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Selected issues on pathogen inactivation in blood components; reports presented at the 33rd Regional Congress of the International Society of Blood Transfusion (ISBT) in Gothenburg, June 17–21, 2023

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Introduction

Pathogen inactivation in blood components was the subject of more than a dozen papers presented at the 33rd Regional ISBT Congress, held in Gothenburg (June 17–21, 2023). The outcome of testing pathogen-reduced blood components was demonstrated both during the Parallel Session 8: Pathogen inactivation and the poster session (Blood products, Pathogen inactivation).

Axel Seltsam of the Bavarian Red Cross, held oral presentation on the current status of the implementation of pathogen inactivation methods in blood components. He stressed the growing interest in inactivation methods as result of the spread of infectious agents from endemic regions an example of which was the recent global COVID-19 pandemic. Thanks to pathogen inactivation systems the transfusion of blood components is now safer and the components are more available. Pathogen inactivation methods reduce the risk of transmitting bacteria (particularily in PC), parasites and viruses. Some of them also inactivate T lymphocytes which is crucial for prevention of Transfusion-Associated Graft-versus-Host-Disease (TA-GvHD). Nowadays, pathogen inactivation systems are routinely used for plasma and PC and their mechanism of action is based on photodynamic and photochemical reactions. Although several methods for pathogen inactivation in whole blood (WB) and in red blood cells (RBCs) are already available none has yet been implemented for routine use. Most advanced and promising are two methods; for pathogen inactivation in whole blood (the Mirasol PRT system) and in RBCs with amustalin and glutathione. In the opinion of some presenting authors the low interest in pathogen inactivation methods observed in some countries may be related to anxiety over potentially lower effectiveness of pathogen-reduced blood components, expensive procedure and possible adverse reactions. However the first clinical trials have already demonstrated sufficient clinical efficacy and an acceptable safety profile of pathogen-reduced blood components, though in some clinical settings more transfusions may be required. The authors stressed the point that although pathogen inactivation was indeed associated with higher workload, the recent economic evaluations and studies of the benefit-risk ratio suggest that implementation of pathogen inactivation for plasma and PC significantly reduces the risk of transfusion-transmitted infection when new pathogens emerge [1]. In another oral presentation, a representative of several French blood transfusion centers (Paris, Marseille, Nantes, Rennes, La Plaine St-Denis) summarized the impact of implementation of 100%

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of pathogen-reduced PC in additive solution on their manufacture and issue as well as the risk of transfusion-transmitted infections. In France, the Intercept system was implemented into routine use in November 2017. In a survey that covered the years 2013–2022, the risk of pathogen transmission was compared before and after the implemention of pathogen inactivation. The percentage of PCs from whole blood (with additive solution) increased from 51% in 2013 to 72% in 2022. In 2019 the storage time of pathogen-reduced PC was extended up to 7 days. In 2018, the percentage of PC for separation was 30%, while in 2022 it already accounted for 96.5%. In 2017, apheresis PCs, were still not subject to separation and in 2022 the percentage was 11%. It was also stressed that implementation of pathogen inactivation for PC extended storage duration which resulted in lower wastage due to shelf life. Although pathogen-reduced PC had lower platelet count and more transfusions were necessary, the total number of transfused PCs per patient increased only slightly. A significant reduction of bacterial transmission with transfused pathogen-reduced PCs was also reported [2].

Poster session

The poster session (*Pathogen inactivation*) included 11 presentations referring to methods of pathogen inactivation in blood components. Four presentations were devoted to the Intercept system. One poster dealt with RBCs obtained from WB pathogen-reduced in the Mirasol PRT system and another referred to PCs pathogen reduced in the Mirasol PRT system. One poster was devoted to comparative studies of the Intercept and the Mirasol PRT systems. Two centers presented pilot studies — one with the Theraflex UV Platelets system, the other with amustalin and glutathione for RBCs. Two posters focused on the Theraflex MB Plasma system. Each of the 11 posters forwarded information on the inactivation system which they used; the collective data for all systems described are presented in form of a Table 1.

Intercept system

For years now the Strasbourg Blood Transfusion Center has been using the Intercept system for pathogen reduction in PCs. Together with Cerus representatives they presented an evaluation study of in vitro quality of buffy-coat PCs treated with prototype light-emitting diode (LED) iluminator used instead of the conventional UVA light emitting diode iluminator. Four study groups of PCs were compared (n = 5 each):

- PC PAS-C (InterSol)/axis (55%/45%) pathogen-reduced with conventional UVA light emitting diode iluminator (INT100 at 320–400 nm, 3.9 J/cm^2);
- PC with PAS-C/axis (55%/45%) pathogen-reduced with a prototype light emitting diode (LED) iluminator (350 nm, 3.3 J/cm^2);
- PC with PAS-E (SSP+)/plasma (55%/45%) pathogen-reduced conventional UVA light emitting diode iluminator;
- PC with PAS-E/plasma (55%/45%) and pathogen-reduced with a prototype light emitting diode (LED) iluminator.

In vitro platelet quality and function were assessed with multiple biochemical and functional tests during 7 days of storage. In all study groups platelet counts were stable throughout the storage period. Integrin αIIbβ3 and glycoprotein VI (GPVI) expression remained stable, while the expression of GPIbα and GPV was lower in all study groups. PCs treated with both UVA and LED light irradiation presented similar glucose consumption, lactate levels, LDH release as well as pH values. The analysis of results revealed that the use of either the prototype light-emitting diode (LED) iluminator or the UVA light emitting diode iluminator (INTERCEPT) had similar effect on the metabolism, activation, viability and hemostatic function of platelets in PCs from buffy-coat stored for 7 days in PAS-C or PAS-E/plasma [3]. In 2021, a report was published in the United States which described a transfusion-transmitted bacterial infection (TTBI) in a recipient of apheresis PC which had been pathogen reduced with the Intercept system. Post-transfusion residual PC screening revealed the presence of three bacteria — *Staphylococcus saprophyticus, Leclercia adecarboxylata and Enterobacter soli.* The stage at which contamination occurred — collection or preparation — was not determined. Assuming that the contamination occurred prior to inactivation, the conclusion is that inactivation with the Intercept system proved ineffective against these pathogens. Cerus therefore designed a study to evaluate the effectiveness of inactivation of pure *E. soli* culture and in a mixture with *L. adecarboxylata* and *S. saprophyticus* cultures. For this purpose, a mixture of *E. soli* culture and a mixture of *E. soli, L. adecarboxylata* and *S. saprophyticus* cultures (1:1:1 ratio) were added to an apheresis platelet unit and then pathogen-reduced using the Intercept system. Samples were collected before and after the bacteria were added,

Table 1. Pathogen inactivation systems for PC and plasma

System/parametr	Theraflex MB Plasma	Intercept	Mirasol PRT	Theraflex UV Platelets
Type of blood component	Single unit of plasma from WB or apheresis	PC Single unit of plasma from WB (pooled) or apheresis PC in plasma or PAS (InterSol, SSP+)	Plasma; PC in plasma or PAS (SSP+)	PC, plasma during testing
CE marking (year)	2000	PC: 2002; plasma: 2006	PC: 2007; plasma: 2008	PC: 2009
Photosensitizer	Methylene blue	Amotosalen hydrochloride (S-59)	Riboflavin (vit. B2)	No photosensitizer used
Photoproducts	Azur A, B, C; thionone	Dimers S-59	Lumichrome, lumiflavin, 2'ketoflavin, 4-ketoflavin, mononucleotide, flavin forms methylflavin	None
Conditions	Visible light (590 or 630 nm) 180 J/cm^2 UVA (320–400 nm) 3 J/cm^2 UV (280–400 nm) 6.24 J/cm^2 UVC (254 nm) 0.2 J/cm^2	UVA (320–400 nm) 3 J/cm^2	UV (280–400 nm) 6.24 J/cm^2	UVC (254 nm) 0.2 J/cm^2
Additional steps	Leukoreduction (Plasmaflex); removal of photosensitizer and photoproducts (Blueflex)	Removal of photosensitizer and photoproducts	Not applicable	Not applicable

as well as after inactivation with the Intercept system. None of the microbiological tests revealed bacterial growth, which only proved the Intercept system to be effective for these pathogens [4]. In another poster presentation the authors stressed the fact that in Europe, the Intercept system is in routine use for pathogen inactivation in both pooled and apheresis PC whereas in the USA — mostly for apheresis PCs (TRIMA® in 100% plasma or AMICUS® in a 65% PAS-3/35% plasma mixture). In 2015, members of the WHO Expert Committee on Biological Standardization (ECBS) in collaboration with the Paul-Ehrlich Institute (PEI), approved an expanded panel of bacterial strains used for evaluation of methods strengthening microbiological safety of blood components (Spindler-Raffel et al, Vox Sang. 2015). The aim of the study presented by Cerus was to evaluate the effectiveness of inactivation of a broad spectrum of bacterial strains that were added to apheresis PCs in plasma, to apheresis PCs in additive solution (65% PAS-3/35% plasma) and to plasma units. The Intercept system was found to inactivate high titers of *K. pneumoniae* and *S. aureus* in plasma. Inactivation was effective for *K. pneumoniae, S. aureus, E. coli* and *S. epidermidis* in PCs. The data show the INTERCEPT system to be effective for inactivation of WHO reference bacterial strains responsible for transfusion-transmitted bacterial infections [5]. A Swedish blood transfusion center presented their own method of pooling and inactivation of unused plasma. The Uppsala University Hospital noted a high percentage of plasma waste (about 25% of the whole 2018-inventory) initially intended for patients with serious injuries (e.g., traffic accidents). Unused plasma units (e.g.when the patient dies) are returned to the hospital blood bank and can still be used up to 7 day storage. Usually, however, they are destroyed. To prevent the big waste, a method was devised to create pools of unused plasma. For this purpose, 100 units of leukocyte-reduced plasma units were frozen (≤ –25°C), thawed after 7 days and then 10 pools (of 10 units each) were prepared. Each pool was then divided into 4 (650 ml) sub-pools each of which was subjected to inactivation in the Intercept system. Following inactivation, each sub-pool was divided into 3 units to finally give 120 units of inactivated plasma (200 ml each) to be frozen and thawed as required. The quality control tests revealed that

freezing and thawing did not significantly affect factor VIII activity and fibrinogen concentration, the value of these parameters however were significantly reduced as result of inactivation (though the values were still acceptable; 69% and 87% of the initial value for factor VIII and fibrinogen content respectively). The authors concluded that pooling 10 plasma units before inactivation may contribute to standardization of the volume and protein content in plasma units and reduction of plasma waste to 12% [6].

Mirasol PRT system — pathogen inactivation in platelet concentrate

Since 1990 when methods of pathogen inactivation in blood components were implemented, transfusions have become much safer. Inactivation however adversely affects the quality of blood components and leads to lower recovery of platelet cells in the recipient's circulatory system. In consequence, for some patients a higher number of transfusions is required. With the aim of improving the quality of platelets in leuko-reduced PCs. Representatives of the Dutch Sanquin Blood Bank decided to check whether such components as pyruvate, glutathione and vitamin C added to the additive solution (PAS-E) favorably affect the post-inactivation quality of PCs. A pool of 10 buffy-coat PCs was divided into two sub-pooles: one with PAS-E (T-PAS+ from Terumo BCT), the other with PAS-E and glutathione, pyruvate and vitamin C. Both sub-pools were inactivated using the Mirasol PRT system and the components were stored for up to 8 days. The analysis of results revealed that pyruvate decreases glucose uptake, lactate formation and promotes better pH maintenance. Both glutathione and vitamin C inhibit activation (CD62P expression) and apoptosis (annexin V). The authors emphasized that pyruvate and antioxidants in the additive solution may mitigate some inactivation effects. Further studies are however required to confirm the beneficial effects of the modified formula of the additive solution. Equally important is to assess whether the modified content of the additive solution improves platelet quality after pathogen inactivation. [7].

Mirasol PRT system — pathogen inactivation in whole blood

Despite years of research, no method of pathogen inactivation in RBCs has yet been implemented into routine use, although red blood cells are the most often transfused blood component. The Mirasol PRT system for pathogen inactivation in whole blood was CE marked in 2015, this however has not increased the interest in this method. The staffs of the Regional Blood Transfusion Center (RCKiK) in Lodz and the Institute of Hematology and Transfusion Medicine in cooperation with Terumo, presented research on the quality of leukoreduced RBCs (PAGSM) obtained from WB inactivated with the Mirasol PRT system (Terumo BCT) with the automated method (Reveos). The study included 40 WB units which were divided into 2 groups: study gtroup (S) — subjected to inactivation and control (C) which obtained/prepared RBCs then stored at 2–6°C for up to 42 days. The expression of CD 44, CD 47 and annexin V antigens was monitored. Comparison of the parameters on storage day 1 and 42 demonstrated no significant differences in CD44, CD47 or annexin V antigen expression, which suggests that the Mirasol system of pathogen inactivation in WB has no effect on the expression of the these antigens in RBCs [8].

Intercept system, vs. Mirasol PRT system

In 2021 the Luxembourg Red Cross implemented some measures to upgrade their self-sufficiency and to reduce PC waste related to shelf life. In their study the quality of pathogen-reduced PCs in the Mirasol PRT system and the Intercept system were compared. The results pointed to the Intercept system which was implemented and the storage time of pathogen-reduced PCs was extended to 6 days. The quality of PCs during the initial period of Intercept system implementation, particularly apheresis PC, did not meet the standard for quality control (QC). It was therefore decided to assess the impact of the introduced changes on QC results, to determine the number of components not meeting the QC standards and to estimate the waste related to shelf life — as well as to determine the average „age" of the PCs issued. For comparison, retrospective data was used (Pathogen-reduced PCs using the Mirasol PRT system) as well as current data (Pathogen-reduced PCs using the Intercept system). In addition, a breakdown was made into pooled PCs (Revos system, Terumo BCT) and apheresis PCs (Trima, Terumo BCT). PCs pathogen-reduced in the Intercept system manifested a higher loss of platelets in the pooled PCs which was attributed to the removal of residual photosensitizing compound and its photoproducts (compound adsorption device, CAD). No loss in

the platelet count was observed in apheresis PC as the PCs subjected to inactivation always had an above — standard platelet count. Moreover, the duration of component issue was extended from 2.73–4.03 days to 3.31–4.26 days (required incubation with CAD).The extension of shelf life and good QC parameters, have no adverse affect on the overall process of PC production and quality. The percentage of waste decreased and no increase in the percentage of non-QC-compliant PCs was recorded [9].

Theraflex MB Plasma System

Two posters were devoted to pathogen inactivation in plasma using the Theraflex MB Plasma System. They referred to the effectiveness of bacterial and viral inactivation in plasma stored in DEHP-free plastic containers [10, 11].

Theraflex UV-Platelets System

Representatives of the Bavarian Red Cross came forward with a study on the implementation of PC pathogen inactivation using the Theraflex UV-Platelets system solely based on the properties of short-wave UVC-light (UVC). The recipients of PCs pathogen reduced with this method are not exposed to as much as trace amounts of chemical compounds. The purpose of the study was process validation and qualitative assessment of pathogen--reduced PCs. 6 duplicate units of apheresis PC were divided into study group (S) — pathogen--reduced with the Theraflex UV Platelet system and a control group (C). All PC units were stored at 22°C with agitation. The quality control parameters were within normal range, the average platelet count in both groups was similar, and both groups also demonstrated similar response to hypotonic shock response and swirling. Although the glucose consumption was higher in the test group, in both cases (C and B) there were traces of glucose on storage day 9 [12].

Pathogen inactivation technology using amustaline (S-303) and glutathione (GSH)

Under intensive investigation are currently pathogen inactivation methods dedicated for erythrocyte-containing blood components. One of them is the chemical method of pathogen inactivation with amustaline (S-303) and glutathione (GSH).

S-303 efficiently crosses through cell membranes and viral capsids and incorporates into the helical regions of nucleic acids to form interstrand cross-linking by di-alkylating group. Among others, S-303 also reacts with phosphates, proteins and water. Glutathione (GSH) is added to prevent such non-specific reactions. Numerous clinical studies have reported the formation of neoantigens and antibodies in susceptible patients following transfusion of RBCs pathogen-reduced with the chemical method (amustalin and glutathione). German scientists have therefore conducted a pilot study to find an optimal method of determining these antibodies. The study demonstrated that in vivo recovery and survival of red blood cells can be traced using Surface acridine or biotin flow cytometric markers. The authors stressed the fact that the acridine marker requires no additional preparation steps [13].

Summary

The 33rd ISBT Congress presented a wide variety of papers on inactivation. The quality of PCs inactivated in the new prototype illuminator (Intercept system) was evaluated. The Intercept system was assessed for the effectiveness of pathogen inactivation against new bacterial strains in the pathogen reduced PCs. A pilot study was conducted to evaluate the benefits of introducing new chemical compounds into the PAS-E additive solution to improve the quality of PCs pathogen--reduced in the Intercept system. There was also a study — rather controversial from the point of view of Polish legal regulations, regarding ways of reducing wastage of plasma units. One study recalled the method of pathogen inactivation in PCs solely with UVC. Although the method (used in the Therflex UV Platelets system) was CE markled in 2009, its application seems to be somewhat limited. Contrary to expectations and rather dissappointing is the fact that so few studies were devoted to pathogen inactivation in whole blood and packed red blood cells. This implies that routine implementation of pathogen inactivation systems for better safety of red-blood cell components is still a long way off. Attention was also focused on new challenges facing blood transfusion service — including the need to change the plasticizer in kits currently used for collection, preparation, inactivation and storage. The variety of topics covered at the conference only confirms the determination to constantly upgrade the parameters of the blood components and to provide safer and better quality blood components.

Conflict of interest: none declared

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