VoxSanguinis
The International Journal of Transfusion Medicine

ISBT International Society of Blood Transfusion

Vox Sanguinis (2020)

ORIGINAL PAPER

DOI: 10.1111/vox.12888

Inactivation of three emerging viruses – severe acute respiratory syndrome coronavirus, Crimean–Congo haemorrhagic fever virus and Nipah virus – in platelet concentrates by ultraviolet C light and in plasma by methylene blue plus visible light

Markus Eickmann,[1,†] Ute Gravemann,[2,†] Wiebke Handke,[2] Frank Tolksdorf,[3] Stefan Reichenberg,[3] Thomas H. Müller[2] & Axel Seltsam[2]

[1]*Institute for Virology, Philipps University Marburg, Marburg, Germany*
[2]*German Red Cross Blood Service NSTOB, Springe, Germany*
[3]*Maco Pharma International GmbH, Langen, Germany*

Vox Sanguinis

Background Emerging viruses like severe acute respiratory syndrome coronavirus (SARS-CoV), Crimean–Congo haemorrhagic fever virus (CCHFV) and Nipah virus (NiV) have been identified to pose a potential threat to transfusion safety. In this study, the ability of the THERAFLEX UV-Platelets and THERAFLEX MB-Plasma pathogen inactivation systems to inactivate these viruses in platelet concentrates and plasma, respectively, was investigated.

Materials and methods Blood products were spiked with SARS-CoV, CCHFV or NiV, and then treated with increasing doses of UVC light (THERAFLEX UV-Platelets) or with methylene blue (MB) plus increasing doses of visible light (MB/light; THERAFLEX MB-Plasma). Samples were taken before and after treatment with each illumination dose and tested for residual infectivity.

Results Treatment with half to three-fourths of the full UVC dose (0·2 J/cm^2) reduced the infectivity of SARS-CoV (≥3·4 log), CCHFV (≥2·2 log) and NiV (≥4·3 log) to the limit of detection (LOD) in platelet concentrates, and treatment with MB and a fourth of the full light dose (120 J/cm^2) decreased that of SARS-CoV (≥3·1 log), CCHFV (≥3·2 log) and NiV (≥2·7 log) to the LOD in plasma.

Conclusion Our study demonstrates that both THERAFLEX UV-Platelets (UVC) and THERAFLEX MB-Plasma (MB/light) effectively reduce the infectivity of SARS-CoV, CCHFV and NiV in platelet concentrates and plasma, respectively.

Key words: ultraviolet light, methylene blue, pathogen inactivation, plasma, platelet concentrates.

Received: 27 September 2019, revised 18 December 2019, accepted 18 December 2019

Introduction

There is a large group of emerging viruses known to be occasionally transmitted by blood or to have properties suggesting their transmissibility by this route. These pathogens include severe acute respiratory syndrome coronavirus (SARS-CoV), Crimean–Congo haemorrhagic fever virus (CCHFV) and Nipah virus (NiV), which have been identified by the World Health Organization (WHO) as major infectious threats with the potential to cause a global pandemic [1–3].

There are different pathogen inactivation techniques that have been developed to reduce or eliminate the

Correspondence: Axel Seltsam, German Red Cross Blood Service NSTOB, Institute Springe, Eldagsener Strasse 38, 31832 Springe, Germany
E-mail: axel.seltsam@bsd-nstob.de
[†]Contributed equally to this work.

threat of infectivity from known and emerging transfusion-transmissible agents [4]. THERAFLEX UV-Platelets (Macopharma, Tourcoing, France) is a novel method for pathogen inactivation treatment of platelet concentrates (PCs) [5–7]. This purely physical system is based on short-wave UVC light, which penetrates the fluid of PCs and inactivates micro-organisms and leucocytes by damaging nucleic acids. THERAFLEX MB-Plasma (Macopharma) is a photodynamic pathogen inactivation procedure for treatment of plasma [8,9]. Plasma units derived from single blood donations are illuminated with visible light in the presence of the phenothiazine dye methylene blue (MB). When plasma is MB/light-treated, singlet oxygen is generated, which leads to the destruction of viral nucleic acids. The MB/light-based method is in routine use in Europe for about 17 years [10].

Both pathogen inactivation systems have been tested *in vitro* to be effective against many different types of viruses, including emerging viruses such as West Nile virus and yellow fever virus [6,8,9,11–19]. In this study, we investigated the capacity of THERAFLEX UV-Platelets and THERAFLEX MB-Plasma systems to inactivate the emerging viruses SARS-CoV, CCHFV and NiV in PCs and plasma, respectively.

Materials and methods

Selection of donors

Selection of volunteer donors was based on local standard practices. Only regular blood donors that fulfilled the requirements for blood donation and had given their informed consent approved by the local ethics committee were included in the study.

Blood component preparation

Plasma-reduced PCs in platelet additive solution SSP^+ (Macopharma) were prepared from pools of five buffy coats as previously described and were stored under agitation at 22 ± 2°C [14]. The target specifications of the PCs were a platelet concentration of approximately $1x10^9$/mL and a plasma content of approximately 35% in accordance with Macopharma's specifications for UVC treatment of PCs. Air was removed from all plasma units and PCs.

Pathogen inactivation methods

Pathogen inactivation of PCs was performed using the THERAFLEX UV-Platelets system (Macopharma) according to the manufacturer's instructions as described previously [14]. All PCs were irradiated with UVC light to a total dose of 0·2 joules per square centimetre (J/cm^2) with constant vigorous agitation to ensure uniform treatment [6].

Pathogen inactivation of plasma units was performed using the THERAFLEX MB-Plasma system (Macopharma) as described previously [14]. Plasma for pathogen inactivation was processed by filtration for leucodepletion, addition of MB and subsequent illumination with visible light to a total dose of 120 J/cm^2 according to the instructions of the manufacturer for this system [20]. The MB removal step, an integral processing step in routine use of the THERAFLEX MB-Plasma system, was omitted for exclusive analysis of the virus inactivation effects of illumination.

Spiking experiments

Virus titres were determined by assessing for virus-induced changes in morphology (cytopathic effects) of indicator cells and calculated according to the Spearman–Kärber method and expressed as the log of the 50% tissue culture infectious dose (log TCID50) [14,21,22]. Titration was performed at the initial sample dilutions at which no cytotoxicity of indicator cells was observed. The effectiveness of virus inactivation was calculated as the log reduction factor (RF) using the formula $RF = \log_{10}A_0 - \log_{10}A_n$, (R, reduction factor; A_0, spiked total virus load before treatment; and A_n, total virus load after treatment). The overall reduction factor was expressed as the sum of RFs for all steps. The limit of detection (LOD) of the assay was defined as the lowest $TCID_{50}$ achievable at non-cytotoxic sample concentrations.

SARS-CoV, strain Frankfurt 1 [23], was grown and assessed in Vero E6 cells (ATCC CCL-22), CCHFV, strain Afg09-2990 [24], was propagated and assessed in Huh7 cells (JCRB 0403), and NiV, strain Malaysia [25], was grown and assayed in Vero 76 cells (ATCC CRL-1587). For preparation of the virus stocks, viral supernatants were collected on days 2–4 of cell culture, when a cell confluence of approximately 80% was achieved, centrifuged, aliquoted and frozen at −80°C until further use in spiking experiments.

PCs (volume: 375 ml; $n = 2$ per virus) and plasma units (volume: 315 ml; $n = 2$ per virus) were spiked 1:10 with supernatant of each virus and treated with UVC and MB/light, respectively. After spiking, PCs and plasma units were still within the specifications of the respective pathogen inactivation method. The light doses were applied incrementally until the full light doses of each treatment were achieved. After each process step, samples were collected and serially diluted for virus titration. In order to test for intrinsic virus inactivation of the blood product, reference samples were collected from each bag before pathogen inactivation treatment, stored at room

temperature and tested at the end of the experiments to account for any intrinsic virus inactivation by the blood product.

Results

The results of the infectivity assays demonstrated that UVC irradiation and MB/light dose-dependently inactivated SARS-CoV, CCHFV and NiV in plasma-reduced PCs and plasma units, respectively. In PCs, at half of the full UVC dose (0·1 J/cm^2) SARS-CoV and CCHFV infectivity levels were below the LOD, while at three-fourth of the full UVC dose (0·15 J/cm^2) also NIV infectivity levels were below the LOD (Table 1). Thus, virus reduction factors ≥3·4 for SARS-CoV, ≥2·2 for CCHFV and ≥4·3 for NiV were achieved with the UVC-based pathogen inactivation system in PCs.

In plasma, already at one-fourth of the full light dose (30 J/cm^2) SARS-CoV, CCHFV and NiV were inactivated to levels below the LOD (Table 2). These results correspond to virus log reduction factors of ≥3·1, ≥3·2 and ≥2·7 that were achieved by MB/Light treatment for SARS-CoV, CCHFV and NiV, respectively, in plasma.

For SARS-CoV, we observed a loss of infectivity of about 1 log lower after spiking in some cases. This significant loss of infectivity was probably caused by non-specific innate immune factors that neutralize viruses in plasma [23,24]. However, virus titres did not further decrease in controls during the course of the experiments. In particular, there were no significant differences between load and reference samples, indicating that the observed virus inactivation was solely caused by the treatment with UVC and MB/light.

Discussion

A major argument for using pathogen inactivation technologies to treat blood components is that they support a proactive approach providing more generalized protection against new and emerging infectious agents which continuously challenge the safety of the blood supply. The conventional reactive approach to wait until the threat from an emerging transfusion-transmitted agent has been identified before responding by modifying donor screening programmes takes time and, ultimately, the response may not be quick enough to prevent the transfusion of contaminated blood products. Because there are hundreds of known emerging or re-emerging human pathogens [26], the manufacturers of pathogen inactivation methods are required to continuously test the inactivation capacity of their systems for new infectious agents.

In this study, the inactivation efficacy of UVC and MB/light was for the first time tested against CCHFV and NiV

Table 1 Inactivation of SARS-CoV, CCHFV and NiV in platelet concentrates by THERAFLEX UV-Platelets[a]

	SARS-CoV				CCHFV				NIV			
	Bag 1		Bag 2		Bag 1		Bag 2		Bag 1		Bag 2	
Light dose (cumulative)	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF	$\log_{10}$ $TCID_{50}$/ml	$\log_{10}$ RF
Virus stock	7·2 ± 0·1		6·9 ± 0·3		6·8 ± 0·2		6·3 ± 0·1		7·5 ± 0·2		7·4 ± 0·3	
Spiked bag	5·8 ± 0·3		6·0 ± 0·2		5·2 ± 0·3		4·6 ± 0·2		6·2 ± 0·3		6·5 ± 0·2	
0·05 J/cm^2	2·7 ± 0·3	3·1	3·0 ± 0·4	3·0	2·4 ± 0·1	2·8	2·4 ± 0·1	2·1	3·9 ± 0·3	2·3	4·1 ± 0·2	2·4
0·1 J/cm^2	≤2·4	≥3·4	≤2·4	≥3·6	≤2·4	≥2·9	≤2·4	≥2·2	≤1·9	≥4·3	2·0 ± 0·1	4·6
0·15 J/cm^2	≤2·4	≥3·4	≤2·4	≥3·6	≤2·4	≥2·9	≤2·4	≥2·2	≤1·9	≥4·3	≤1·9	≥4·6
0·2 J/cm^2	≤2·4	≥3·4	≤2·4	≥3·6	≤2·4	≥2·9	≤2·4	≥2·2	≤1·9	≥4·3	≤1·9	≥4·6
Ref. sample	5·9 ± 0·2	−0·1	5·9 ± 0·3	0·1	5·4 ± 0·2	−0·2	4·9 ± 0·2	−0·3	6·2 ± 0·3	−0·1	6·3 ± 0·2	0·2

[a]$TCID_{50}$, 50% tissue culture infectious dose; RF, reduction factor; Ref. sample, pretreatment reference sample.

Table 2 Inactivation of SARS-CoV, CCHFV and NiV in plasma by THERAFLEX MB-Plasma[a]

	SARS-CoV				CCHFV				NiV			
	Bag 1		Bag 2		Bag 1		Bag 2		Bag 1		Bag 2	
Light dose (cumulative)	log_{10} $TCID_{50}$/ml	log_{10} RF	log_{10} $TCID_{50}$/ml	log_{10} RF	log_{10} $TCID_{50}$/ml	log_{10} RF	log_{10} $TCID_{50}$/ml	log_{10} RF	log_{10} $TCID_{50}$/ml	log_{10} RF	log_{10} $TCID_{50}$/ml	log_{10} RF
Virus stock	7·4 ± 0·2		7·1 ± 0·2		6·5 ± 0·3		6·0 ± 0·2		7·5 ± 0·2		7·5 ± 0·2	
Spiked bag	5·4 ± 0·3		5·6 ± 0·3		5·2 ± 0·2		5·1 ± 0·2		6·1 ± 0.2		6·4 ± 0·2	
30 J/cm^2	≤2·4	≥3·1	≤2·4	≥3·2	≤1·9	≥3·2	≤1·9	≥3·2	≤3·4	≥2·7	≤3·4	≥3·0
60 J/cm^2	≤2·4	≥3·1	≤2·4	≥3·2	≤1·9	≥3·2	≤1·9	≥3·2	≤3·4	≥2·7	≤3·4	≥3·0
90 J/cm^2	≤2·4	≥3·1	≤2·4	≥3·2	≤1·9	≥3·2	≤1·9	≥3·2	≤3·4	≥2·7	≤3·4	≥3·0
120 J/cm^2	≤2·4	≥3·1	≤2·4	≥3·2	≤1·9	≥3·2	≤1·9	≥3·2	≤3·4	≥2·7	≤3·4	≥3·0
Ref. sample	5·4 ± 0·3	0·0	5·7 ± 0·3	−0·1	5·1 ± 0·2	0·1	5·2 ± 0·2	−0·1	6·2 ± 0·2	−0·1	6·3 ± 0·2	0·1

[a]$TCID_{50}$, 50% tissue culture infectious dose; RF, reduction factor; Ref. sample, pretreatment reference sample.

or other members of the *Nairoviridae* and *Paramyxoviridae* families. SARS-CoV was also included in the study to confirm the efficacy of the two pathogen inactivation systems for coronaviruses, as has previously been demonstrated for MERS-CoV [14]. The results of this study show that both pathogen inactivation systems effectively inactivated all three viruses spiked into the PC and plasma samples, even at light dose increments below the full doses recommended by the manufacturers. One limitation of this study is that the number of replicates was small due to safety constraints – laboratory studies with these zoonotic viruses must be performed in accordance with the highest biosafety requirements. In addition, large-volume plating which would have allowed increasing the sensitivity of the assay and consequently improving the log reduction value could not be performed.

SARS-CoV is an enveloped, positive-sense single-stranded RNA coronavirus. It emerged in 2002 in China and spread to 29 additional countries and is thought to be an animal virus that spread to humans from civets most likely infected by bats [27]. Similar to Middle East respiratory syndrome coronavirus (MERS-CoV), the main route of human-to-human transmission of SARS-CoV is nosocomial transmission. However, transmission between family members has also been observed, suggesting that SARS-CoV might continue to spread via transmission by infected persons returning from affected areas. Transmission by blood transfusion has not been described yet. Nevertheless, the high mortality of the disease and the not yet fully understood transmission mechanisms of SARS-CoV pose a potential threat to the safety of the blood supply [27]. Interestingly, the detection of low-level viremia in asymptomatic patients during an SARS-CoV outbreak suggests a theoretical risk of transmission via blood products. As a precautionary measure, the World Health Organization introduced a recommendation for the deferral of blood donations from donors potentially exposed to SARS-CoV, and the Australian Red Cross Blood Service amended its donor screening questionnaire to include questions to identify persons with SARS-CoV-related symptoms [28].

Crimean–Congo haemorrhagic fever virus is an enveloped, negative-sense single-stranded RNA virus of the *Nairoviridae* family. CCHFV often results in a mild, non-specific febrile illness but may occasionally cause severe haemorrhagic disease. This disease was first identified in the Crimean region of the former Soviet Union in 1944 and is a significant public health concern. CCHFV occurs across a wide geographic region, including Europe, Asia and Africa, and may expand into new regions [29]. The virus is usually transmitted to humans through contact with infected ticks and animal blood, but it is also transmissible from human to human via exposure to infected

blood and other body fluids. Although no cases of CCHFV transmission by blood transfusion have been reported to date, incidences of hospital-acquired CCHFV infection due to contaminated medical instruments have been documented [30]. These cases are strongly reminiscent of the transmission routes of many other transfusion-relevant viruses.

NiV is an enveloped, single-stranded negative-sense virus that belongs to the *Paramyxoviridae* family. It was reported for the first time in the Malaysian population in 1998 and reappeared on different occasions in Asia. The NiV disease spectrum ranges from asymptomatic infection to acute respiratory illness and fatal encephalitis [31]. NiV is a zoonotic virus transmitted to humans from animals such as bats or pigs, but it can also be transmitted through contaminated foods or directly person-to-person through close contact with virus-containing body fluids and excretions [32]. The available data, particularly the findings on viral load in different body fluids, are too limited to provide a full understanding of its transmission routes [33]. Transfusion has not been implicated as a potential transmission pathway to date. The incubation period of up to 14 days and the occurrence of latent infections with subsequent reactivation of NiV months and even years after exposure suggest that infected persons may be overlooked by donor screening programmes. However, the potential transfusion risk may be limited by the fact that asymptomatic and mild NiV infections are rare.

Future studies are needed to determine whether SARS-CoV, CCHFV and/or NiV can be transmitted through transfusion. If one or more of these viruses is transfusion transmissible, its threshold concentration to elicit disease must be examined to determine whether the capacity of these pathogen inactivation technologies to inactivate the respective virus in plasma and PCs is sufficient to prevent transfusion transmission. Interpreting pathogen load in relationship to infectivity and inactivation efficacy is generally a very complex task [34]. When attempting to do so, it is important to consider that because quantitative polymerase chain reaction (qPCR), the most commonly used approach, measures viral load by detecting a small fragment of the viral genome, the results may not reflect infectivity and that qPCR usually overestimates the titre of circulating infectious agents [34]. In contrast, infectivity assays, which were used in this and previous studies [5,12–14], determine the inactivation capacity of a pathogen inactivation method based on intact, functional viral units. Nevertheless, the log reduction factors observed in this study and the safety margins calculated from the inactivation levels achieved using only a fraction of the standard light dose suggest that the THERAFLEX UV-Platelets and THERAFLEX MB-Plasma pathogen inactivation technologies may effectively reduce the potential risk of SARS-CoV, CCHFV and NiV and related viruses for platelet or plasma transfusion.

Acknowledgements

We would like to thank Katharina Kowalski for excellent technical assistance and the staff of the blood collection and blood preparation departments for their support.

Conflict of interest

FT and SR are employees of Macopharma, manufacturer and distributor of the THERAFLEX pathogen inactivation (PI) system. UG, WH, THM and AS received project grants from the German Red Cross Blood Services and Macopharma for the development of the UVC-based PI technology for platelets. ME has no conflicts of interest to disclose.

Author contributions

M. Eickmann designed the study, interpreted the data and co-wrote the manuscript. U. Gravemann designed the study, performed the *in vitro* experiments and analysed the data. W. Handke performed the *in vitro* experiments and analysed the data. F. Tolksdorf interpreted the data and edited the manuscript. S. Reichenberg interpreted the data and edited the manuscript. T. H. Müller interpreted the data and edited the manuscript. A. Seltsam designed the study, interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the German Red Cross Blood Services (Deutsche Forschungsgemeinschaft der Blutspendedienste des Deutschen Roten Kreuzes) and Macopharma S.A.S.

References

1 Stramer SL, Hollinger FB, Katz LM, *et al.*: Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion* 2009; **49**:1S–29S

2 Stramer SL: Current perspectives in transfusion-transmitted infectious diseases: emerging and re-emerging infections. *ISBT Sci Ser* 2014; **9**: 30–6

3 Bahar B, Schulz WL, Gokhale A, *et al.*: Blood utilisation and transfusion reactions in adult patients transfused with conventional or pathogen-reduced platelets. *Br J Haematol* 2019. https://doi.

org/10.1111/bjh.16187 [Epub ahead of print].
4 Seltsam A, Muller TH: Update on the use of pathogen-reduced human plasma and platelet concentrates. *Br J Haematol* 2013; **162**:442–54
5 Mohr H, Steil L, Gravemann U, *et al.*: A novel approach to pathogen reduction in platelet concentrates using short-wave ultraviolet light. *Transfusion* 2009; **49**:2612–24
6 Seltsam A, Muller TH: UVC irradiation for pathogen reduction of platelet concentrates and plasma. *Transfus Med Hemother* 2011; **38**:43–54
7 Kim S, Handke W, Gravemann U, *et al.*: Mitochondrial DNA multiplex real-time polymerase chain reaction inhibition assay for quality control of pathogen inactivation by ultraviolet C light in platelet concentrates. *Transfusion* 2018; **58**:758–65
8 Mohr H, Lambrecht B, Selz A: Photodynamic virus inactivation of blood components. *Immunol Invest* 1995; **24**:73–85
9 Seghatchian J, Walker WH, Reichenberg S: Updates on pathogen inactivation of plasma using Theraflex methylene blue system. *Transfus Apher Sci* 2008; **38**:271–80
10 Politis C, Kavallierou L, Hantziara S, *et al.*: Haemovigilance data on the use of methylene blue virally inactivated fresh frozen plasma with the Theraflex MB-Plasma System in comparison to quarantine plasma: 11 years' experience. *Transfus Med* 2014; **24**:316–20
11 Williamson LM, Cardigan R, Prowse CV: Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? *Transfusion* 2003; **43**:1322–9
12 Faddy HM, Fryk JJ, Prow NA, *et al.*: Inactivation of dengue, chikungunya, and Ross River viruses in platelet concentrates after treatment with ultraviolet C light. *Transfusion* 2016; **56**:1548–55
13 Fryk JJ, Marks DC, Hobson-Peters J, *et al.*: Reduction of Zika virus infectivity in platelet concentrates after treatment with ultraviolet C light and in plasma after treatment with methylene blue and visible light. *Transfusion* 2017; **57**:2677–82
14 Eickmann M, Gravemann U, Handke W, *et al.*: Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. *Transfusion* 2018; **58**:2202–7
15 Arnason NA, Johannson F, Landro R, *et al.*: Pathogen inactivation with amotosalen plus UVA illumination minimally impacts microRNA expression in platelets during storage under standard blood banking conditions. *Transfusion* 2019; **59**:3727–3735
16 Castro E, Girones N, Guerrero N, *et al.*: The effectiveness of UVC pathogen inactivation system on reducing the Trypansosoma cruzi and Leishmania infantum burden in platelets. *Vox Sang* 2008; **95**:290
17 Castro E, Gonzalez LM, Rubio JM, *et al.*: The efficacy of the ultraviolet C pathogen inactivation system in the reduction of Babesia divergens in pooled buffy coat platelets. *Transfusion* 2014; **54**:2207–16
18 Gravemann U, Handke W, Lambrecht B, *et al.*: Ultraviolet C light efficiently inactivates nonenveloped hepatitis A virus and feline calicivirus in platelet concentrates. *Transfusion* 2018; **58**:2669–74
19 Gravemann U, Handke W, Muller TH, *et al.*: Bacterial inactivation of platelet concentrates with the THERAFLEX UV-Platelets pathogen inactivation system. *Transfusion* 2019; **59**:1324–32
20 Seghatchian J, Struff WG, Reichenberg S: Main properties of the THERAFLEX MB-plasma system for pathogen reduction. *Transf Med Hemother* 2011; **38**:55–64
21 Spearman C: The method of "right and wrong cases" ("constant stimuli") without Gauss's formulae. *Br J Psychol* 1908; **2**:277–82
22 Kaerber G: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol* 1931; **162**:480–3
23 Agafonov AP, Gus'kov AA, Ternovoi VA, *et al.*: Primary characterization of SARS coronavirus strain Frankfurt 1. *Dokl Biol Sci* 2004; **394**:58–60
24 Olschlager S, Gabriel M, Schmidt-Chanasit J, *et al.*: Complete sequence and phylogenetic characterisation of Crimean-Congo hemorrhagic fever virus from Afghanistan. *J Clin Virol* 2011; **50**:90–2
25 Wild TF: Henipaviruses: a new family of emerging Paramyxoviruses. *Pathol Biol (Paris)* 2009; **57**:188–96
26 Woolhouse ME, Haydon DT, Antia R: Emerging pathogens: the epidemiology and evolution of species jumps. *Trends Ecol Evol* 2005; **20**:238–44
27 de Wit E, van Doremalen N, Falzarano D, *et al.*: SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016; **14**:523–34
28 Dunstan RA, Seed CR, Keller AJ: Emerging viral threats to the Australian blood supply. *Aust N Z J Public Health* 2008; **32**:354–60
29 Bente DA, Forrester NL, Watts DM, *et al.*: Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. *Antiviral Res* 2013; **100**:159–89
30 Conger NG, Paolino KM, Osborn EC, *et al.*: Health care response to CCHF in US soldier and nosocomial transmission to health care providers, Germany, 2009. *Emerg Infect Dis* 2015; **21**:23–31
31 Sharma V, Kaushik S, Kumar R, *et al.*: Emerging trends of Nipah virus: A review. *Rev Med Virol* 2019; **29**:e2010
32 Ang BSP, Lim TCC, Wang L: Nipah virus infection. *J Clin Microbiol* 201856(6):e01875-17
33 Nikolay B, Salje H, Hossain MJ, *et al.*: Transmission of nipah virus - 14 years of investigations in Bangladesh. *N Engl J Med* 2019; **380**:1804–14
34 McCullough J, Alter HJ, Ness PM: Interpretation of pathogen load in relationship to infectivity and pathogen reduction efficacy. *Transfusion* 2019; **59**:1132–46