Inactivation of Middle East respiratory syndrome coronavirus (MERS-CoV) in plasma products using a riboflavin-based and ultraviolet light-based photochemical treatment

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BACKGROUND: Middle East respiratory syndrome coronavirus (MERS-CoV) has been identified as a potential threat to the safety of blood products. The Mirasol Pathogen Reduction Technology System uses 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 MERS-CoV when tested in human plasma.

STUDY DESIGN AND METHODS: MERS-CoV (EMC strain) was used to inoculate plasma units that then underwent treatment with riboflavin and UV light. The infectious titers of MERS-CoV in the samples before and after treatment were determined by plaque assay on Vero cells. The treatments were initially performed in triplicate using pooled plasma (n = 3) and then repeated using individual plasma units (n = 6).

RESULTS: In both studies, riboflavin and UV light reduced the infectious titer of MERS-CoV below the limit of detection. The mean log reductions in the viral titers were $\geq$4.07 and $\geq$4.42 for the pooled and individual donor plasma, respectively.

CONCLUSION: Riboflavin and UV light effectively reduced the titer of MERS-CoV in human plasma products to below the limit of detection, suggesting that the treatment process may reduce the risk of transfusion transmission of MERS-CoV.

The Middle Eastern respiratory syndrome coronavirus (MERS-CoV) was first identified in 2012 and was most likely a zoonotic transmission event from camels.[1] MERS-CoV causes severe lower respiratory tract infections that can result in pneumonia and multiorgan failure, particularly in patients with underlying comorbidities and the elderly.[1,2] As of September 27, 2015, the Saudi Arabian Ministry of Health has reported 1250 cases of MERS-CoV infection and 535 fatalities.[3] The largest outbreak of MERS-CoV outside the Arabian Peninsula occurred in the Republic of Korea (May-July 2015); and, according to the World Health Organization, there were 186 confirmed cases and 36 deaths.[4] Transmission of MERS-CoV has been extensively documented in hospital settings in Saudi Arabia and Korea once the index case enters the health care system.[2] The greatest impact of MERS-CoV to date has been abroad, but two documented cases in travelers have been reported in the United States.[5] Thus, it is reasonable to expect that MERS-CoV will continue to emerge in the United States as travelers and health care workers enter the country or return home.

ABBREVIATIONS: ELP = extended-life platelet; MERS-CoV = Middle Eastern respiratory syndrome coronavirus; PRT = pathogen-reduction technology.

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Although nosocomial MERS-CoV infections have occurred, there is no evidence to date that MERS-CoV has been transmitted through transfusion of a blood product in a health care setting. This is similar to what was observed during the 2003 outbreak of severe acute respiratory syndrome (SARS), which is caused by another member of the coronavirus family, SARS-CoV. In that outbreak, viral RNA was detected in symptomatic patient serum[6]; however, to date, no blood transfusions have been implicated in viral transmission.[7] It is unclear whether the viral RNA present in serum indicates a possible asymptomatic viremic phase, which could lead to a possible transfusion transmission. Despite the lack of direct evidence of transfusion transmission risk with MERS-CoV and other coronaviruses, MERS-CoV has been identified as an agent that poses an existing or potential threat to blood safety by the AABB.[1] Currently, there are no MERS-CoV-specific screening questions or donor deferral periods recommended by the AABB.[8]

The Mirasol Pathogen Reduction Technology (PRT) System was developed to provide an added layer of protection to help prevent the transmission of existing and emerging pathogens, such as MERS-CoV, through the transfusion of human blood products. The Mirasol PRT System is a broad-spectrum pathogen-reduction process that has been shown to effectively reduce the infectivity of blood-borne bacteria, parasites, and viruses.[9-12] The system combines riboflavin, a photosensitizer, and ultraviolet (UV) light to irreversibly damage nucleic acids in blood products. The resulting DNA/RNA damage prevents pathogens and white blood cells from replicating, but it does not affect nonreplicating cells like platelets or red blood cells; and it maintains acceptable plasma protein quality post-treatment.[13-15] The purpose of this study was to evaluate the efficacy of the Mirasol PRT System on the reduction of MERS-CoV (EMC strain) in human plasma products using both pooled plasma and individual-donor plasma units.

MATERIALS AND METHODS

Plasma products

All plasma products were collected at an accredited blood bank under institutional review board approval and were shipped to Terumo BCT. The units consisted of recovered plasma, which were frozen to ≤ -20°C within 8 to 24 hours of collection. Two sets of plasma were used for these experiments. A preliminary study with a limited number of replicates was performed to determine whether riboflavin and UV light treatment inactivated MERS-CoV and was performed using pooled plasma units (n = 3). A verification study using more replicates followed in which individual donor plasma units (n = 6) were used.

Riboflavin and UV light process for plasma products

The riboflavin and UV light process has been previously described in detail.[9,12] Briefly, 200 mL of human plasma was dispensed into extended-life platelet (ELP) illumination/storage bags and then mixed with 35 mL riboflavin solution (500 μmol/L riboflavin in 0.9% sodium chloride, pH 4.0-5.0 [Terumo BCT]). After the riboflavin-plasma mixture was inoculated with virus, the samples were placed into the Mirasol Illuminator (Terumo BCT) for UV treatment. The plasma units were exposed to 6.24 J/mL of energy. Because the MERS-CoV virus is a BSL-3 level pathogen and the Mirasol Illuminator was used in a BSL-2 level laboratory, the filled plasma bags were briefly immersed in bleach before illumination to ensure that no live virus was adhering to the outside of the ELP bag; this did not affect the light transmission properties of the ELP bag.

Virus reduction studies

The virus reduction studies were performed at Colorado State University. For the preliminary study, 200 mL of pooled plasma product with riboflavin was inoculated with 13 mL MERS-CoV stock (2×10^7 plaque forming units [PFU]/mL). After mixing, a sample was obtained and held at ambient temperature as the pretreatment sample. After treatment with riboflavin and UV light, a second sample was obtained as the posttreatment sample. Each pretreatment and posttreatment sample was serially diluted from 10^{-1} to 10^{-6} in BA-1 medium (minimum essential medium supplemented with bovine serum albumin), and the viral titer was tested in duplicate using a plaque assay on Vero cells. The neat dilution was not plated, because previous work demonstrated that the plasma had a cytotoxic effect on Vero cells (data not shown).

For the verification study, each 200-mL single-donor plasma unit with riboflavin was inoculated with 15 mL MERS-CoV (3.6×10^7 PFU/mL). A 1-mL to 2-mL sample was removed from each unit before and after treatment to measure the virus titer. The pretreatment samples were serially diluted from 10^{-1} to 10^{-6} in BA-1 medium, and each dilution was tested once using a plaque assay on Vero cells. The posttreatment samples were diluted to 10^{-1} and tested in triplicate using a plaque assay on Vero cells to increase assay sensitivity. Again, the neat dilution was not plated because previous work demonstrated that the plasma had a cytotoxic effect on Vero cells (data not shown).

Briefly, for the plaque assay, confluent Vero cell monolayers were grown in six-well plates, and each well was inoculated with 0.1 mL of diluted plasma sample. The plates were rocked every 10 to 15 minutes for 45 minutes and then overlaid with 0.8% agarose in medium, and incubated for 2 days at 37°C, 5% CO_2. After 2 days, a second overlay containing 0.005% neutral red was added, and the

TABLE 1. Log Reduction in Middle East respiratory syndrome coronavirus titers after pathogen-reduction technology treatment: pooled plasma

Replicate no.†	Viral load, log PFU/mL*		Log reduction
	Pretreatment	Posttreatment	
1	6.24	≤2.18	≥4.06
2	6.23	≤2.18	≥4.05
3	6.27	≤2.18	≥4.09
Average	6.25	≤2.18	≥4.07

*Posttreatment titers were at the limit of detection for the assay.
†Replicates consist of pooled plasma units spiked with a known quantity of Middle East respiratory syndrome coronavirus.
PFU = plaque forming units.

TABLE 2. Log reduction in Middle East respiratory syndrome coronavirus titers after pathogen-reduction technology treatment: single-donor plasma

Unit no.	Viral load, log PFU/mL*		Log reduction
	Pretreatment	Posttreatment	
1	6.46	≤2.00	≥4.46
2	6.45	≤2.00	≥4.45
3	6.48	≤2.00	≥4.48
4	6.49	≤2.00	≥4.49
5	6.36	≤2.00	≥4.36
6	6.28	≤2.00	≥4.28
Average ± SD	6.42 ± 0.08	≤2.00 ± N/A	≥4.42 ± 0.08

*Posttreatment titers were at the limit of detection for the assay.
PFU = plaque forming units; SD= standard deviation; N/A = not applicable.

plaques were counted the following day. The virus titer was determined based on plaque number and dilution.

MERS-CoV culture protocol

The virus used in these experiments was a low-passage human isolate of MERS-CoV (strain HCoV-EMC/2012) propagated in Vero cells cultured in Dulbecco's modified Eagle medium and fetal bovine serum, as described previously.[16] The virus stock was maintained at −80°C until it was thawed for use.

Calculation of limit of detection

When the posttreatment samples were negative for the presence of virus, the limit of detection had been reached. All 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:

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

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

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 = 0.05); V is the total volume of the treated product (plasma + riboflavin + virus); and v is the volume used for viral enumeration (volume inoculated/well in mL) × (number of replicate wells) × (lowest dilution inoculated).

RESULTS

The inactivation of the MERS-CoV virus by riboflavin and UV light was evaluated. MERS-CoV represents an emerging pathogen that is a cause for concern regarding transfusion transmission. For all of the work performed, an in vitro plaque assay was used to measure the infectious viral load.

The efficacy of riboflavin and UV light against the MERS-CoV was first evaluated in triplicate using a pooled plasma sample (Table 1). The mean reduction in the log viral titer was ≥4.07. Notably, across all three replicates, the assay reduced the observed viral titer below the limit of detection (≤2.18 log PFU/mL).

Having demonstrated the efficacy of riboflavin and UV light in pooled plasma, the net reduction of MERS-CoV in individual-donor plasma units (n = 6) was assessed. The results are summarized in Table 2. The mean ± standard deviation reduction in viral titer was ≥4.42 ± 0.08 logs. Like in the pooled plasma, the virus titer was reduced to the limit of detection (≤2.0 log PFU/mL) in all six of the individual-donor plasma units.

DISCUSSION

Although there are no documented cases of transfusion transmission for MERS-CoV, it is one of the pathogens cited as a specific agent of concern by the AABB.[1] The use of a riboflavin and UV light-based treatment reduced the infectious titer of MERS-CoV to below the limit of detection in all of the plasma units tested. The mean >4.4 log reduction in the MERS-CoV titer exceeded the European Committee of Blood Transfusion recommendations stating that, to be considered effective, a pathogen-reduction process should reduce screened pathogens by at least 3 logs.[17] A study conducted by Corman and colleagues indicated that MERS-CoV-infected patients may have viral loads up to 5 or 6 log RNA copies/mL in their serum.[18] However, those authors also indicated that this virus did not appear to be infectious in cell culture,[18] suggesting that transfusion transmission is unlikely.

Although the observed reduction of MERS-CoV was only evaluated in plasma products, it can safely be assumed to extend to platelet products on the basis of previous research using the Mirasol PRT System.[9] It has been demonstrated that pathogen-reduction systems

cause minor changes to platelets that do not compromise the hemostatic efficacy of platelet transfusion; however, they also can decrease the risk of graft-versus-host disease and improve shelf life.[19] Taken together, the results from this study suggest that riboflavin and UV light might be able to reduce the likelihood of MERS-CoV transfusion transmission in both platelet and plasma products should infectious virus be present in blood from an asymptomatic donor.

Over 68 pathogens have been identified by the AABB as "of concern" for blood safety based on the likelihood of transmission transfusion and clinical disease in the transfusion recipient.[1] The most pressing concerns relate to diseases with known transfusion transmission events and severe or fatal outcomes (e.g., dengue virus, *Babesia* parasites, and Creutzfeldt-Jakob prions).[1] Targeted screening processes that identify blood products containing potentially transmissible pathogens have markedly improved the overall safety of transfusions. However, although it is rare, transfusion transmission of even well-known pathogens like human immunodeficiency virus (HIV) still occurs in the United States.[20] In resource-limited setting like Africa, as many as 10% to 15% of new HIV cases can be linked to transfusion-transmitted infections.[21] Targeted screening programs are limited in part by the incremental nature of updating the pathogen list and by our knowledge of each pathogen. A recent estimate is that, of the 1407 known human pathogens, 58% are zoonotic, and 13% are considered emerging or re-emerging pathogens.[22] Known pathogens are not the only concern for blood safety: one estimate is that 5.3 novel viruses are discovered each year and that from 60% to 70% of these viruses have animal origins.[1] Targeted screening on this scale is not economically or logistically feasible and cannot keep pace with the emerging threats to blood safety. Pathogen-reduction systems have the potential to be a complementary approach to blood screening programs and may have the potential to replace some screening assays, like cytomegalovirus and West Nile virus. Riboflavin and UV light already have established efficacy against a broad array of viral pathogens (14 different viruses), including influenza A, HIV, and West Nile virus.[9,12] The results from the current study support the finding that riboflavin and UV light effectively inactivate coronaviruses in addition to the broad spectrum of other enveloped and nonenveloped viruses already tested.

CONCLUSIONS

Riboflavin and UV light effectively reduced the titer of MERS-CoV in human plasma products to below the limit of detection using an in vitro cell culture model, expanding the list of pathogens that are effectively reduced by the technology. In addition, should infectious MERS-CoV be discovered in the blood of asymptomatic donors, then riboflavin and UV light would reduce the likelihood of transfusion transmission in both platelet and plasma products.

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CONFLICT OF INTEREST

SD and SM are employees of Terumo BCT, the manufacturer of the technology described in this article. RB has no conflicts of interest to declare.

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