Chapter 8

Vitamin B2 and Innovations in Improving Blood Safety

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Additional information is available at the end of the chapter

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Abstract

Although transfusion of blood components is becoming increasingly safe, the risk of transmission of known and unknown pathogens persists. The application of vitamin B2 (riboflavin) and UV light to pathogen inactivation has several appealing factors. Riboflavin is a naturally occurring vitamin with a well-known and well-characterized safety profile. This photochemical-based method is effective against clinically relevant pathogens and inactivates leukocytes without significantly compromising the content and the efficacy of whole blood or blood component. This chapter gives an overview of the innovative technology for pathogen inactivation, the Mirasol® pathogen reduction technology (PRT) System, based on riboflavin and UV light, summarizing the mechanism of action, toxicology profile, pathogen reduction performance and clinical efficacy of the process.

Keywords: riboflavin, pathogen reduction technology, blood transfusion, safety

1. Introduction

The collection, processing, transfusion of whole blood, red blood cells, platelets, plasma, and infusion of fractionated plasma components are essential medical practices, often required for the preservation of life and for the treatment of disease. Although the transfusion/infusion of these components is a vital therapy, transfusions are still associated with some risk for transmission of disease to the patient [1].

Worldwide measures to reduce the risk of transmission of diseases to recipients through blood have been continuously implemented and improved [2]. Blood safety improvements include donor's questionnaire, self-deferrals and donation screening methods designed to detect possible contaminating agents in blood. Serological testing and nucleic acid testing

have become staples of modern blood banking and have greatly reduced the risk of disease transmission by blood product transfusion. Yet, growing socio-political changes of contemporary society together with environmental changes challenge the practice of blood transfusion with a continuous source of unforeseeable threats with the emergence and re-emergence of blood-borne pathogens [2, 3].

In the last two decades, several pathogen reduction/inactivation technologies (PRT) have been developed to allow treatment of blood products with the intent of reducing the levels of infectivity and eventually inactivating white blood cells that can cause immunological complications to blood recipients. PRT methods involve physicochemical disruption of pathogen structural elements, mostly applied to the production of plasma-derived fractionated products or photochemical modification of nucleic acids to prevent replication, applicable to labile blood components like platelet concentrates, therapeutic plasma and eventually red cell concentrates [4, 5].

One of these PRT technologies, the Mirasol PRT System, uses riboflavin or vitamin B2 as a photochemical sensitizer and relies on the association of riboflavin with nucleic acid and activation with UV-light to generate a photochemical reaction that modifies guanine residues and thus prevents replication processes. This method creates irreversible damage via electron transfer processes at the sites where riboflavin-guanine base chemistry occurs [6].

Flavins are present in all biologic fluids and tissues. The most common biologically important flavins are riboflavin and its nucleotides: riboflavin-5′-phosphate (flavin mono-nucleotide, FMN), and the intramolecular complex of FMN with adenosine-5′-monophosphate (flavin adenine dinucleotide, FAD) [7]. Riboflavin in its coenzyme form is a component of many oxidation-reduction reactions and of energy production. It is essential for growth and tissue repair in all animals from protozoa to man [8] unlike fat-soluble vitamins, which are stored in body fat, riboflavin is a water-soluble vitamin and excess amounts are rapidly excreted. Because there are no physiological stores of riboflavin and excretion is constant, frequent dietary intake is important to maintain sufficient concentration and in the case of excess, return to normal levels is commensurate with renal function [9].

The choice of riboflavin as photosensitizer in the Mirasol PRT System was reinforced by its well-documented safety profile, being widely used as food coloring in the United States, where it is "generally regarded as safe" by the FDA [10]. Neonates, including preterm and very low birth weight (VLBW) infants, requiring nutritional supplementation due to immature gastrointestinal and metabolic systems, commonly undergo parenteral nutrition with a multivitamin preparation which includes vitamin B12, thiamine, folate and riboflavin [11]. The FDA concluded in their review that the LD_{50} is orders of magnitude greater than the Recommended Daily Allowance (RDA); additionally, no reports on carcinogenicity, mutagenicity or teratogenicity associated with riboflavin have been reported to the agency [12]. In Europe, it has been approved by the Scientific Committee on Food [13]. Furthermore, an anti-neoplastic action of riboflavin photoproducts to hematological malignancies and solid tumors has been postulated, whereas high dose of riboflavin has been suggested for migraine prophylaxis [14–16].

2. Vitamin B2 and UV light: the chemistry

Riboflavin (RB) has absorption maxima at 220, 265, 375, and 446 nm in water and is yellow-orange in color. When aqueous solutions containing RB are exposed to sunlight, RB is converted into lumichrome (LC) under neutral conditions, and into lumiflavin (LF) in alkaline solutions [17, 18]. LC is also a known metabolic breakdown product of RB in the human body [19]. Flavin systems are known to be photochemically active, and the products of flavin photochemistry are known [17, 19, 20].

The mechanism of pathogen reduction using RB likely involves three potential pathways: Type I Photochemistry [47, 48] Type II Photochemistry, [21] and the effects of UV light alone. The contribution of each of these three pathways to the Mirasol PRT System pathogen reduction process has been described in the literature.

The reported mode of action of RB in the reduction of pathogens is postulated to be based in part on the ability of RB to interact with nucleic acids and to undergo chemistry with those nucleic acids upon exposure to light. This chemistry is believed to involve both oxygen-dependent and oxygen-independent (electron transfer) processes. It has been described thoroughly in the chemical literature over the past several decades [22–30]. The use of UV light with platelets and plasma also affords a third contributor to pathogen kill via the direct action of light.

2.1. Action spectra and absorbance spectra

Figure 1 depicts the action spectrum of RB and lambda phage minus light alone (yellow) over-laid with the absorbance profile of RB in PBS (pink) and absorbance of DNA in PBS (blue). At wavelengths lower than 300 nm, RB acts to shield the effects on DNA due to the

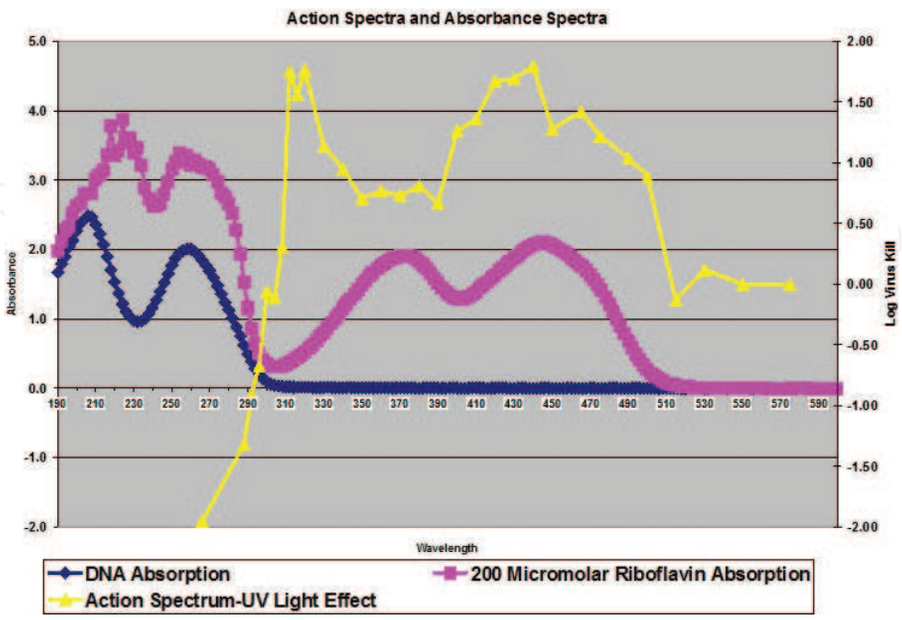

Figure 1. Action spectra and absorbance spectra of riboflavin with lambda phage.

direct action of UV light. Greater levels of inactivation in the presence of RB occur at wavelengths between 300 and 350 nm compared to the prediction due to the absorbance profile of free RB in solution. This is also observed for wavelengths higher than 500 nm. In the region of 308–575 nm, in order to achieve the same magnitude of log reduction that was observed between 266 and 304 nm, the energy required for the experiments between 308 and 575 nm was increased 50-fold from 0.1 to 5.0 J/mL. There is no inactivation of lambda phage with light of wavelength ≥330 nm in the absence of RB.

The action spectrum (AS) do not correlate perfectly with the absorption curve of either RB or LC in PBS over the entire wavelength regime. There appears to be essentially an identical amount of viral inactivation at 355 and 500 nm, although the optical densities (of solutions containing the same concentration of RB) differ by a factor of five at these two wavelengths. The phage reduction obtained at 320 nm and at 500 nm is greater than that expected based on the absorption spectrum of RB in PBS at these wavelengths. The effect is clearly seen in **Figure 1** which plots lambda phage inactivation achieved in the presence of RB at various concentrations minus that realized in its absence.

2.2. RB sensitized modification of nucleic acids

Several studies have been conducted in order to examine the ability of RB sensitization to modify nucleic acids [31]. The DNA fragmentation studies in leukocytes and bacteria utilized chemical agents that bind to portions of the DNA strand, which have been severed or broken because of chemical modification. The fragments that are produced leave regions that can be chemically tagged with a fluorescence probe and subsequently measured to provide an estimate of the extent of fragmentation that has occurred. Single-strand breaks throughout the nucleic acid sequence can be identified in this way. More complete breaks leading to denaturation of the nucleic acid can also be monitored by gel electrophoresis. In the latter case, the complete denaturation of the nucleic acid can be followed by examining migration patterns on polyacrylamide gels [31, 32]. This assay looks for much more severe and complete nucleic acid degradation than single-strand breaks.

In one set of studies, the level of DNA fragmentation occurring in white cell DNA was determined using a flow cytometric assay (Trevigen Apoptotic Cell System (TACS) assay. The level of DNA fragmentation obtained was significantly increased in the presence of RB. Similar observations were made for samples of plasmid DNA and for DNA isolated from *Escherichia coli* following treatment in the presence and absence of RB [31]. These combined studies demonstrate a sensitizing effect, with respect to nucleic acid damage, which RB imparts to samples treated with UV light. These observations are consistent with literature reports for RB.

Cadet and co-workers have evaluated the chemistry involved in the formation of specific lesions induced in nucleic acids by RB and light. These lesions differ from those induced by exposure to light alone in that chemically distinct oxidized species of guanine where residues are formed. This chemistry DNA fragmentation in isolated white cell DNA following exposure to light in the presence and absence of RB was evaluated because of the fact that mammalian systems do not normally contain enzymatic systems capable of repairing these types of lesions. This is in stark contrast to the predominant lesion (thymine-thymine dimers formed) upon exposure of nucleic acids or agents containing nucleic acids to light alone [33–35].

These studies identify the precise site of the lesions induced in nucleic acids treated with monochromatic 266, 308, or 355 nm light from either an excimer or Yttrium Aluminum Garnet (YAG) laser in the presence and absence of RB. The results demonstrate that in the presence of RB, the predominant modifications occur to guanine bases, as evidenced by the formation of 8-oxodGuo. The extent of the oxidized guanines formed in the presence of RB is far in excess of those observed upon exposure to light alone. These results are consistent with the literature reports of Cadet and co-workers of the mechanism of action of RB with regard to nucleic acid chemistry [24]. The results were contrasted to those using UV light alone in the absence of RB, and suggest that the addition of RB to the system specifically enhances the damage to DNA induced by UV light alone.

2.3. Phage reactivation

Virus reactivation is a phenomenon, which is known to occur through the use of host cell nucleic acid repair mechanisms. In the context of virus inactivation, the desired end target for these treatments is the prevention of virus replication. It is also desirable, in this context, to prevent repair of damaged virus particles because such repairs may render non-infectious agents capable of transmitting the disease when re-infused. This may be accomplished by generating either an extent of damage that the host system cannot repair or a type of damage that the host system does not have the capability of repairing.

Studies of the inability of bacteriophage to repair the lesions (Weigle reactivation) induced by RB and light as contrasted to the observations with light exposure alone have been conducted [31]. These studies confirm that the rescue of DNA damaged phage does not occur to the same extent when RB is present in samples exposed to light. These observations are consistent with the data suggesting that the presence of RB and UV light selectively enhances damage to the guanine bases in DNA or RNA. These data also suggest that this type and extent of damage to nucleic acids of virus in the presence of RB makes it less likely to be repaired by normal repair pathways available in host cells [36]. This result is essential for a system intended to assure the highest and most complete levels of pathogen inactivation attainable.

In summary, the Mirasol PRT process works through three independent mechanisms of action in rendering pathogens inactive. These include oxygen dependent chemistry induced by the combination of RB and light, electron transfer chemistry induced by the direct interaction of excited RB molecules with nucleic acid base pairs (primarily guanine bases) leading to oxidation products, and effects that are due to the action of UV light alone. In essence, the presence of RB in this system enhances the effects, which are due to UV light alone, creating a condition of greater sensitivity of the pathogen to the UV light to which the sample is exposed (photosensitization effect). The combination of these three modes affords broad and extended levels of pathogen inactivation with this process.

3. Toxicology and safety

Although the safety of RB has been extensively studied, there were no reports that directly supported its use in the Mirasol PRT System. Therefore a comprehensive preclinical safety

evaluation program in support of the Mirasol PRT System, designed to investigate all potential sources of concern, was conducted as part of the overall development program. In vivo animal and in vitro toxicity studies were performed using RB, lumichrome and photolyzed RB (see **Table 1**).

To obtain a consistent test article in as humane a fashion as possible for those studies, species-specific plasma was used rather than platelets. The photochemistry of RB yields equivalent photoproduct profiles in plasma products and in platelet products (which consist mainly of plasma). The absence of platelets eliminates the possibility of detecting toxic alterations to the platelet surface; however, that issue was addressed in the neoantigenicity and ^{14}C-RB binding studies.

3.1. Systemic toxicity

No toxicologically significant findings were observed in any of the studies of acute toxicity. In the repeated-dose toxicity study, the levels of RB and lumichrome in blood samples from animals receiving Mirasol PRT-treated products were below the limits of quantification, as were the levels in blood samples from animals receiving untreated plasma. These results were consistent with the observed rapid clearance of RB after IV administration, both in the literature [9] and in the pharmacokinetic study with ^{14}C-RB in Mirasol PRT-treated products. RB and its photoproducts naturally occur in human blood, see **Figure 2**. All photoproducts were found to be present in apheresis platelet products that had not undergone any photochemical treatment, although at a much lower concentration. The presence of these agents in human blood, the ubiquitous nature of RB exposure, its presence in human diets and the ability of humans to metabolize it and manage its inherent photochemistry suggests a low risk profile for this product.

3.2. Developmental toxicity and genotoxicity

No developmental toxicity was observed in the embryo-fetal development study. All fetuses were examined for malformations and developmental variations. No mutagenicity was

• Acute Toxicity*	Negative
• Neoantigenicity*	Negative
• Ames Mutagenicity#	Negative
• CHO Clastogenicity#	Negative
• Cytotoxicity#	Negative
• Reproductive Toxicity*	Negative
• Subchronic Toxicity*	Negative
• MMN Genotoxicity*	Negative
• Blood Compatibility#	Passed
• Leachables and Extractables#	Passed

Table 1. In vivo* and in vitro# toxicology.

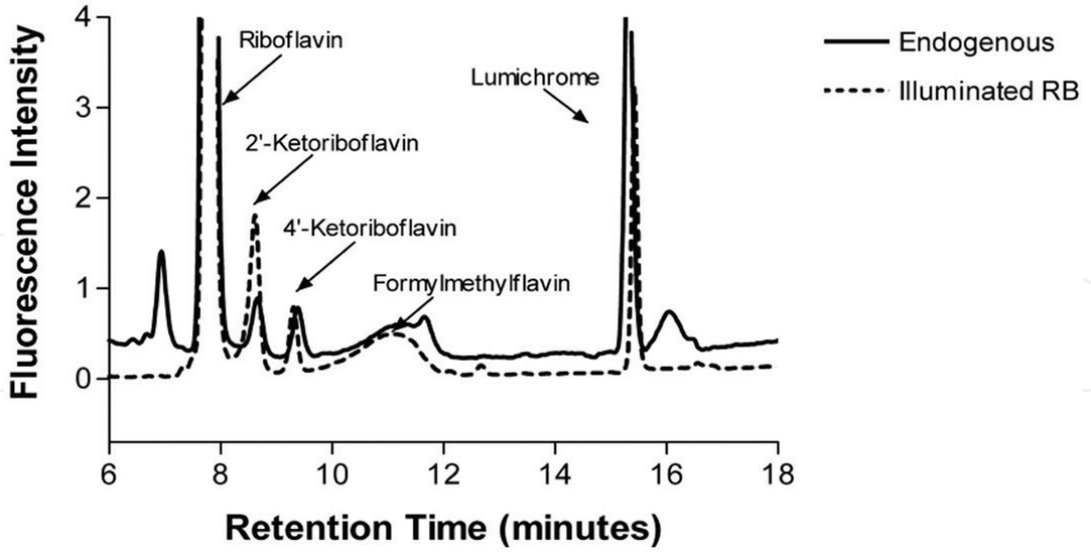

Figure 2. Riboflavin and its photoproducts are naturally present in human blood; no new compounds are formed after Mirasol treatment.

observed in the Ames test for treated or control human platelets, or for lumichrome. The in vitro and in vivo tests for clastogenicity in mammalian cells (chromosomal aberration in cultured CHO cells and micronucleus test in mouse bone marrow cells, respectively) were also performed with Mirasol PRT-treated products. Human platelets treated with the Mirasol PRT System gave negative results in all genotoxicity experiments.

3.3. Neoantigenicity and cytotoxicity

Results of studies using ^{14}C-labeled RB and exposure of platelets and plasma to UV light did not demonstrate any detectable binding of RB or its photoproducts to platelets or to plasma proteins. No evidence of neoantigenicity was observed with the Ouchterlony assay, indicating that no new antigens were formed during treatment with the Mirasol PRT System. Treatment with the Mirasol PRT System did not result in greater immunoglobulin G binding than what was observed in comparison with untreated controls, when assessed with the Capture-P assay. In the tests of lumichrome cytotoxicity, and of the cytotoxicity of Mirasol PRT-treated products, no cytotoxicity was observed.

3.4. Hemocompatibility

In tests of hemocompatibility, no hemolysis was observed. In functional assessments, when mixed with thrombocytopenic whole blood, the function of Mirasol PRT-treated platelets was well preserved, in comparison with controls [37]. Treated platelets displayed no evidence of hyperactivation or hypercoagulability.

3.5. Pharmacokinetics of photolyzed ^{14}C-RB in rats

After a single IV administration of Mirasol treated plasma containing photolyzed ^{14}C-RB, the radioactivity was well distributed from the whole blood to tissues selected for assay within

the first hour postdose. Most of the excreted urinary radioactivity was recovered by 12 h postdose, and more than half of all radioactivity was excreted in urine. Blood levels of radioactivity declined rapidly post-dose, as expected from studies of RB metabolism and excretion in humans [9]. Measurements of the radioactivity associated with the ^{14}C-RB-treated plasma indicated rapid initial apparent distribution (and/or clearance) from the systemic circulation that appeared to be complete within the first 8 to 48 h postdose.

3.6. Leachables and extractables

The leachables and extractables analyses detected no polymeric material in either test or control platelet products. The Mirasol illumination/storage bag does not contain the plasticizer di (2-ethylhexyl)phthalate (DEHP), and testing verified that this plasticizer was not present in treated and stored products. No toxicologically relevant concentrations of metals were found. These results correlate with those from the biocompatibility testing of the Mirasol illumination/storage bag elements—all elements were biocompatible.

4. Safety of blood

Blood transfusion safety is considered by the World Health Organization an integral part of each country's national health care policy and infrastructure [38]. In the last four decades safety of blood has been positively impacted by technological, economic and social improvements [2]. Improvements in blood processing and storage as per good manufacturing practices (GMP), introduction of policies discouraging paid blood donation and successive addition of screening tests for known transmissible pathogens, as Hepatitis B virus (HBV), Human Immunodeficiency viruses 1 and 2 (HIV-1/2) and Hepatitis C virus (HCV) are among the most successful measures to increase quality and safety of blood transfusion worldwide [39, 40]. From the late nineties onwards, introduction of nucleic acid testing (NAT) was able to minimize the window period of detection of these three viruses in asymptomatic blood donors to single days [41, 42].

Yet, in the last 20 years attention has been drawn to blood safety threats by recently known and/or re-emergent pathogens such as, Severe Acute Respiratory Syndrome virus (SARS), West-Nile Fever virus (WNV), Chikungunya virus (CHIKV), Dengue virus (DENV) or most recently ZIKA virus (ZIKV). Epidemics of these diseases are geographical or seasonal in nature and may not necessarily require universal reactive measures [2, 43, 44].

These unpredictable threats, as well as the long-recognized risk of bacterial transmission through platelet transfusion, may be effectively countered through the novel proactive approach with broad applicability and effectiveness, the pathogen inactivation/reduction technology (PRT) [4]. PRT has first been used to treat plasma and focused on destroying the structural elements of potential pathogens by the solvent-detergent method [45]. By the mid-nineties, the nucleic acid binding properties of Methylene Blue (MB) became exploited in a pathogen inactivation system for fresh frozen plasma using visible light [46].

Though quite effective for the treatment of plasma, neither of these methods could be used for cellular blood products. Two newer technologies have been developed, both using UV light and two distinct chemical compounds to enable irreversible breakage of nucleic acids and blocking further replication of cells and pathogens. One technology uses amotosalen hydrochloric acid or S-59 as the photoactive-compound, which together with its photoproducts need to be removed from the blood component post-illumination due to its high toxic profile [47]. The second system uses riboflavin, a natural vitamin (vitamin B2) of which both photoproducts and catabolites are found endogenously in the normal blood and therefore do not need to be eliminated from the blood component before transfusion [6, 48, 49].

5. The Mirasol PRT process

Pathogen reduction is a proactive strategy to mitigate the risk of transfusion-transmitted infections. The Mirasol PRT System consists of an illumination/storage bag, RB solution, and an Illuminator that delivers UV light to cause permanent damage to nucleic acids of pathogens and leukocytes (see **Figure 3**). The system has been shown to be effective against clinically relevant pathogens [50, 51] and inactivates leukocytes [52] without significantly compromising the efficacy of the product [53–55] or resulting in product loss. The process involves transferring the blood component to the Illumination/Storage bag and adding 35 ± 5 mL of RB solution (500 μM). The product is then placed into the Mirasol Illuminator and exposed to UV light. After illumination, the final PRT treated product can be transfused immediately or stored without the need for additional filtration or processing. Treated plasma products are transferred to a storage bag appropriate for freezing. The Mirasol PRT System has been developed with the flexibility of treating plasma and platelet components, as well as whole blood.

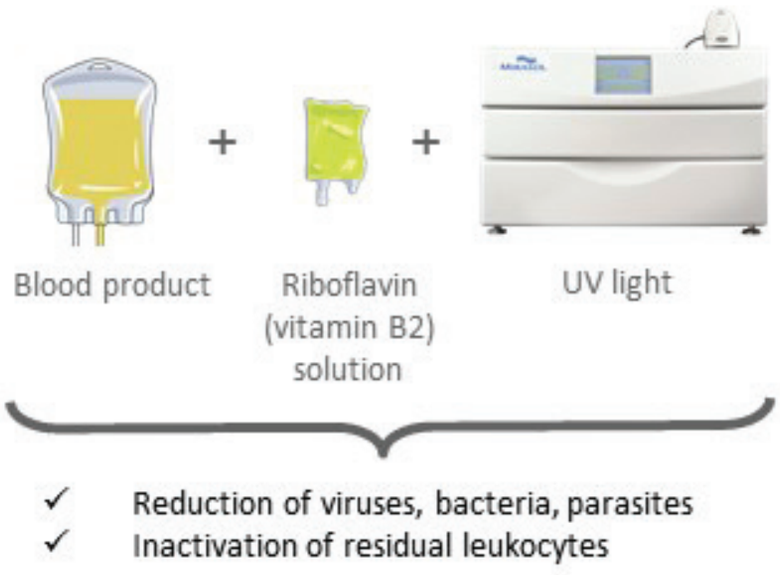

Figure 3. Mirasol PRT System.

5.1. Platelets and plasma

PRT-treated plasma products have been on the market in Europe for more than a decade and were issued a CE Mark in 2007 and 2008 for platelets and plasma respectively. FFP intended for transfusion to patients with multiple coagulation factor deficiencies (e.g. massive transfusion), emergency reversal of warfarin as well as for therapeutic plasma exchange must contain adequate functional levels of coagulation factors and other therapeutically valuable proteins. Protein levels should be as close as possible to those found in fresh plasma. Blood component processing can affect the quality of plasma products, particularly labile coagulation factors such as factors V and VIII.

Mirasol-treated FFP shows high overall protein retention under a broad range of blood banking conditions. Mirasol-treated FFP meets the European guidelines [56], showing on average factor VIIIc levels of 0.8 ± 0.2 IU/ml post treatment. Protein content meets guidelines even when whole blood is held overnight at room temperature and plasma is separated up to 18 h or frozen up to 24 h after collection. Additionally, anticoagulant factors such as protein C and protein S are well preserved after treatment with a 96% retention reported post treatment for both proteins. Extended storage of treated plasma at −30°C for up to 2 years does not significantly decrease protein quality [57].

Platelet products derived from apheresis or whole-blood can be treated with the system and products can be stored either in plasma or platelet additive solutions (PAS) for up to 7 days under standard blood banking conditions. It is critical that Mirasol-treated platelets remain viable and hemostatically effective. A series of in vitro studies have been performed to assess platelet quality after Mirasol treatment, and a correlation between in vitro parameters and in vivo performance was established [58]. In these studies pH and lactate production rate were found to be most strongly correlated with the in vivo recovery and survival of Mirasol-treated platelets. Glucose consumption rate and swirl also showed some correlation with these in vivo parameters, though to a lesser extent. P-selectin, pO_2 and pCO_2 expression in Mirasol-treated platelets, however, were poorly correlated with in vivo platelet recovery and survival. Changes in cell quality parameters do occur, cellular metabolism is up-regulated in treated platelets, and treatment induces some degree of platelet activation. However, shear-induced adhesion is maintained in Mirasol-treated platelets, and mitochondrial function is preserved [37, 59].

5.2. Whole blood: military and developing world

The Mirasol system was further developed for the treatment of whole blood, providing a single pathogen reduction and leukocyte inactivation step, followed by the use of the product as whole blood or pRBCs. The treatment of RBCs or whole blood has been more challenging due to the absorption of light by hemoglobin. Although the peak absorption of hemoglobin (400–450 nm) is outside the spectral region of the Mirasol lamp output, the UV light energy dose delivered to units of whole blood is normalized for RBC volume (J/ml_{RBC}). In vitro cell quality studies have verified that adequate quality and functionality of the RBCs and plasma components post treatment and throughout 21 days of storage is preserved [49]. In addition, crossmatch compatibility of the products is preserved. PRT treatment of whole blood has received CE marking in

2015 and is a significant step forward ensuring blood safety where whole blood transfusions are routine, such as sub-Saharan Africa and in far-forward combat situations.

6. Pathogen reduction performance

The Mirasol PRT System pathogen reduction process has been evaluated for performance against several pathogens. **Table 2** summarizes the pathogen reduction results. The data show reduction factors ranging from 2 to 6 log (99.0–99.9999% reduction) for each pathogen tested with the Mirasol PRT System. Log reduction values reported in the table were calculated by determining the number of virus particles present in infectious form prior to treatment and the number of virus particles present after treatment. The level of log reduction is reported as the starting titer expressed in units of 10× per mL minus the level after treatment expressed as the titer in 10× per mL. Because volume was constant in the samples before and after treatment, the unit of volume cancels, resulting in a reported value of log reduction.

For example, a sample containing 1,000,000 infectious virus particles per mL would of course have 10^6 virus particles per mL. If after treatment, only 100 particles per mL were measured in tissue culture infectivity assays, this would correspond to 10^2 virus particles per mL. The log reduction reported for this system would be 10^4 or 4 logs. This corresponds to a reduction in virus level of 99.99%. Because values are reported in log units, 100% reduction is never achievable.

Despite the fact that bacterial contamination of blood products poses one of the greatest risks for transfusion, there are currently no standards in place that establish a panel of species to test or a method to evaluate technology for pathogen reduction. A panel based on published hemovigilance studies incorporating those species responsible for the majority of morbidity and mortality in transfusion-associated reactions was utilized to guide study targets. Two complementary test methods were developed, as described below, to measure bacterial reduction performance.

To assess bacterial reduction efficiency of the system, two complementary test methods, known as "High Spike Bacterial Titer" and "Low Spike Bacterial Titer" tests, for bacterial reduction have been developed to measure the Mirasol PRT System performance. Both methods involve inoculation of known titers of bacteria (a "spiking" study) into platelet products followed by PRT treatment and subsequent measurement of the presence of bacteria. The objective of the High Spike Bacterial Titer experiments is to determine the overall bacterial reduction ability of the system against a severely contaminated platelet product. These studies may not, however,

Pathogen type	Typical performance
Viruses (enveloped, non-enveloped; intracellular, extracellular)	~2 to 6 log (99.0–99.9999%)
Parasites (Malaria, Chagas, Babesiosis, Leishmaniasis, Scub typhus)	≥ 3.0 to ≥ 5.0 (≥ 99.9% to ≥ 99.999%)
Bacteria (Gram +, Gram −)	~2 to 5 log (99.0–99.999%)

Table 2. Pathogen reduction performance.

represent a clinically relevant finding in that viable bacteria remaining after treatment may grow to high titers through the storage period. The objective of the "Low Spike Bacterial Titer Experiments" is to spike a platelet product with a more clinically relevant bacterial titer, treat the product using the Mirasol PRT System, and evaluate the platelet product using a standard culture system through a 7-day storage period to determine if it has remained culture negative, indicating that the platelet product meets release criteria for transfusion. The system demonstrated 98% effectiveness in these studies against a broad range of bacteria [60]. The combined data from these studies demonstrates the bacterial reduction capability of the system under conditions that are still substantially higher challenges than may be anticipated in an actual clinical setting.

7. Clinical performance

There have been 11 completed clinical studies with the Mirasol PRT System for Platelets stored in 100% plasma or platelet additive solution (PAS). There are two ongoing clinical studies in the United States, one study with platelets and one with RBCs derived from Mirasol-Treated Whole Blood. Primary outcome measure in most clinical studies has been levels of circulating platelets in thrombocytopenic patient's blood after prophylactic transfusion. Both CI (count increment) and CCI (corrected count increment) are accepted as surrogate markers of platelet transfusion efficacy, but they do not necessarily account for platelet function or bleeding outcomes in patients and they rely upon the assumption that a sufficient number of circulating, intact platelets will provide protection against bleeding. Patient factors and platelet product variability have been shown in published studies to affect increments, limiting the sensitivity of the CCI as a clinical efficacy measure. The CCI at one and 24 h after transfusion is decreased in patients receiving PRT treated products, compared to patients receiving control products. In two recent clinical trials Grade 2 or higher bleeding was the primary endpoint [55, 66]. Although lower CCIs were observed in these 2 studies, no difference in clinically meaningful bleeding in thrombocytopenic patients was observed.

The clinical evaluation of the Mirasol Whole Blood system includes a clinical trial in patients assessing the incidence of transfusion transmitted *Plasmodium* spp. infection that was conducted in Kumasi, Ghana [61]. Treatment of whole blood reduced significantly the incidence of transfusion-transmitted malaria. The safety profile and clinical outcomes were similar between test and control groups.

8. Current adoption for routine use

Since 2007, when the Canadian Consensus Conference on Pathogen Inactivation (PI) concluded that a proactive approach in accordance to the precautionary principle would reduce the theoretical risk and help sustain public confidence in the blood supply, many national and international committees, such as the Advisory Committee on Blood Safety and Availability (ACBSA), USA and the European Committee on Blood Transfusion of the Council of Europe discussed the accumulating evidence about the efficacy and safety of PRT [62, 63].

PRT treatment of blood components is regarded as the next step to increase blood safety and support the credibility of blood institutions and health policy makers. However, there is a lack of consistency in the decision-making criteria used by regulatory bodies and blood operators regarding PRT implementation. The European Directorate for the Quality of Medicines & Healthcare of the Council of Europe in its Guide to the Preparation, Use and Quality Assurance of Blood Components, 19th Edition defines properties and requirements for therapeutic plasma, platelet concentrates and cryoprecipitate treated with PRT [56], yet PRT treatment of blood components is mandated in very few countries in Europe. Belgium, Switzerland and France have made the use of pathogen-inactivation mandatory for the treatment of platelet concentrates. Plasma treated by PRT is mandatory in Belgium, whereas the use of solvent/detergent treated plasma is more widespread in Europe but not mandated by national agencies.

The Mirasol PRT system has been gradually adopted in Europe, Asia and Latin-America. A hemovigilance program, based on the collection of passive hemovigilance data of Mirasol-treated components in multiple blood transfusion centers in Europe started in 2010. By 2015 data about 94,509 transfused platelet concentrates and 96,115 plasma transfusions were recorded in the program [64]. By 2017 over 750,000 disposable treatment sets have been distributed worldwide and 225,000 transfusion data have been recorded in the hemovigilance program.

9. Future

It is reasonable to envisage a future when all labile blood components will be PR treated to ensure a safe and sustainable blood supply in accordance with regional transfusion best practices. PR treatment of WB represents the most efficient implementation path to achieve this goal. It has been recently demonstrated through a clinical trial in a malaria-endemic country that a WB PR technology based upon riboflavin and UV light does reduce the risk of transfusion-transmitted malaria [61]. RBCs derived from PR-treated WB have shown good quality and recovery in health subjects and are currently being evaluated in a pivotal clinical trial [65].

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Parts of the chapter were taken from previously published papers by Goodrich RP and co-workers et al., and we have the permission to re-use it.

Conflict of interest

Marcia Cardoso and Susanne Marschner are employees of Terumo BCT, the manufacturer of the Mirasol PRT System.

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