of UV-B irradiation for approximately 2 to 4 minutes (1.2–2.4 J/cm²).59 An added advantage of the extra UV-B irradiation step is more effective viral inactivation. Thionine-based pathogen inactivation systems are currently under development and clinical testing.

**ONGOING DEVELOPMENT OF PATHOGEN INACTIVATION—WHOLE BLOOD AND RED BLOOD CELLS**

Pathogen inactivation of components containing red blood cells presents a particularly challenging dilemma. Methods using photoactivation must do so in the red wavelength region of the light spectrum above that of hemoglobin to avoid absorption or scattering of the light by the red cell. Many potential methods of pathogen inactivation easily alter or disrupt the red cell membrane resulting in decreased red cell survival, hemolysis, or immunogenicity.

**S-303**

S-303 (Helinx), a small molecule designed for pathogen inactivation treatment of red blood cells, is an alkylating agent derived from a quinacrine mustard that belongs to a class of “frangible anchor linker effectors” compounds (Figure 3). Frangible anchor linker effectors compounds contain an intercalator group that inserts into the helical region of DNA and RNA, an effector group that permits covalent attachment of nucleic acids, and a central frangible bond that orchestrates the degradation of the compound.87 S-303 is a positively charged planar structure that easily intercalates into the helical regions of the negatively charged nucleic acids (Figure 4). The process does not depend on light for activation. Frangible anchor linker effectors compounds are activated by a shift from a lower pH storage environment to the higher neutral pH of red blood cells causing hydrolysis and generating S-300, the primary degradation product; cross-linking of the DNA and RNA ensues. S-300 is rapidly metabolized and excreted leaving no detectable parent compound by mass spectroscopy. The remaining free degradation products are absorbed and removed by a compound removal step. S-303 binds to other proteins and cell membranes as well as to nucleic acids, and up to 20% can potentially remain bound to the surface or contained within the red cells.

S-303 has demonstrated pathogen inactivation of a wide range of viruses, bacteria, and protozoa.88 No unexpected toxicities have been described. Assays for red cells storage lesions (extracellular potassium leakage, plasma free hemoglobin, adenosine triphosphate, 2,3-diphosphoglycerate, glucose, and lactate) are comparable to control red cells. The red cell function appears to be normal, and in vivo51 Cr-labeled survival studies exceed the standard of 75% at 24 hours.

Two randomized, controlled phase III trials involving patients either undergoing first-time cardiovascular surgery or with hemoglobinopathies were in progress when antibodies to residual red cell bound S-303 were discovered in 2 subjects.89 The trials were suspended as a consequence of these findings. Additional studies have revealed that 1% of patients and healthy donors that had never been exposed to S-303 had naturally occurring antibodies that reacted with S-303 treated red blood cells. Modifications have been made to the S-303 treatment process to reduce the amount of red cell bound S-303 in attempts to eliminate immunoreactivity and immunogenicity.90 Preliminary finding indicate that red cells from the modified S-303 treatment process were crossmatch-compatible with the anti–S-303 antibodies formed after exposure to the original S-303 formulation as well as the anti–S-303 antibodies found in the patients and donors never exposed to S-303. The antibodies do not appear to impair transfusion or to pose any clinical problem.

**PEN110**

PEN110 is an INACTINE compound capable of inactivating a broad spectrum of viruses, bacteria, protozoa, and mycoplasma in red blood cells (Table 4).91–98 INACTINE is a low-molecular-weight, rapidly soluble, positively charged ethylenediamine oligomer with high selectivity for nucleic acid guanine bases (Figure 3).99 It covalently binds negatively charged phosphate groups and undergoes activation resulting in a reactive species with an aziridino group that forms a covalent bond with the N-7 position of guanine. Subsequent alkylation opens the imidazole ring structure resulting in breakage of the nucleic acid strands and disruption of replication and transcription (Figure 4).

Red blood cells are leukoreduced prior to the addition of PEN110 at a concentration of 0.1% (vol/vol). The mix-

<table>
<thead>
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<th>Enveloped</th>
<th>Infectivity Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immunodeficiency 1/2</td>
<td>ss-RNA</td>
<td>+</td>
<td>5.57</td>
</tr>
<tr>
<td>BVDV (surrogate for hepatitis C)</td>
<td>ss-RNA</td>
<td>+</td>
<td>&gt;5</td>
</tr>
<tr>
<td>West Nile</td>
<td>ss-RNA</td>
<td>+</td>
<td>5–7</td>
</tr>
<tr>
<td>PPV (surrogate for human erythro B19)</td>
<td>ss-DNA</td>
<td>–</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

* Red blood cells were treated with 0.1% PEN110 at 22 ± 2°C for up to 22 ± 2 hours. BVDV indicates bovine viral diarrhea virus; PPV, porcine parvovirus; ss, single stranded; ds, double stranded; +, presence of an envelope; and −, no envelope.

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Pathogen Inactivation—Bryant & Klein 729
ture is incubated at room temperature for 6 hours, followed by dilution of the PEN110 to less than 50 ng/mL by washing with up to 6 L of 5% dextrose in normal saline. The red cells are suspended in a preservative solution and stored at 4°C for 42 days from collection.93

Red cell membrane integrity seems to be maintained in PEN110 treated red cells. Hemolysis was less than 1% after 42 days of storage at 4°C. In a single-center, phase I, 2-arm crossover randomized trial, PEN110 treated or control autologous red cells were transfused to 12 healthy subjects after 28 days of storage.99 Red cell recovery was comparable in each group. There were no detected alterations of red cell surface antigens, and no neoantigen formation was identified during subsequent testing. However, as seen with S-303 treatment, phase III clinical trials were halted when antibody formation was reported in repeatedly transfused patients with sickle cell disease. There were no clinical consequences observed in the cases involving antibody formation. The nature of immune responses and specificities of the antibodies formed in the PEN110 trials have not been reported. All clinical studies have been suspended.

The future of PEN110 and S-303 as pathogen inactivation systems for red blood cells is uncertain until the immunoreactivity and immunogenicity are investigated further and resolved.

**Riboflavin/UV-A Light**

Riboflavin-based pathogen inactivation systems for red cells are currently under development. If found to be a successful means of pathogen inactivation of red blood cells, riboflavin may serve as the one material to inactivate pathogens in 3 blood components (red cells, platelets, and plasma).

**Dimethylmethlene Blue**

Dimethylmethene blue is another photactive phenothiazine dye like MB that has been investigated as a potential pathogen inactivation agent for red cells.100–102 Dimethylmethene blue is more hydrophobic and has a greater affinity for nucleic acids than MB. Dimethylmethene blue plus visible light has been shown to inactivate RNA and DNA viruses in leukocytes and red cells. Early clinical studies demonstrated only small decreases in adenosine triphosphate and 2,3-diphosphoglycerate levels posttreatment, but hemolysis exceeded 1% and in some studies ranged as high as 40%. Recent studies have shown that the composition of the posttreatment additive storage solution strongly affects the red cell integrity after photochemical treatment.

In the dimethylmethene blue pathogen inactivation process, leukoreduced red cells are washed twice with normal saline and resuspended in an additive solution to a hematocrit of 30% to facilitate increased light penetration and uniform light distribution.103 The suspended red cells were incubated with 25 µM dimethylmethene blue for 10 minutes prior to illumination for 16 minutes with agitation at a wavelength greater than 610 nm to prevent light absorption by hemoglobin. After illumination, the red cells are washed and suspended in a storage solution. The additive solution into which the cells are diluted and resuspended prior to illumination has little effect on red cell integrity.104 In contrast, the additive solution used for posttreatment storage strongly affects the red cell integrity. The presence of citrate or suroce prevents hemolysis of the phototreated cation-permeable red cells by counteracting the osmotic activity of hemoglobin. Even with specialized storage solutions, the hemolysis still approaches 1%. Modification of the method to better preserve red cell integrity will be necessary if this agent is to enter clinical trials.

**Flexible Photosensitizer Dyes**

Different photosensitizing dyes have provided the basis of several pathogen inactivation methods for red blood cells. In theory, these dyes attach and penetrate viral membranes and capsids by binding and intercalating into the viral nucleic acids while leaving the nucleus-free red blood cells unharmed. However, these dyes are capable of attaching to red cell membrane lipids, glycoproteins, and proteins. The bound photosensitizer along with free photosensitizer in solution can cause reactive oxygen species to damage the red cell membrane during phototreatment. Developing a method that reduces red cell damage from the free and bound photosensitizers while maximizing the pathogen inactivation properties of the intercalating dyes remains a major challenge.

Most photosensitizing dyes are rigid with fixed aromatic rings that liberate oxygen radicals on phototreatment regardless of whether they are free in solution or bound to a substrate. A new group of flexible photosensitizers contain aromatic ring systems separated by single bonds capable of rotating when the photosensitizer is not firmly bound to a substrate (Figure 3).105,106 When the unbound photosensitizing dye is illuminated, the dye absorbs the light energy and dissipates it as heat energy through the twirling of the flexible bonds. Reactive oxygen species, therefore, are not generated. When the flexible photosensitizer is bound, it becomes fixed and rigid so that oxygen radicals are generated on phototreatment. To be even more efficient and less damaging to red cells, the optimal flexible photosensitizer should be designed to minimize red cell affinity and binding.

Thiopyrylium (TP) is a flexible photosensitizing dye capable of intercalating into nucleic acids and becoming photoactivated in the bound state (Figure 4).105 Although TP has a strong affinity for red cell membranes, the use of a chemical additive with a similar structure could serve as a competitive inhibitor and limit the attachment of TP to the red cells. Dipyridamole is a red cell–specific antioxidant that has been shown to reduce the photochemical damage of red cells and is already licensed for intravenous use as a platelet antiaggregant. Dipyridamole resembles TP structurally and appears to block the binding of TP to some (30.5% ± 12.4%) but not all sites on the red cell membrane. Thiopyrylium phototreatment in the presence of the competitive inhibitor dipyridamole proves effective in inactivating numerous intracellular viruses to the limit of detection, reducing intracellular HIV by 6.2 log, and inactivating many gram-positive and gram-negative bacteria from 5.4 to greater than 7.1 log (Table 5). The photoinduced red cell hemolysis in the combined TP and dipyridamole pathogen inactivation system was limited to 0.3% in units with Erythrosol additive. As noted previously, the impermeable citrate ion in the Erythrosol additive was responsible for maintaining the external osmotic pressure and offsetting the internal osmotic pressure from hemoglobin.104 Phototreated red cells exhibited acceptable levels of extracellular pH, adenosine triphosphate, glucose utilization rates, lactate production, and
4.106 Because only about 21% of thiazole orange binds to that acts as a photosensitizer only when bound (Figure zole orange, an asymmetrical intercalating cyanine dye activating pathogens in red blood cell suspensions is thia-
tassium leakage was increased compared with controls.105
morphology scores when tested at 42 days; however, po-
tassium efflux, and similar levels of adenosine triphos-
Tritated from 5.4 to 7.1 log of selected viruses and 2.3 to great-
Thiazole orange has been shown to photoacti-
the red cells, a competitive inhibitor or quencher is not
Thus far, published studies of both TP/dipyridamole
Another flexible photosensitizing dye capable of inac-
tivating pathogens in red blood cell suspensions is thia-
These technologies are generally expensive and have the
Another flexible photosensitizing dye capable of inactivating pathogens in red blood cell suspensions is thiazole orange, an asymmetrical intercalating cyanine dye that acts as a photosensitizer only when bound (Figure 4).106 Because only about 21% of thiazole orange binds to the red cells, a competitive inhibitor or quencher is not required. Thiazole orange has been shown to photocactivite from 5.4 to 7.1 log of selected viruses and 2.3 to greater than 7.1 log of tested bacteria (Table 5). After 42 days of storage, treated red cells exhibited acceptable but slight-

<table>
<thead>
<tr>
<th>Viral Pathogens</th>
<th>Genome</th>
<th>Enveloped</th>
<th>Thiopepyriliun/Dipyridamole</th>
<th>Thiazole Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immunodeficiency 1/2</td>
<td>ss-RNA</td>
<td>+</td>
<td>&gt;6.8 extracellular; 6.2 ± 1 intracellular</td>
<td>&gt;6.5 extracellular; &gt;6.3 intracellular</td>
</tr>
<tr>
<td>DHBV (surrogate for hepatitis B)</td>
<td>ds-DNA</td>
<td>+</td>
<td>&gt;4.8 extracellular</td>
<td></td>
</tr>
<tr>
<td>BVDV (surrogate for hepatitis C)</td>
<td>ss-DNA</td>
<td>+</td>
<td>&gt;5.3</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Vesicular stomatitis</td>
<td>ss-DNA</td>
<td>+</td>
<td>&gt;7.2</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>Pseudorabies (surrogate for herpes)</td>
<td>ds-DNA</td>
<td>+</td>
<td>&gt;5.9 extracellular</td>
<td>5.4 ± 0.7</td>
</tr>
</tbody>
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Table 5. Inactivation of Viruses and Microbial Pathogens by Treatment of Red Blood Cells With Flexible Photosensitizer Dyes

<table>
<thead>
<tr>
<th>Microbial Pathogens</th>
<th>Gram</th>
<th>Aerobes Vs Anaerobes</th>
<th>Thiopepyriliun/Dipyridamole</th>
<th>Thiazole Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Neg</td>
<td>Aerobe</td>
<td>7.1 ± 0.5</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Neg</td>
<td>Aerobe</td>
<td>6.2 ± 1.0</td>
<td>3.5 ± 2.5</td>
</tr>
<tr>
<td>Serratia liquefaciens</td>
<td>Neg</td>
<td>Aerobe</td>
<td>6.8 ± 0.2</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Neg</td>
<td>Aerobe</td>
<td>5.4 ± 1.0</td>
<td>2.3 ± 1.2</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Neg</td>
<td>Aerobe</td>
<td>5.9 ± 0.6</td>
<td>6.4 ± 2.0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Pos</td>
<td>Aerobe</td>
<td>&gt;6.3</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Pos</td>
<td>Aerobe</td>
<td>&gt;7.7</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>Deinococcus radiodurans</td>
<td>Pos</td>
<td>Aerobe</td>
<td>&gt;6.8</td>
<td></td>
</tr>
</tbody>
</table>

* DHBV indicates duck hepatitis B virus; BVDV, bovine viral diarrhea virus; ss, single stranded; ds, double stranded; +, presence of an envelope; −, no envelope; Neg, gram negative; Pos, gram positive; TP, thiopyrilium; TO, thiazole orange; 1, 80 μmol/L TP and 1.1 J/cm² red light; 2, 7.5 μmol/L TP and 1.1 J/cm² red light; 3, 2 μmol/L TP and 1.1 J/cm² red light; 4, 100 μmol/L TP and 1.1 J/cm² red light; 5, 15 μmol/L TP and 1.1 J/cm² red light; 6, 100 μmol/L TP and 1.1 J/cm² red light; 7, 80 μmol/L TP and 7.9 J/cm² cool white light.

SUMMARY

Human blood is essential for life and has proved to be a valuable therapeutic agent for numerous medical disorders and treatment strategies. However, allogeneic blood is an inherently risky biologic source material. Among the dangers is transmission of a wide range of pathogens. Donor selection and blood testing have reduced this risk dramatically and will remain the cornerstone of blood safety programs. Nevertheless, infectious units still elude screening and testing; testing errors and product release errors are probably impossible to eliminate. The largest current infectious risks involve pathogens for which we do not test, for which we have no test, or for which demographic screening and testing are inadequate. The greatest fear concerns the emergence of a “new” transmissible agent, particularly one that has not previously been associated with human disease, has a long silent period, can infect others by secondary spread, and is highly lethal—as was the case with HIV. Ideally, pathogen inactivation techniques would provide an additional safeguard. That has been the experience in the plasma fractionation industry.

No single pathogen reduction method will likely be effective for every class of agent and for every blood component. Some combination of techniques that remove and inactivate infectious agents will probably be needed. These technologies are generally expensive and have the
potential to profoundly escalate the cost of blood, but this cost may be partially offset by the elimination of some testing markers. However, if potent pathogen inactivation techniques that preserve blood function and do not evidence some new toxic risk can be created, the developed world, which embraces the myth of zero-risk transfusion, will likely adopt them almost regardless of cost. For the developing world, in which low-risk blood donors are at a premium and elegant testing methods are often not feasible, a good but not perfect pathogen reduction method, especially if relatively inexpensive and easy to implement, could save millions of lives.

We acknowledge the expertise of Lydia V. Kibiuk, Medical Illustrator from the National Institutes of Health Medical Arts and Events Management, Division of Medical Arts and Printing Services for her assistance with Figure 4.

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