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Mirasol PRT System inactivation efficacy evaluated in platelet concentrates by bacteria-contamination model

Ocena efikasnosti inaktivacije bakterija pomoću Mirasol PRT sistema u koncentrovanim trombocitima primenom modela bakterijske kontaminacije

Miodrag Jocić*, Miroljub Trkuljić*, Dragana Jovičić*, Nemanja Borovčanin*, Milena Todorović†, Bela Balint*‡

***Military Medical Academy,** Institute of Transfusiology, **Belgrade, Serbia;** †**Clinical Center of Serbia,** Clinic for Hematology, **Belgrade, Serbia; University of Belgrade,** ‡Institute for Medical Research, **Belgrade, Serbia**

Abstract

Background/Aim. Bacterial contamination of blood components, primarily platelet concentrates (PCs), has been identified as one of the most frequent infectious complications in transfusion practice. PC units have a high risk for bacterial growth/multiplication due to their storage at ambient temperature (20 ± 2°C). Consequences of blood contamination could be effectively prevented or reduced by pathogen inactivation systems. The aim of this study was to determine the Mirasol pathogen reduction technology (PRT) system efficacy in PCs using an artificial bacteria-contamination model. **Methods.** According to the ABO blood groups, PC units (n = 216) were pooled into 54 pools (PC-Ps). PC-Ps were divided into three equal groups, with 18 units in each, designed for an artificial bacteria-contamination. Briefly, PC-Ps were contaminated by *Staphylococcus epidermidis*, *Staphylococcus aureus* or *Escherichia coli* in concentrations 10^2 to 10^7 colony forming units (CFU) per unit. Afterward, PC-Ps were underwent to inactivation by Mirasol PRT system, using UV (λ = 265–370 nm) activated riboflavin (RB). All PC-Ps were assayed by BacT/Alert Microbial Detection System for CFU quantification before and after the Mirasol treatment. Samples from non-inactivated PC-P units were tested after preparation and immediately following bacterial contamination. Samples from Mirasol treated units were quantified for CFUs one hour, 3 days and 5 days after inactivation. **Results**. A complete inactivation of all bacteria species was obtained at CFU concentrations of 10^2 and 10^3 per PC-P unit through storage/investigation period. The most effective inactivation (10^5 CFU per PC-P unit) was obtained in *Escherichia coli* setting. Contrary, inactivation of all the three tested bacteria species was unworkable in concentrations of ≥ 10^6 CFU per PC-P unit. **Conclusion**. Efficient inactivation of investigated bacteria types with a significant CFU depletion in PC-P units was obtained – 3 Log for all three tested species, and 5 Log for *Escherichia coli*. The safety of blood component therapy, primarily the clinical use of PCs can be improved using the Mirasol PRT system.

Key words:
blood platelets; platelet transfusion; bacterial infections; treatment outcome; riboflavin; ultraviolet rays.

Apstrakt

Uvod/Cilj. Bakterijska kontaminacija hemoprodukata, prvenstveno koncentrovanih trombocita (KT), jedna je od najčešćih infektivnih komplikacija u transfuzijskoj praksi. Zbog skladištenja na sobnoj temperaturi (20 ± 2°C), KT predstavljaju veliki rizik od umnožavanja bakterija. Posledice bakterijske kontaminacije mogu biti efikasno sprečene ili smanjene upotrebom sistema za inaktivaciju patogena u različitim hemoproduktima. Cilj ovog rada bio je procena efikasnosti Mirasol sistema za redukciju patogena (PRT) u KT korišćenjem modela arteficijalne bakterijske kontaminacije. **Metode**. U skladu sa krvnim grupama ABO, jedinice KT (n = 216) spojene su u 54 pula (P-KT) koji su bili podeljeni u tri jednake grupe, u svakoj po 18 jedinica, namenjenih za arteficijalnu bakterijsku kontaminaciju. Jedinice P-KT bile su kontaminirane bakterijama *Staphylococcus epidermidis*, *Staphylococcus aureus* i *Escherichia coli* u koncentracijama od 10^2 do 10^7 CFU po jedinici. Potom su P-KT bili podvrgnuti inaktivaciji sistemom Mirasol PRT, korišćenjem riboflavina aktiviranog UV zracima (λ = 265 – 370 nm). Svi P-KT su testirani sistemom *BacT/Alert Microbial Detection* na prisustvo CFU pre i posle postupka Mirasol. Uzorci iz neinaktivisanih jedinica PKT testirani su posle pripremanja i neposredno

Correspondence to: Bela Balint MD, PhD, Military Medical Academy, Institute for Transfusiology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: beciusb@yahoo.com

posle kontaminacije bakterijama. Uzorci iz jedinica tretiranih Mirasolom ispitivani su na prisustvo CFU jedan sat, odnosno 3 i 5 dana nakon inaktivacije. **Rezultati**. Tokom perioda čuvanja/istraživanja postignuta je kompletna inaktivacija bakterija svih vrsta u koncentracijama od 10^2 i 10^3 CFU po P-KT. Najefikasnija inaktivacija (10^5 CFU po P-KT) postignuta je pri ispitivanju bakterije *Escherichia coli*. Nasuprot tome, inaktivacija kod sve tri vrste bakterija nije bila efikasna u koncentracijama bakterija $\geq 10^6$ CFU po P-KT. **Zaključak.** Efikasna inaktivacija ispitivanih bakterija sa bitnim smanjenjem CFU u P-KT – 3 Log postignuta je za sve tri vrste bakterija i 5 Log za bakteriju *Escherichia coli*. Bezbednost terapije krvnim komponentama, prvenstveno klinička primena KT, može biti unapređena korišćenjem sistema Mirasol PRT.

Ključne reči:
trombociti; transfuzija trombocita; infekcija, bakterijska; lečenje, ishod; vitamin b2; ultravioletni zraci.

Introduction

The use of various inactivation techniques clearly reduces pathogen occurrence in collected blood. The Mirasol pathogen reduction technology (PRT) system is based on the treatment by ultraviolet (UV) illuminated/activated riboflavin (RB), resulting in inactivation of white blood cells (WBC) and pathogens at the molecular level due to irreversible photochemically induced damage of nucleic acids. These photochemical mechanisms inhibit nucleic acid replication and decrease incidence of potential transfusion side effects or complications [1–5].

Generally, the risk of transfusion-associated infections – applying bacteria contaminated platelet concentrates (PCs) is about 1,000 times greater than the hazard of transfusion-related HIV, hepatitis C or B virus and human T-lymphotropic virus transmission [6, 7]. The most important sources of bacterial contamination of collected blood are the donor skin [8–11] or asymptomatic donors – low-level or transient bacteremia in chronic bacterial infections, as well as a recovery from a disease [12–15]. Seldom, the source of bacteria can be a nonsterile equipment for collection or devices for processing of harvested blood units [16–18]. The prevalence of bacterial contamination is relatively high in PCs – from 0.14% to 1.41% – since their storage temperature (22 ± 2°C) favors bacteria growth/multiplication [6, 19]. Consequently, PCs are the most common cause of transfusion-associated bacterial morbidity and mortality. However, the rate of blood contamination is higher than the incidence of bacterial infections because their clinical manifestation depends on numerous factors, such as patient's general condition, antibiotic therapy, quantity and type of bacteria, etc [20–22].

Strategies to reduce the risk of transfusion-associated bacterial infections include superior donor selection [23], improved preparation and disinfecting of venepunction field [24–27], redirecting the initial blood stream into satellite bag at the start of collection [25, 26], improved processing procedure safety and reduced storage time [28, 29], reevaluation/optimization of thresholds and criteria for transfusion supportive treatment [30, 31], as well as the use of different pathogen inactivation systems [1, 5–7, 32].

The aim of this study was to evaluate the Mirasol PRT system efficacy in artificial bacteria-contamination model and to predict the importance of its application in prevention of potential infectious complications of PC clinical use.

Methods

The study included 216 units of buffy coat derived PCs; the volume was 62.4 ± 8 mL in average. The units of PC were separated from whole blood collected by a CPD/SAGM quadruple bag system (Macopharma, France) within 6 hours after donation, using a T-ACE II blood processor (Terumo, Japan). According to the ABO blood groups, PC units were pooled into 54 pools (PC-Ps; four PCs *per* PC-P unit). After that PC-Ps were divided into three equal groups, with 18 PC-P units in each (mean PC-P volume was 256.6 ± 14 mL). PC-Ps were stored at ambient temperature (22 ± 2°C) for 2 hours and then were filtered using an Imugard III-PL (Terumo, Japan).

The PC-P units were artificially contaminated by three different bacteria species. In brief, into the units of the first PC-P group *Staphylococcus epidermidis* (isolated from the skin), in the second group *Staphylococcus aureus* (ATCC# 25923), and in the units of the third group *Escherichia coli* (ATCC# 25922) were inoculated. The initial bacteria concentration for all the three species was 0.5 McF (1.5 × 108 CFU / mL). Initial suspensions were diluted (six different dilutions were applied) and inoculated into the units of PC-P groups regarding all the three species in the same way. Therefore, the final counts of inoculated CFUs were 102 to 107 *per* PC-P unit.

Before bacterial contamination, samples were taken (1st sample; sterility control) from the PC-P units and investigated by a BacT/Alert Microbial Detection System (Biomerieux, France). After contamination, from PC-P units samples were taken also to confirm contamination success (2nd sample; contamination checking). All PC-P units underovment inactivation by the Mirasol PRT system (CaridianBCT, USA) – that is using UV (λ = 265 – 370 nm) activated RB according to the manufacturer's instructions. Concisely, a sterile solution contains RB (500 µmol / L) in a 0.9% sodium chloride solution (pH range: 4.0–5.0). A volume of 35 ± 5 mL of this solution is added to PC-P units to produce a final concentration 57–60 µmol / L. The illuminator delivers the required UV light dose (6.24 J / mL) to the contents of an illumination bag (Mirasol Platelet Illumination/Storage set), based on product volume and measured flux rate [3].

The units are then returned to platelets shaker up to the moment of the investigations that followed. Finally, the samples from the inactivated units one hour, 3 days and 5 days

after the Mirasol inactivation and storage at 20 ± 2°C (3rd, 4th and 5th samples) were investigated for CFU units.

Results

The results of the PC-P testing before and after the contamination with bacteria *Staphylococcus epidermidis* (six different concentrations), and after inactivation of pathogens using the Mirasol PRT system, are presented in Table 1.

Testing relating to contamination of PC-Ps with bacteria *Staphylococcus aureus* and bacteria *Escherichia coli* in different concentrations is shown in Tables 2 and 3, respectively.

In the samples from PC-Ps contaminated with *Staphylococcus aureus* in the concentration of 10^4 CFU *per* PC-Ps, we proved the presence of the said bacteria after the storage period of three and five days (4th and 5th samples, respectively), despite the negative results of the first sample taken one hour after the Mirasol PRT inactivation.

Table 1

Inactivation efficiency of the Mirasol PRT after platelet concentrates contamination with *Staphylococcus epidermidis*

PC-P number	CFU *per* PC-P unit	Bacteria presence in the sample				
		initial*	contaminated**	inactivated–1$^{\Psi}$	inactivated–2$^{\Psi\Psi}$	inactivated–3$^{\Psi\Psi\Psi}$
J1004 1000001		Ø	+	Ø	Ø	Ø
J1004 1000002	10^2	Ø	+	Ø	Ø	Ø
J1004 1000003		Ø	+	Ø	Ø	Ø
J1004 1000004		Ø	+	Ø	Ø	Ø
J1004 1000005	10^3	Ø	+	Ø	Ø	Ø
J1004 1000006		Ø	+	Ø	Ø	Ø
J1004 1000007		Ø	+	Ø	Ø	Ø
J1004 1000008	10^4	Ø	+	Ø	Ø	Ø
J1004 1000009		Ø	+	Ø	Ø	Ø
J1004 1000010		Ø	+	+	+	+
J1004 1000011	10^5	Ø	+	Ø	Ø	Ø
J1004 1000012		Ø	+	+	+	+
J1004 1000013		Ø	+	+	+	+
J1004 1000014	10^6	Ø	+	+	+	+
J1004 1000015		Ø	+	+	+	+
J1004 1000016		Ø	+	+	+	+
J1004 1000017	10^7	Ø	+	+	+	+
J1004 1000018		Ø	+	+	+	+

*1st sample – before bacterial contamination; **2nd sample – immediately after bacterial contamination; $^{\Psi}$3rd sample – one hour after inactivation; $^{\Psi\Psi}$4th sample – day 3 after inactivation; $^{\Psi\Psi\Psi}$5th sample – day 5 after inactivation.

Table 2

Inactivation efficiency of the Mirasol PRT after platelet concentrations contamination with *Staphylococcus aureus*

PC-P number	CFU *per* PC-P unit	Bacteria presence in the sample				
		initial*	contaminated**	inactivated–1$^{\Psi}$	inactivated–2$^{\Psi\Psi}$	inactivated–3$^{\Psi\Psi\Psi}$
J1004 1000019		Ø	+	Ø	Ø	Ø
J1004 1000020	10^2	Ø	+	Ø	Ø	Ø
J1004 1000021		Ø	+	Ø	Ø	Ø
J1004 1000022		Ø	+	Ø	Ø	Ø
J1004 1000023	10^3	Ø	+	Ø	Ø	Ø
J1004 1000024		Ø	+	Ø	Ø	Ø
J1004 1000025		Ø	+	Ø	+	+
J1004 1000026	10^4	Ø	+	Ø	Ø	Ø
J1004 1000027		Ø	+	Ø	Ø	+
J1004 1000028		Ø	+	+	+	+
J1004 1000029	10^5	Ø	+	+	+	+
J1004 1000030		Ø	+	Ø	+	+
J1004 1000031		Ø	+	+	+	+
J1004 1000032	10^6	Ø	+	+	+	+
J1004 1000033		Ø	+	+	+	+
J1004 1000034		Ø	+	+	+	+
J1004 1000035	10^7	Ø	+	+	+	+
J1004 1000036		Ø	+	+	+	+

*1st sample – before bacterial contamination; **2nd sample – immediately after bacterial contamination; $^{\Psi}$3rd sample – one hour after inactivation; $^{\Psi\Psi}$4th sample – day 3 after inactivation; $^{\Psi\Psi\Psi}$5th sample – day 5 after inactivation.

The samples of contaminated PC-Ps with *Staphylococcus epidermidis* in the concentration of 10^4 CFU *per* PC-P were also sterile after the Mirasol PRT inactivation process and during a storage period, while in bacterial concentration of 10^5 CFU *per* PC-P, only one PC-P was sterile.

The highest degree of pathogen reduction has been made in PC-Ps contaminated by *Escherichia coli* inoculation. In concentrations of bacteria ≤ 10^5 CFU *per* PC-Ps, the sam-

Table 3

Inactivation efficiency of the Mirasol PRT after platelet concentrate contamination with *Escherichia coli*

PC-P number	CFU *per* PC-P unit	Bacteria presence in the sample				
		initial*	contaminated**	inactivated–1$^{\Psi}$	inactivated–2$^{\Psi\Psi}$	inactivated–3$^{\Psi\Psi\Psi}$
J1004 1000037		Ø	+	Ø	Ø	Ø
J1004 1000038	10^2	Ø	+	Ø	Ø	Ø
J1004 1000039		Ø	+	Ø	Ø	Ø
J1004 1000040		Ø	+	Ø	Ø	Ø
J1004 1000041	10^3	Ø	+	Ø	Ø	Ø
J1004 1000042		Ø	+	Ø	Ø	Ø
J1004 1000043		Ø	+	Ø	Ø	Ø
J1004 1000044	10^4	Ø	+	Ø	Ø	Ø
J1004 1000045		Ø	+	Ø	Ø	Ø
J1004 1000046		Ø	+	Ø	Ø	Ø
J1004 1000047	10^5	Ø	+	Ø	Ø	Ø
J1004 1000048		Ø	+	Ø	Ø	Ø
J1004 1000049		Ø	+	+	+	+
J1004 1000050	10^6	Ø	+	+	+	+
J1004 1000051		Ø	+	+	+	+
J1004 1000052		Ø	+	+	+	+
J1004 1000053	10^7	Ø	+	+	+	+
J1004 1000054		Ø	+	+	+	+

***1st sample – before bacterial contamination; **2nd sample – immediately after bacterial contamination; $^{\Psi}$3rd sample – one hour after inactivation; $^{\Psi\Psi}$4th sample – day 3 after inactivation; $^{\Psi\Psi\Psi}$5th sample – day 5 after inactivation.**

ples were sterile during the whole storage period. However, pathogen inactivation was not successful with bacterial concentrations ≥ 10^6 CFU per PC-Ps.

The results show that all PC-P units (n = 54) were sterile before testing (1st sample), as well as that the contamination of units by all the three bacteria species in all concentrations – from 10^2 to 10^7 – was confirmed (2nd sample). There was a complete inactivation of bacteria in concentrations of 10^2 and 10^3 CFU per PC-P (the degree of reduction was 2 and 3 Log) during the storage period (3rd, 4th and 5th samples) for all the three types of bacteria.

Summarily, in our study using the Mirasol PRT system bacterial depletion rank was 3–5 Log for all the three of bacteria species.

Discussion

The bacteria presence in PCs is often the result of their inadequate removal from the skin of donors (venepunction field), not diagnosed donor's bacteremia and possible blood contamination during collection and processing [33–36]. Bacterial contamination of PCs, associated with adverse transfusion reactions showed that most commonly isolated Gram-positive bacteria from donor's skin (*Staphylococcus epidermidis* and *Staphylococcus aureus*) were found in more than 70% of published cases of sepsis associated with PC transfusion [6, 35, 37]. Contrary to this, some published data showed that Gram-negative bacterias, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Serratia marcescens* are most commonly isolated pathogens in transfusion associated sepsis. Fatal outcome was the result of infection with Gram-negative bacterias in 63% of cases in comparison with 37% of fatal outcome after infections with Gram-positive bacterias [6, 35, 37].

To assess the efficacy of bacterial reduction by the Mirasol PRT system, two types of experiments known as "high spike bacterial titer" and "low spike bacterial titer" tests were performed [1]. Both methods involve inoculation of the known number of bacteria before inactivation of pathogens, and subsequently prove the presence or quantification of remaining bacteria (CFU) and calculate degree of their reduction. The aim of the experiments with high-titer bacteria inoculation was to determine the full potential of the Mirasol PRT system in the terms of reduction of a large number of bacteria in PCs. Contrary, in studies with inoculation of low, but clinically significant titer of bacteria, after the Mirasol inactivation of pathogens (0.5–2 Log CFU per mL), evaluation of PCs usefulness for transfusion was performed using standard systems to detect contamination during the whole storage period [1, 38].

Based on these facts, our pathogen inactivation model examined the Mirasol treatment efficacy in PCs, previously contaminated with different bacterial species most frequently associated with bacterial adverse transfusion complications – *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli*. Inoculation prepared with a various bacterial concentrations (range: 10^2 to 10^7 CFU *per* PC-P unit). Checking the maximum capacity of the Mirasol PRT system for the degree of pathogen inactivation was testing by inoculation of high bacterial concentrations in the PCs. Evaluation of the Mirasol efficacy in the prevention of potential infectious complications after transfusion of contaminated PC units was performed due to inoculation of lower bacteria's concentrations – mimicring the conditions regularly seen in clinical practice.

For the period of storage bacteria can growth/multiply quickly, as in our study with PC-Ps contaminated with *Staphylococcus aureus* species in the concentration of 10^4 CFU *per* PC-P in two PC-Ps. Despite the reduction of bacteria's number after the Mirasol PRT system inactivation to undetectable degree (negative result in the 3rd sample), there was a multiplication of the remaining viable bacteria to

detectable levels (confirmed in two PC-P units at 3rd and/or 5th days).

Concerning the literature data, fresh PCs are contaminated with less than 100 bacteria *per* product [1, 20]. The number of inoculated bacteria can vary from low concentrations (100–1,000 times higher than clinically relevant concentrations) to high, when their number is approximately 10,000–100,000 times bigger than in typical clinical conditions. In our model, we achieved the degree of pathogen reduction from 3–5 Log which represents an additional high-level safety for patients receiving PCs. Impossibility to complete inactivation of viable bacteria number in concentrations $\geq 10^6$ CFU *per* PC-P unit has no importance, because in clinical practice we do not regularly see such a large number of bacteria in fresh blood products. Finally, the obtained degree of pathogen reduction/inactivation in our research model was in accordance with the studies of other authors [1, 4, 38], as well as the manufacturer's instructions.

The advantage of the Mirasol PRT system, unlike other systems developed to inactivate pathogens in blood products, is in the fact that after illumination of product with UV light during 6–10 minutes (6.24 J / mL), these products are immediately ready for clinical use. Therefore, there is no need for subsequently removing RB and its metabolites from blood products, since it is a vitamin, already present in the body of the recipient.

Conclusion

In this study efficient pathogen inactivation (CFU depletion) was obtained in investigated PC-P units – 3 Log for all the three tested bacteria species and 5 Log for *Escherichia coli*. Thus, the safety of blood component therapy – predominantly the clinical use of PCs – can be significantly improved (lower morbidity/mortality rate) by using the Mirasol PRT system.

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