Review Article

THERAFLEX ultraviolet C (UVC)-based pathogen reduction technology for bacterial inactivation in blood components: advantages and limitations

Torsten J. Schulze[1], Ute Gravemann[1], Axel Seltsam[2]

[1]German Red Cross Blood Service NSTOB, Springe, Germany; [2]Bavarian Red Cross Blood Service, Nuremberg, Germany

Contributions: (I) Conception and design: TJ Schulze, A Seltsam; (II) Administrative support: None; (III) Provision of study materials or patient: None; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Torsten J. Schulze. Institut Springe, Eldagsener Straße 38, 31832 Springe, Germany. Email: torsten.schulze@bsd-nstob.de.

Abstract: While viral screening of blood products has significantly decreased transfusion-related viral infection rates, the risk of receiving bacterially contaminated blood products, especially platelet concentrates has not significantly decreased. Pathogen reduction technologies (PRTs) offer a solution. They can be applied in order to sterilize blood products and, thus, increase the transfusion safety. PRTs can also inactivate emerging viruses that may spread due to travel, trade and climate change. An ultraviolet C (UVC)-based PRT (THERAFLEX UV-Platelets, Maco Pharma, Mouvaux, France) is a robust, safe and reliable UVC-based PRT that effectively inactivates viruses, bacteria, parasites and residual leukocytes in platelet concentrates. In particular, it has a strong inactivation capacity for transfusion-relevant bacteria (log_{10} reduction factors ranging from 4 to 7), as shown in studies using the WHO International Reference Repository of Platelet Transfusion-Relevant Bacterial Reference Strains. It is however crucial for the PRTs to treat blood products as soon as possible, before contaminating bacteria can grow exponentially and overwhelm the pathogen reduction (PR) capacity of the PRT. Time-to-treatment studies are critical for determining the optimal time to start PR treatment. THERAFLEX UV-Platelets is currently the only technology that provides such specific data. This UVC-based PRT requires no photosensitizer and, consequently, no additional processing time for the removal of such chemicals. Hence, it can be performed immediately after platelet collection or preparation resulting in faster delivery of the blood products. Moreover, its simpler configuration provides added safety by eliminating potential handling and transportation errors that may lead to post-reduction contamination.

Keywords: Pathogen reduction technology (PRT); bacteria; ultraviolet C light (UVC light); platelets (PLT); whole blood

Received: 07 June 2021; Accepted: 09 November 2021; Published: 30 September 2022.
doi: 10.21037/aob-21-44
View this article at: https://dx.doi.org/10.21037/aob-21-44

Introduction

The risk of viral transfusion transmitted infection (TTI) could be dramatically decreased by different means, such as testing and donor deferral in at-risk cases. Apart from contamination levels below the detection limits, one can generally assume that if the donor tests negative, the blood product will also be negative. Nonetheless, bacterial contamination remains a constant threat in transfusion medicine (1-10) because TTIs cannot be prevented by simply testing blood donors for bacteria. Contaminating bacteria mainly enter into blood bags during venipuncture. Generally speaking, due to their initial low numbers they may not be detectable at the time when blood is drawn for the cultivation from blood components, such as red blood

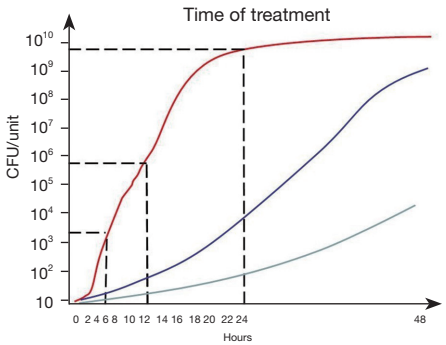

Figure 1 Growth curve analysis of various bacteria seeded at 10–100 CFU/unit (approx. 10^{-2}–10^{-1} CFU/mL) and treated with pathogen reduction technology at different time points. Shown are the growth curves for slowly growing bacteria (green), bacteria with an intermediate growth rate (blue), and fast-growing bacteria (red), which can expand to 10^6 CFU/mL within 12 hours. CFU, colony-forming units.

cells (RBCs) and platelets (PLT). By the time of transfusion, their numbers may have increased to levels capable of causing severe complications, including sepsis. Bacterial growth kinetics vary greatly with respect to strain, ambient temperature and type of blood component. Particularly when stored at room temperature, PLT preparations are at a very high risk of high-titer contamination. Bacterial growth curve simulations indicate that, from a low starting population of 10–100 colony-forming units (CFU) per unit, the maximum time between PLT product preparation and pathogen reduction technology (PRT) treatment, which must be observed in order to guarantee product safety and sterility, is 24 hours in the case of slowly growing bacteria and 12 hours for the majority of bacterial strains with an intermediate growth rate (*Figure 1*). However, if contaminated with fast-growing species (e.g., *Escherichia coli* or *Staphylococcus aureus)* that double in number every 60 minutes, earlier pathogen reduction (PR) is crucial to ensure product sterility: from a low initial count of 100 CFU/unit, fast-growing bacteria may increase to counts of 6.4×10^3 CFU per PLT unit within 6 hours, 4.1×10^5 CFU per PLT unit within 12 hours, and 1.7×10^9 CFU per unit within 24 hours (*Figure 1*). These simulations imply that PRT with a very high inactivation capacity of more than seven $\log_{10}$ reduction steps for the respective contaminating bacterial species may be able to achieve sufficient sterility if applied to the PLT within 12 hours, but would be overwhelmed by the bacterial load if used even slightly later. Consequently, the time point of PR is highly relevant to the bacterial safety of pathogen-reduced PLT. Therefore, the capacity of a PRT to inactivate bacteria in PLT concentrates strongly depends on the timing of treatment.

Different measures to prevent bacterial contamination are available. Sophisticated pre-transfusion testing strategies to prevent bacterial contamination of PLT concentrates have recently been published (11-14). Cold storage, for example, dramatically decreases the growth of bacteria that usually contaminate PLT preparations, but reduces PLT recovery and survival (15-19). Consequently, cold-storage PLT concentrates may be more suitable for therapeutic PLT transfusions than for prophylactic transfusions (20,21). PRTs offer a universal approach to reducing bacterial contamination. The PRT based on the addition of amotosalen followed by ultraviolet A (UVA) irradiation (320–400 nm) is a photochemical technology that works via the irreversible modification of nucleic acids in pathogens and leukocytes: Upon irradiation amotosalen is crosslinked between nucleic acid base pairs (INTERCEPT, Cerus Corp, Concord, CA, USA) (22-25).

The PRT based on the addition of riboflavin and subsequent UVA/ultraviolet B (UVB) irradiation (265–370 nm) is a photodynamic technology that works via the promotion of an irreversible modification of nucleic acids in pathogens and leukocytes in the presence of riboflavin (Mirasol, Terumo BCT, Lakewood, CO, USA) (25-28). Ultraviolet C (UVC)-based PRT (THERAFLEX UV-Platelets, Maco Pharma, Mouvaux, France), on the other hand is a purely physical PRT that uses UVC light (254 nm) alone, without a photosensitizing agent, to directly and irreversibly disrupt the integrity of nucleic acids (29). All of these technologies aim to inhibit nucleic acid amplification and to reduce or eliminate the infectivity of blood products due to disease-causing bacteria, viruses and protozoa.

THERAFLEX UV-Platelets UVC-based PRT

Effects of UVC irradiation

The germicidal effect of UVC light is already utilized for surface sterilization. When used to inactivate pathogens in blood products, however, one must strike a balance between exerting the germicidal properties of UVC and maintaining the functional integrity of the blood products. THERAFLEX UV-Platelets works by exposing blood products to UVC at a wavelength of 254 nm—a level

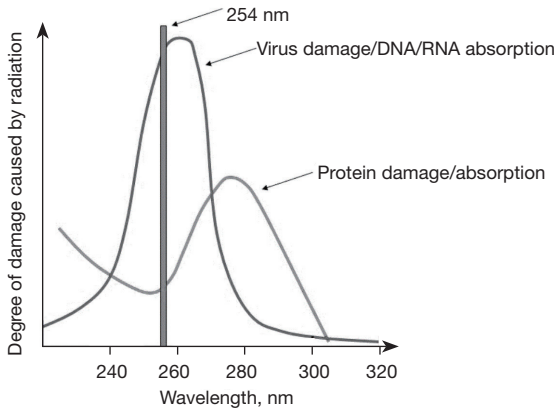

Figure 2 Relationship between UVC wavelength, DNA/RNA absorption and protein damage modified after (29). Damage capacity of UVC light at a wavelength of 254 nm: UVC light induces peak DNA/RNA absorption at 260 nm and the lowest level of protein absorption at 250 nm—this is crucial for maintaining the functional integrity of PLT. UVC, ultraviolet C; PLT, platelets.

that is close to the optimum wavelength for peak DNA/RNA absorption (260 nm) and the lowest possible protein absorption and damage (*Figure 2*). The latter property is crucial for conserving the delicate structure of PLT during UVC treatment (30,31).

UVC-induced chemical reactions in the nucleic acids of the contaminating pathogens result in the formation of cyclobutane-pyrimidine and pyrimidine-pyrimidone dimers, thus effectively blocking the elongation of nucleic acid transcripts (32,33). When cells are targeted, the number of alterations on bases may overwhelm the DNA repair capacity and lead to apoptosis and related programmed cell death processes (34,35). Because mature RBCs and mature PLT do not bear a nucleus that is required for cellular activity and function, disease-causing pathogens (e.g., bacteria, viruses and protozoa) in these blood products can be effectively inactivated while maintaining PLT and coagulation factor function (36,37). However, it is common to all available methods for PR of PLT that they cause or exert some effect on the integrity of PLT. *In vitro* studies have consistently shown increased metabolic activity and moderate activation of PLT after treatment with UVC and other PRT technologies (38-43). In addition, reduced recovery of PRT-treated PLT in transfused patients suggests an impact on PLT viability (44-52). Therefore, it is important for each PRT method to define the optimal conditions and protocols to find the right balance between effective pathogen inactivation and functional preservation (43). White blood cells (WBC), however, are susceptible to apoptosis, so leukocytes, which could cause graft-versus-host disease (GvHD) in vulnerable patients, are also inactivated by UVC (53-63).

PR procedure

THERAFLEX UV-Platelets is a PRT that is solely based on UVC light and does not utilize a photosensitizer, so that no additional steps for the removal of such substances are needed before transfusion of the treated blood components (64,65). In brief, whole blood-derived or apheresis PLT suspended in SSP+ Platelet Additive Solution (Maco Pharma, Mouvaux, France) are transferred to a UVC-permeable bag and irradiated with UVC light using an irradiation device (MACOTRONIC UV, Maco Pharma, Mouvaux, France). The large irradiation bag results in a small filling volume, providing a PLT concentrate with a small layer thickness and a big surface during light exposure. UVC light is applied from both sides at a dose of 0.2 J/cm^2 while simultaneously agitating the bag. Due to the relatively low penetration depth of UVC light, vigorous mixing of the PLT unit is required to expose every blood compartment to UVC light at the surface of the bag. UVC exposure time is less than one minute. After UVC treatment, the PLT are transferred into a storage container, ready for transfusion without any further processing. In its current version, the UVC-based PRT process uses a plasma concentration of 30–40% for effective pathogen inactivation and maintenance of product quality.

Bacterial inactivation capacity

The standard method of determining the inactivation capacity of PRTs for PLT concentrates and other blood products is by measuring the reduction in virus titers achieved by the system (66). However, bacterial growth poses a different challenge for inactivation studies. Appropriate bacteria spike protocols have been established in previous studies with other PRT systems (67-69). In contrast to viral particles, bacteria can rapidly multiply in blood units and accumulate to titers that may overwhelm the capacity of the PRT within a relatively short time span (70-72). Consequently, recent recommendations for the validation of PRTs include the conditions of intended use, aiming at achieving a safe and sterile blood product through timely pathogen inactivation (73). These recommendations propose that validation tests be conducted using pooled

PLT from at least three different donors to exclude donor-related effects, keeping the initial dose of bacteria as low as possible (1–100 CFU/unit) to mimic real contamination scenarios, and to use sufficiently large samples (>8 mL) for sterility testing after PR treatment.

Studies for evaluation of the inactivation capacity of the UVC-based PRT (THERAFLEX UV-Platelets, Maco Pharma) were assessed while strictly adhering to the recent recommendations. These tests were performed using a wide variety of transfusion-relevant bacteria, including the recently published and enlarged WHO reference repository of PLT transfusion-relevant bacterial reference strains (74-76). Moreover, different strains of the species were tested to check for potential intra-strain differences in UVC susceptibility, and clinical isolates from contaminated PLT concentrates were also tested. The results revealed that UVC treatment effectively inactivated all bacterial strains in the investigated PLT concentrates (*Table 1*). In brief, PLT concentrates of a volume between 325 and 375 mL were inoculated with the aforementioned bacterial suspensions. The yielded bacterial titers were between 10^5 CFU/mL and 10^7 CFU/mL. PLT concentrates were treated up to the full UVC light dose of 0.2 J/cm^2. In parallel, "hold samples" that were inoculated with bacteria and left untreated did not show any bacteria inactivation by the blood product itself, excluding any kind of self-sterilizing effect (56).

Bacterial titers can develop very differently within a PLT concentrate, depending on the inherent growth characteristics of the bacterial species and its response to the blood component and the additive solution. Some strains are highly susceptible to this environment and show self-sterilizing behavior, whereas some sterilizing effects may also be attributed to donor-related characteristics (37,77). Other strains grow slowly and reach significant titers only at the end of their shelf life. The most dangerous strains are those that are nearly undetectable at the time of a preparation and then rapidly grow to numbers that overwhelm the inactivation capacity of a PRT (*Figure 1*). Thus, it had to be determined what interval between preparation and UVC illumination safely and effectively ensures the sterility of pathogen-reduced PLT concentrates over the entire storage period (56,65).

In brief, in these time-to-treatment experiments two PLT concentrates were pooled and then inoculated with the respective bacterial suspension using a target titer of 100 CFU/unit. This concentrate was then split into a control and a test PLT concentrate. From the test PLT concentrate bacterial titers were determined after 6 or 8 hours and the PLT concentrate was then subjected to UVC-irradiation according to the THERAFLEX-UV-Platelets protocol. Sterility testing was performed during storage time, using a culture system (BacT/ALERT, bioMérieux, Marcy l'Etoile, France) (56).

The time-to-treatment experiments showed that UVC treatment consistently achieved PLT concentrate sterility for up to 7 days of storage when performed within 6 hours after spiking. In order to identify the possible limits of this PRT, titers of fast-growing bacteria—*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*—were also determined in PLT concentrates UVC-treated 8 hours after preparation. This additional time before pathogen inactivation impaired the sterility of the PLT concentrate in single cases: 11 out of 12 *Escherichia coli* and *Streptococcus pyogenes* samples were still sterile 7 days after PRT. The above data demonstrate that timely inactivation is the key to preventing the transmission of bacteria to PLT recipients (*Table 2*).

Advantages and limitations

THERAFLEX UV-Platelets has an easy and quick procedure. Because it does not require any photosensitizers, it has a short hands-on time along with a short overall processing time. Since UVC treatment is performed after standard PLT collection and preparation, it can easily be integrated into the blood product supply chain without altering the manufacturing process significantly. According to the current specifications, the THERAFLEX UV-Platelets technology must be applied to plasma-reduced PLT for efficient PR.

When PLT are stored, bacteria may adhere to blood bag and tubing systems via fibrin caps or other organized biofilms (78-82). Penetration of photosensitizers used for PRT may then be impaired, making subsequent UV treatment ineffective, leading to break-through contamination. THERAFLEX UV-Platelets uses different bags in sequence, so the initial adherence of bacteria to the bag cannot impact the final preparation. The PLT are transferred from a first storage bag to an irradiation bag, and then to a final storage bag. Moreover, THERAFLEX UV-Platelets only uses UVC light for PR, making the accessibility of a biofilm to a photosensitizer irrelevant. The system uses relatively large irradiation bags, which are agitated rapidly during the irradiation process to ensure efficient mixing and complete penetration of UVC light. The bag system is designed so as to prevent niches or

Table 1 Inactivation capacity of THERAFLEX UV-Platelets for bacteria (N=3–6) (56)

Species	Strain	N	Log_{10} reduction factor at full UVC dose (0.2 J/cm²)*
Enterobacter cloacae	PEI-B-P-43	3	6
Escherichia coli	PEI-B-P-19	6	7
Klebsiella pneumoniae	PEI-B-P-08	6	6
Morganella morganii	PEI-B-P-91	3	7
Proteus mirabilis	PEI-B-P-55	3	7
Pseudomonas fluorescens	PEI-B-P-77	3	7
	ATCC 17569	3	≥5
Serratia marcescens	PEI-B-P-56	3	6
	ATCC 43826	6	≥5
Staphylococcus aureus	PEI-B-P-63	3	6
	ATCC 25923	3	5
	Clinical isolate	3	4
Staphylococcus epidermidis	PEI-B-P-06	6	4
Streptococcus bovis	PEI-B-P-61	3	7
	ATCC 33317	3	4
Streptococcus dysgalactiae	PEI-B-P-71	3	4
	ATCC 35666	3	4
Streptococcus pyogenes	PEI-B-P-20	4	4
	DSM 11728	6	4
	DSM 25953	6	4
	ATCC BAA-1064	6	5
	Clinical isolate	6	5
Listeria monocytogenes	ATCC 19115	6	5
Acinetobacter baumannii	ATCC 17961	3	5
Streptococcus agalactiae	ATCC 13813	3	≥6
Streptococcus pneumoniae	ATCC 33400	3	5
Bacillus cereus	PEI-B-P-57	3	3
Bacillus thuringiensis	PEI-B-P-07	3	4
Propionibacterium acnes	ATCC 6919	6	5

*, in some cases, the mean log_{10} reduction factors could not be exactly determined after full-dose UVC treatment with THERAFLEX UV-Platelets (and was thus expressed as "≥") as the bacterial titers of some species reached the limit of detection of the plating assay at this UVC dose (56). UVC, ultraviolet C.

reservoirs where pathogens could collect and escape from UVC light exposure.

THERAFLEX UV-Platelets is so far the only system that provides a systematic evaluation of the timing of bacterial PR needed to ensure product sterility (*Table 2*). Data show that this system not only has a high capacity to effectively inactivate transfusion-relevant bacteria in PLT concentrates, but also ensures the sterility of PLT

Table 2 Time to treatment: sterility of UVC-treated platelet concentrates 6 or 8 hours after bacterial spiking (56)

Species	Origin/strain	Sterility of UVC-treated PLT concentrates (N/N) depending on time to treatment after spiking	
		6 hours	8 hours
Bacillus cereus†	PEI-B-P-57	12/12	n.t.
Bacillus thuringiensis†	PEI-B-P-07	12/12	n.t.
Escherichia coli	PEI-B-P-19	12/12	11/12
Klebsiella pneumoniae	PEI-B-P-19	12/12	12/12
Enterobacter cloacae	PEI-B-P-43	12/12	n.t.
Morganella morganii	PEI-B-P-91	12/12	n.t.
Proteus mirabilis	PEI-B-P-55	12/12	n.t.
Pseudomonas fluorescens	PEI-B-P-77	12/12	n.t.
Serratia marcescens	PEI-B-P-56	12/12	n.t.
Staphylococcus aureus	PEI-B-P-63	12/12	n.t.
Staphylococcus epidermidis	PEI-B-P-06	12/12	12/12
Streptococcus bovis	PEI-B-P-61	12/12	n.t.
Streptococcus pyogenes	PEI-B-P-20	12/12	11/12
Streptococcus dysgalactiae	PEI-B-P-71	12/12	n.t.

These experiments were done with 12 replicates (two series of six) for each bacterial strain. †, vegetative forms only. n.t., not tested; UVC, ultraviolet C; PLT, platelet.

concentrates contaminated with less PI-sensitive bacteria when applied early after PLT preparation (56).

Leukocyte and parasite inactivation experiments have shown that UVC light effectively damages ribonucleic acid inside blood cells (36,59). The high capacity of UVC to inactivate leukocytes and intracellular parasites like Plasmodium falciparum suggests that the THERAFLEX UV-Platelets system may also be effective against intracellular bacteria and viruses, although this remains to be proved in scientific experiments.

After implementation of the INTERCEPT PR method for PLT concentrates in routine use in the US, isolated cases of sepsis after transfusion of pathogen-reduced PLT concentrates have been reported over the last years, and some even had a fatal outcome (83-85). While it must be underscored that hemovigilance data do not provide evidence of systemic flaws, the investigators demonstrated that the patients in question did contract the bacteria from the PLT concentrates, and it is striking that the bacteria involved in the cases with a fatal outcome (*Acinetobacter baumanii complex*, *Leclercia adecarboxylata* and *Staphylococcus saphrophyticus*) were effectively inactivated by seven $\log_{10}$ steps in inactivation experiments. In view of the fact that *Acinetobacter baumanii* and *Staphylococcus saphrophyticus* are environmental strains rather than part of the usual skin flora, contamination after amotosalen/UV light inactivation seemed likely. Later analysis of the bags revealed invisible leaks that could only be detected by air pressure tests. Abrasion-related damage to the bags during transport or agitation was determined to be the most likely cause of the undetected leaks. The same bacteria, *Acinetobacter baumanii* and *Staphylococcus saphrophyticus*, were also implicated in three previous cases of sepsis after transfusion of pathogen-reduced PLT. Since PR was performed after 16 hours in these cases, high bacterial contamination with titers above the inactivation capacity of the PR system could be discussed as a cause. On the other hand, the amotosalen/UV-based system has a complex bag system consisting of one bag for the photosensitizer amotosalen, one bag for illumination, one bag for the removal of amotosalen and photoproducts, and one final storage bag for the PLT concentrate, making this system vulnerable to handling, transportation and storage-related damage. The UVC-based PRT system requires only the PLT concentrate to

be UVC-treated, a big irradiation bag and the final storage bag. The irradiation process itself takes less than one minute. Afterwards, the UVC-treated PLT are transferred into the storage bag. The UVC-based PRT does neither require addition nor removal of photoactive substances. While post-PRT damage can occur on any PLT bags of any PRT system, it seems plausible that the THERAFLEX UV-Platelets system's simple procedure may be less susceptible to material damage and production errors that could cause undetectable leakage.

All PRTs for blood components are limited in their efficacy to inactivate spores. Previous studies have shown that UVC inactivates vegetative bacteria much better than spores (64,86,87). Thus, PLT concentrates contaminated by spore-forming bacteria (e.g., *Bacillus spp.)* may contain viable spores after PR. Surviving spores in PLT concentrates could then develop into vegetative forms and grow to clinically relevant numbers during storage (88). However, the relevance and levels of spores in bacterially contaminated PLT concentrates are largely unknown. Storage of PLT products at room temperature provides good growth conditions for bacteria, but may not favor the production of high numbers of spores. More research on the sporulation of bacteria in PLT concentrates is required to better address potential safety issues related to the insufficient inactivation of spores by a PRT.

As already emphasized above, high titers of bacteria can overwhelm the inactivation capacity of the THERAFLEX UV-Platelets system at the time of treatment. This problem is a significant challenge to all PRTs that can only be adequately resolved by treating PLT concentrates very early after preparation.

The preparation of pooled PLT concentrates from whole blood donations usually takes more time from blood collection to PR than the preparation of apheresis PLT concentrates. Therefore, bacteria infiltrating the collection bag during whole blood donation may have more time to adjust to the additive solution and environment within the blood bag and multiply than bacteria contaminating apheresis PLT concentrates. However, current data suggests that the initial bacterial burden of pooled PLT concentrates at the time of preparation is comparable to that of apheresis PLT concentrates after donation. Previous studies have shown that bacteria are significantly eliminated by the preparation procedure for random donor PLT concentrates (77,89-91). Therefore, it might be reasonable to define the maximum allowable time between PLT collection and PR treatment in terms of the time of preparation of the respective pooled or apheresis PLT concentrate. Time-to-treatment experiments using blood banking conditions and bacterial contamination levels that mimic routine use have shown that a maximal interval of 6 hours between PLT preparation and UVC treatment is sufficient to guaranty sterility for both PLT product types. It would, of course, be desirable to have more experimental data on the growth kinetics and location of bacteria during the manufacturing process of whole blood-derived pool PLT concentrates and on the inactivation capacities of the available PRTs for contaminated pooled PLT concentrates under different PLT concentrate production, bacterial contamination and treatment timing conditions. However, controlling the experimental settings of such studies is very complex because additional confounding factors, such as donor-specific interactions, elimination of bacteria by WBCs and the choice of additive solution, need to be considered.

Perspectives

The investigated UVC light-based PRT effectively inactivates viruses, bacteria, parasites and alloreactive T-cells in PLT concentrates while maintaining PLT function (54-58,60,63,92-95). A phase I study of the safety and tolerability of autologous UVC-irradiated PLT concentrates in healthy volunteers did not reveal any adverse reactions or immunization against treated PLT concentrates, among other clinical parameters (96). Recently, the CAPTURE study (Clinical Assessment of Platelets Treated with UVC in Relation to Established Preparations), a phase III randomized, double-blind, parallel controlled non-inferiority trial comparing pathogen-reduced pooled and apheresis PLT with conventional pooled and apheresis PLT was completed (97). The application for marketing authorization of the THERAFLEX UV-Platelets system is currently under evaluation by the responsible authority in Germany.

The THERAFLEX technology was originally developed for PLT but is also suitable for plasma, RBCs and whole blood. Proof of principle of UVC treatment for the inactivation of pathogens in plasma units and RBCs has been demonstrated (98,99). PRTs for the treatment of whole blood would be a major step towards increasing the bacterial safety of blood components. Whole blood could be pathogen-reduced early in the manufacturing process, thus significantly reducing the time for contaminating bacterial growth. Situations in which the bacterial load could exceed the inactivation capacity of the PRT would then be less

likely to occur. Moreover, such technology would have significant practical and economic advantages because the treatment of a single unit of whole blood could yield up to three different pathogen-reduced blood components: plasma, PLT and RBCs.

Bacterial inactivation capacity research conducted in the course of new PRT systems development or existing systems modification should include other tests in addition to the classical experiments for the determination of $\log_{10}$ reduction capacity. To be meaningful, the study design must also consider the clinical setting. In accordance with recommendations of the Transfusion Transmitted Infectious Disease Bacteria Working Party of the ISBT (TTID-B), focused research should be conducted under conditions which simulate routine clinical use while the sterility of the investigated blood products is the only clinically relevant outcome. Key elements of this approach include the use of transfusion-relevant bacteria (ideally, WHO International Reference Repository of Platelet Transfusion-Relevant Bacterial Reference Strains), different sources of blood products to balance the influence of donor-specific parameters, and sensitive tests for the detection of residual bacteria (73,100,101).

Acknowledgments

The authors would like to thank Anke Wenk for her assistance in the preparation of the manuscript.
Funding: TJS, UG and AS received grants from the Research Foundation of the German Red Cross Blood Services (Deutsche Forschungsgemeinschaft der Blutspendedienste des Deutschen Roten Kreuzes) and Maco Pharma for development of the investigated UVC-based PI technology for platelets.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Sandra Ramirez-Arcos) for the series "Bacterial Contamination of Platelet Components" published in *Annals of Blood*. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://aob.amegroups.com/article/view/10.21037/aob-21-44/coif). The series "Bacterial Contamination of Platelet Concentrates" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the manuscript and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/aob-21-44
Cite this article as: Schulze TJ, Gravemann U, Seltsam A. THERAFLEX ultraviolet C (UVC)-based pathogen reduction technology for bacterial inactivation in blood components: advantages and limitations. Ann Blood 2022;7:28.

