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Inactivation of severe acute respiratory syndrome coronavirus 2 in plasma and platelet products using a riboflavin and ultraviolet light-based photochemical treatment

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Vox Sanguinis

Background and Objective Severe acute respiratory distress syndrome coronavirus-2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is a member of the coronavirus family. Coronavirus infections in humans are typically associated with respiratory illnesses; however, viral RNA has been isolated in serum from infected patients. Coronaviruses have been identified as a potential low-risk threat to blood safety. The Mirasol Pathogen Reduction Technology (PRT) System utilizes riboflavin and ultraviolet (UV) light to render blood-borne pathogens noninfectious, while maintaining blood product quality. Here, we report on the efficacy of riboflavin and UV light against the pandemic virus SARS-CoV-2 when tested in both plasma and platelets units.

Materials and Methods Stock SARS-CoV-2 was grown in Vero cells and inoculated into either plasma or platelet units. Those units were then treated with riboflavin and UV light. The infectious titres of SARS-CoV-2 were determined by plaque assay using Vero cells. A total of five (n = 5) plasma and three (n = 3) platelet products were evaluated in this study.

Results In both experiments, the measured titre of SARS-CoV-2 was below the limit of detection following treatment with riboflavin and UV light. The mean log reductions in the viral titres were ≥3·40 and ≥4·53 for the plasma units and platelet units, respectively.

Conclusion Riboflavin and UV light effectively reduced the titre of SARS-CoV-2 in both plasma and platelet products to below the limit of detection in tissue culture. The data suggest that the process would be effective in reducing the theoretical risk of transfusion transmitted SARS-CoV-2.

Key words: blood, COVID-19, pathogen reduction, riboflavin, SARS-CoV-2, ultraviolet.

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Introduction

In early December 2019, an unusual cluster of pneumonia cases caused by an unknown agent was observed in Wuhan, China [1,2]. The unknown agent, now known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [3], is the causative agent of coronavirus disease 2019 (COVID-19). Initially declared a public health emergency on 30 January 2020, the World Health Organization eventually reclassified COVID-19 as a pandemic on 11 March 2020, citing the alarming rate at which the disease was spreading. The number of infected individuals

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that will contract this agent will almost certainly continue to rise until significant herd immunity has developed. SARS-CoV-2 is the seventh known human coronavirus, which also includes Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV), having mortality rates of 35·5% and 10%, respectively[4]. The other four species of coronaviruses have mainly been associated with causing cold-like symptoms [5]. The sudden appearance of SARS-CoV-2 in humans is believed to have been the result of a zoonotic transmission event, although the proximate intermediate host remains unknown [6,7].

COVID-19 has a range of symptoms that includes fever, fatigue, dry cough, aches, and laboured breathing to acute respiratory distress and possibly death. It has also been reported that many infected individuals remain asymptomatic [8,9], which has complicated public health efforts to contain the spread of the virus. Unknown are the effects this virus may have on national blood supplies; however, the 2003 outbreak of SARS-CoV was shown to have a negative impact [10]. To date, there have been no known transfusion transmitted cases of SARS-CoV or MERS-CoV; however, the AABB considered MERS-CoV as an agent of concern [11]. During the 2003 outbreak of SARS-CoV, viral RNA was detected in the serum of symptomatic patients [12-14], and in another study, symptomatic MERS-CoV-infected patients showed viral loads up to 5–6 log RNA copies/ml in their serum [15]. The author of MERS-CoV study also indicated that they were not able to recover infectious virus from those patient samples using cell culture, suggesting that transfusion transmission is a low risk [15]. Similar to the previous coronavirus outbreaks, viral RNA has been recovered from symptomatic SARS-CoV-2 patients [16,17]. In late January 2020, the Wuhan Blood Center began using real-time PCR to screen all blood donations and retrospectively found viral RNA in 4 asymptomatic donors [18]. However, the US FDA is not recommending the use of laboratory screening tests of asymptomatic donors given that respiratory viruses, including coronaviruses, are not known to be transmitted by blood transfusion. To date, there have been no reported cases of transfusion transmission of SARS-CoV-2 [19].

The Mirasol Pathogen Reduction Technology (PRT) System was created to provide an additional layer of safety to blood products by reducing the risk of transfusion transmission of both known and emerging pathogens. The technology uses a UV light source and riboflavin (vitamin B2) in combination to cause irreversible damage to nucleic acids (RNA/DNA). Due to its mode of action, the riboflavin and UV process selectively renders viruses, bacteria and parasites, along with donor white cells, unable to replicate, while maintaining acceptable quality levels for platelets, red blood cells and plasma proteins [20-22]. Beyond having proven effectiveness at reducing infectious titres in a variety of viruses, bacteria, and parasites [23-27], the riboflavin and UV process has specifically demonstrated a high level of effectiveness against MERS-CoV [28]. The purpose of this study was to evaluate the efficacy of the Mirasol PRT System on the reduction of SARS-CoV-2 in both human plasma and platelet products.

Materials and methods

Plasma products (n = 5)

Whole blood products collected in CPD were acquired from an accredited blood bank after institutional review board (IRB) approval and shipped to Terumo BCT. The whole blood products were held overnight at room temperature and then separated on an automated blood processing system to create PF24 plasma (plasma frozen within 24 h after phlebotomy to ≤–20°C). The units were stored at ≤–20°C until needed.

Platelet products (n = 3)

Leukoreduced apheresis platelet products suspended in plasma and collected in ACD were acquired from an accredited blood bank under an IRB-approved protocol and shipped to Terumo BCT. Platelet products were allowed to rest for minimum of 2 h before being placed onto a platelet incubator/ shaker at 22°C ± 2. Prior to use platelets products were evaluated for positive swirl and to ensure the incoming cell count was within 800–2100 x 10^3/μl.

Riboflavin and UV light process for platelet and plasma products

The riboflavin and UV light process has been previously described in detail [24,27]. Briefly, 200 ml ± 5 of either plasma or platelets was dispensed into an extended life platelet (ELP) Illumination/Storage bags (Terumo BCT, Lakewood, CO, USA) and then mixed with 35 ml of riboflavin solution (500 μmol/l riboflavin in 0·9% sodium chloride, pH 4·0 to 5·0 [Terumo BCT, Larne, Ireland]). A fixed product volume was used to simplify the amount of virus required for each unit. After units were spiked with virus, they were placed into the Mirasol Illuminator (Terumo BCT) for UV treatment. The units were exposed to 6·24 J/ml of energy.

SARS-CoV-2 culture protocol

SARS-CoV-2 (isolate USA-WA1/2020) was acquired through BEI Resources. The virus was propagated in Vero cells (ATCC #CCL-81, Manassas, VA, USA) that were cultured in Dulbecco modified Eagle medium with high glucose (MilliporeSigma, St. Louis, MO, USA) and 2% fetal bovine serum (Peak Serum, Wellington, CO, USA). After clarification by centrifugation, the virus stock was supplemented to 10% with fetal bovine serum, frozen, and maintained at −80°C until thawed for use.

Virus reduction studies

The virus reduction studies were performed at Colorado State University by staff trained to operate the Mirasol Illuminator. All treatments occurred within a biosafety level 3 laboratory. For each 200 ml unit of either PF24 plasma or apheresis platelets containing riboflavin, a total of 12 ml of SARS-CoV-2 virus in media was added. After mixing, a pretreatment sample was obtained and held at ambient temperature until the completion of the treatment (<10 min). Following treatment with riboflavin and UV light, a post-treatment sample was collected. Samples were frozen and stored at −80°C until the plaque assay could be performed. Each of the pretreatment samples was serially diluted from 10^{-1} to 10^{-7} in sterile phosphate-buffered saline (PBS), and the dilutions were plated in duplicate by plaque assay on Vero cells. Each of the post-treatment samples was serially diluted from 10^{-0} to 10^{-2} in PBS, and 6 replicate wells were plated for each dilution. The number of replicate wells in the post-treatment samples was increased to provide greater assay sensitivity to detect low levels of virus, if present.

To perform the plaque assay, confluent Vero cell monolayers were grown in 6-well tissue culture plates and each well was inoculated with 0·3 ml of the appropriate diluted sample. The plates were rocked every 10–15 min for 45 min and then overlaid with 0·5% agarose (Life Science Products, Frederick, CO, USA) in media and incubated at 37°C, 5% CO_2. After 2 days, a second overlay containing 0·005% neutral red (MP Biomedicals, Irvine, CA, USA) was added and the plaques were counted the following day. The virus titre was determined based on the total plaque count and then corrected for dilution and the volume plated.

Calculation of limit of detection

When no virus is detected in the post-treatment samples at the lowest dilution tested, the limit of detection for the assay has been reached [29]. All of the values at the limit of detection were considered less than or equal to the calculated limit of detection. The theoretical limit of detection was calculated using the following equations:

$$N = \frac{\log(P)}{\log\left(1 - \frac{v}{V}\right)} \quad (1)$$

$$\mathrm{LOD} = \log\left(\frac{N}{V}\right) \quad (2)$$

where N is the lowest number of particles in the product that can be detected with 1-P confidence; P is the probability that a virus will be undetected (95% confidence of detecting a virus, $P = 0{\cdot}05$); V is the total volume of the treated product (platelet or plasma + riboflavin + virus); and v is the volume used for viral enumeration (volume inoculated per well in ml) × (number of replicate wells) × (lowest dilution inoculated).

Results

A total of five ($n = 5$) PF24 plasma units were evaluated using riboflavin and UV light against SARS-CoV-2. The average starting titre of the five units was 4·62 log PFU/ml, and the mean log reduction was ≥3·40 log (Table 1). All five treated units were reduced to the limits of detection (≤1·22 log PFU/ml). The measured titre of the viral inoculum was 6·04 log PFU/ml, and the theoretical pretreatment titre based on the measured stock titre is 4·73 log PFU/ml. The theoretical pretreatment titre was in agreement with the average measured starting titre indicating that there was no neutralization of the virus by native immune components in the plasma products, nor was there an effect of sample handling on the measured viral titre.

A follow-up study in platelets was also performed. Three apheresis platelet products were inoculated with stock virus and treated with the riboflavin and UV process. The average pretreatment titre was 4·77 log PFU/ml, and the mean log reduction was ≥4·53 log (Table 2). As with the plasma study, the virus titre was reduced to the limit of detection (≤0·25 log PFU/ml) in all three donor platelet units. The measured titre of the viral inoculum was 6·11 log PFU/ml, and the theoretical pretreatment titre based on the measured stock titre is 4·80 log PFU/ml. Again, the theoretical pretreatment titre was in agreement with the average measured starting titre indicating that sample handling did not affect the measured viral titre, nor was there any indication that native immune components in the platelet products neutralized the virus.

The overall log reduction observed in both studies was limited by the titre of the viral stock. In the plasma study,

Table 1 Log reduction of SARS-CoV-2 after PI– PF24 plasma units

Unit number	Pretreatment viral load (Log PFU/ml)	Post-treatment viral load (Log PFU/ml)	Log reduction
1	4·58	*≤1·22*	≥3·36
2	4·79	*≤1·22*	≥3·57
3	4·48	*≤1·22*	≥3·26
4	4·85	*≤1·22*	≥3·63
5	4·38	*≤1·22*	≥3·16
Average	4·62	*≤1·22*	≥3·40
SD	0·20	N/A	N/A
Stock Virus Titre	6·04 log PFU/ml		
Theoretical Pretreatment Titre[a]	4·73 log PFU/ml		

Titres shown in italic font are at the limit of detection for the assay.

PFU, plaque forming unit.

[a]Theoretical pretreatment titre is based on a ~5% (12 ml) viral inoculum with a final product volume of 247 ml (200 ml plasma + 35 ml RB + 12 ml stock virus).

Table 2 Log reduction of SARS-CoV-2 after PI – single-donor platelet units

Unit number	Pretreatment viral load (Log PFU/ml)	Post-treatment viral load (Log PFU/ml)	Log reduction
1	4·93	*≤0·22*	≥4·71
2	4·65	*≤0·30*[a]	≥4·35
3	4·74	*≤0·22*	≥4·52
Average	4·77	*≤0·25*	≥4·53
SD	0·14	*N/A*	N/A
Stock Virus Titre	6·11 log PFU/ml		
Theoretical Pretreatment Titre[b]	4·80 log PFU/ml		

Note: Titres shown in italic font are at the limit of detection for the assay.

PFU, plaque forming unit.

[a]One of the six wells in the 10^0 post-treatment sample was contaminated and removed from the final titre calculation.

[b]Theoretical pretreatment titre is based on a ~5% (12 ml) viral inoculum with a final product volume of 247 ml (200 ml platelet + 35 ml RB + 12 ml stock virus).

the log reduction was further limited due to the 10^0 dilution exhibiting cytotoxicity in the post-treatment samples. This dilution was not included in the limit of detection calculation for the plasma study. Additionally, one well of the six wells plated at 10^0 for the post-treatment sample in unit #2 of the platelet study was contaminated. This well was removed from the study, and this is reflected in the post-treatment titre calculation for this unit.

Discussion

While there have been no documented cases of transfusion transmission of SARS-CoV-2 to date [19], the pandemic this virus is causing illustrates the precarious nature of blood safety and its reliance on donor selection (donor questionnaire and health evaluation) and donor testing to prevent transfusion transmission of infectious diseases. Undoubtedly, these two approaches have prevented countless numbers of transfusion transmitted diseases; however, the COVID-19 pandemic has reinforced known weaknesses to the current blood safety strategy. During a pandemic, widespread donor deferrals can create both regional and national blood shortages, large populations of asymptomatic viremic donors may significantly increase the risk of transfusion transmitted diseases, and testing needs may outpace the ability of the marketplace to supply tests leaving blood units potentially unscreened for an outbreak agent. To combat potential blood shortages caused by the COVID-19 pandemic, the FDA has recently issued revised guidance documents easing traditional donor deferral periods, for example for certain malaria exposures, from 12 to 3 months [30,31]. However, this emergency response potentially brings in new donors who may add a risk of transfusion-transmissible infectious diseases given these donors may not be as well characterized as routine donors [32-34]. The implementation of an effective pathogen inactivation (PI) method can

alleviate some of the above risks by adding in a proactive layer of blood safety that could lessen the need for widespread deferrals, reduce infectious titres in asymptomatic donors and provide time for screening tests to be approved and delivered to the market.

Based upon early case reports of positive clinical outcomes when used in conjunction with supportive care and anti-viral therapy, there has been significant interest in using COVID-19 convalescent plasma as a therapeutic for infected patients [35-38]. Convalescent plasma is drawn from recovered patients, who might also be first-time donors or come from a high-risk population, thus again collecting products from these donors may come with additional risk. Including PI may be regarded as a prudent safety measure for convalescent plasma, particularly where it is to be used prophylactically for at-risk populations such as healthcare workers, first responders and those potentially exposed in localized outbreaks (nursing homes, ocean vessels, homeless shelters, incarceration facilities, etc.). PI would help guard against the residual risk of co-infections as well as a possible super-infection with the pandemic agent [39]. Importantly, the available evidence also suggests that PI does not have a deleterious effect on antibody function [40,41].

Overall, PI methods have the ability to provide a proactive layer of blood safety through their broad-based effectiveness against a wide range of known pathogens [42], as well as potential efficacy against unknown pathogens. During the first few months of a large outbreak or pandemic, they can provide a crucial first line of defence against transfusion transmission of an outbreaking agent along with reducing residual risk of co-infections when using convalescent plasma to treat both infected patients and at-risk populations. It should be noted though that PI methods also come with their limitations including that a donor's peak viremia may exceed a PI method's reduction capacity, that blood product types and specifications that can be treated with a given PI system may be limited, and that no technology has been shown to be universally successful against all classes of pathogens. However, in the midst of a pandemic or large-scale outbreak and a potential worsening blood supply situation, PI could play a vital role in maintaining a safe blood supply.

Conclusions

Riboflavin and UV light effectively reduced the titre of SARS-CoV-2 in both human platelet and plasma products to below the limit of detection using an *in vitro* plaque assay. These data complements previously collected data with MERS, which was also reduced to below the limit of detection, and show this technology is effective against multiple coronavirus species. Although the risk of transfusion transmission for SARS-CoV-2 is suspected to be low, implementing a PI technology like riboflavin and UV light may provide a crucial first line of defence against a future rapidly spreading agent that could theoretically be transmitted via blood transfusions.

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Conflict of interests

S.K., S.Y. and N.D. are employees of Terumo BCT, the manufacturer of the technology described in this article. I.R., L.H. and R.B. have no conflicts of interest to declare.

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Authorship

This manuscript template is based on the International Committee of Medical Journal Editors (ICMJE) recommendations dated December 2014. The ICMJE recommends that authorship be based on the following 4 criteria:

(1) Substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work;
(2) Drafting the work or revising it critically for important intellectual content;
(3) Final approval of the version to be published;
(4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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