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Amustaline (S-303) treatment inactivates high levels of Chikungunya virus in red-blood-cell components

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Vox Sanguinis

Background and objectives Chikungunya virus (CHIKV) infections have been reported in all continents, and the potential risk for CHIKV transfusion-transmitted infections (TTIs) was demonstrated by the detection of CHIKV RNA-positive donations in several countries. TTIs can be reduced by pathogen inactivation (PI) of blood products. In this study, we evaluated the efficacy of amustaline and glutathione (S-303/GSH) to inactivate CHIKV in red-blood-cell concentrates (RBCs).

Material and Methods Red-blood-cells were spiked with high level of CHIKV. Infectious titres and RNA loads were measured before and after PI treatment. Residual CHIKV infectivity was also assessed after five successive cell culture passages.

Results The mean CHIKV titres in RBCs before inactivation was $5.81 \pm 0.18 \log_{10}$ 50% tissue culture infectious dose (TCID₅₀)/mL, and the mean viral RNA load was $10.49 \pm 0.15 \log_{10}$ genome equivalent (GEq)/mL. No CHIKV TCID was detected after S-303 treatment nor was replicative CHIKV particles and viral RNA present after five cell culture passages of samples obtained immediately after S-303 treatment.

Conclusion Chikungunya virus was previously shown to be inactivated by the PI technology using amotosalen and ultraviolet A light for the treatment of plasma and platelets. This new study demonstrates that S-303/GSH can inactivate high titres of CHIKV in RBCs.

Key words: NAT testing, pathogen inactivation, red cell components, Transfusion-transmissible infections.

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Introduction

Chikungunya virus (CHIKV) is a single-stranded RNA arbovirus belonging to the *Alphavirus* genus within the *Togaviridae* family [1]. CHIKV is transmitted by the bite of infected *Aedes* mosquitoes; however, non-vector-borne transmission has also been reported including materno-fetal [2] and accidental infection of healthcare workers through cutaneous puncture [3, 4].

Chikungunya virus was first described in Africa in 1952, but its circulation in humans may have preceded its isolation due to its misidentification as dengue virus (DENV) [5]. CHIKV attracted international attention when it emerged on islands in the Indian Ocean in 2005 [6]. This emergence was associated with mutations of the virus responsible for increased adaptation to the mosquito vector *Aedes albopictus* [7], which is a competent vector responsible for outbreaks and sporadic infections in temperate areas such as Europe where *Aedes aegypti* does not exist [8, 9]. The first CHIKV outbreak in Europe was reported in Italy in 2007 [8], and the virus was reintroduced on a regular basis in the southern parts of Europe

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over the past decade. Recent cases of autochthonous transmission in France and Italy confirmed that favourable conditions can lead to the colonization of new geographic areas [9]. CHIKV was first reported to be responsible for outbreaks on islands and in countries of the Pacific in 2011 [10], in the Americas in 2013 [11], and autochthonous cases were reported within the continental United States (Florida) in 2014 [12]. CHIKV is now present on all continents [11, 13].

Acute phase CHIKV infection mainly results in high fever, bilateral arthralgia and myalgia, back pain, headache, fatigue and occasional rash [11, 13]. Acute complications involve the central nervous system. Late phase complications consist of recurrent and persisting arthralgia and arthritis [11, 13–15].

Transfusion-transmitted infections (TTIs) have not only been reported for arboviruses belonging to the *Flavivirus* genus including DENV [16, 17], Zika (ZIKV) [18], West Nile (WNV) [19], Yellow fever vaccine virus [20], Saint Louis encephalitis virus [21] and tick-borne encephalitis viruses (TBEV) [22] but also for Ross River virus (RRV) which, like CHIKV, belongs to the *Alphavirus* genus [23].

While a confirmed TTI has not yet been reported for CHIKV, the potential risk for CHIKV TTI is supported by several factors: the high attack rate of infections during outbreaks (up to 48% in Guadeloupe) [24], the presence of asymptomatic infections [25, 26], the high CHIKV RNA loads found in pre-symptomatic and asymptomatic blood donors (up to 10^9 copies/mL) [27] and primate TTI with viral persistence in a macrophage reservoir [28].

One of the mitigation strategies to prevent arbovirus TTIs is pathogen inactivation (PI) of blood components. PI technologies inactivate a broad range of pathogens including bacteria, parasites and viruses [29]. Licensed PI systems are available in many areas including Europe (INTERCEPT™, Theraflex Mouvaux, France and Mirasol™

Lakewood, CO, USA) and the United States (INTERCEPT™ and Octaplas™) for treatment of plasma and platelets, but no licensed system is yet available for the treatment of red blood cells (RBCs) [30]. Inactivation of CHIKV has been demonstrated in plasma [31–33] and platelets [31, 32, 34, 35] using different systems. Recently, we demonstrated that the INTERCEPT™ Blood System using amustaline (S-303) and glutathione (GSH) was able to inactivate high levels of DENV and ZIKV in RBCs [30, 36].

The INTERCEPT Blood System for RBCs utilizes S-303 to form covalent adducts with nucleic acids resulting in the inactivation of pathogens. S-303 then decomposes by hydrolysis to the non-reactive compound S-300 (Fig. 1). The natural tripeptide GSH is added to S-303 to quench undesirable side reactions [30].

This study combines infectivity detection assays with genome amplification assays to estimate the genome equivalents associated with the infectious viral titres being reduced by PI treatment. The comparison of genome amplification assays with infectivity detection assays is meant to bridge measurements of functional cell infectivity, with the typical measurements of viral markers in blood donors, performed mainly by nucleic acid amplification methodologies. Treatments with nucleic acid-targeting approaches, like S-303/GSH, have been shown to partially inhibit the amplification of nucleic acids [37, 38]. The ability to inhibit amplification correlates with the size of the amplicons tested and the frequency of the nucleic acid modification. Some of the peculiarities of the two approaches are that detection of a small number of amplicons cannot guarantee infectivity, and the inactivation of a virus to the limit of detection of the infectivity assays does not result in complete abrogation of genome amplification. Employing both measurements in the same samples allows nonetheless comparisons of

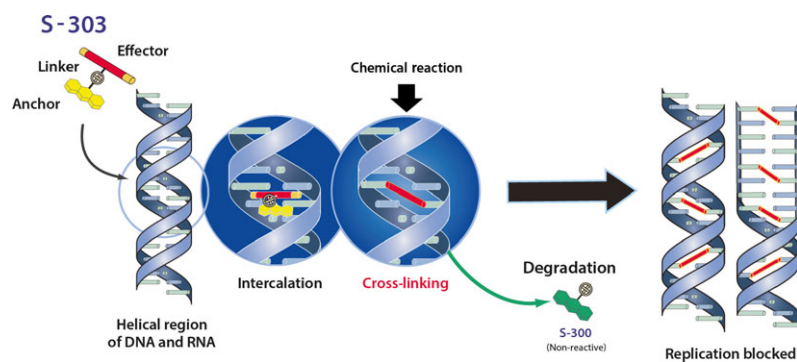


Fig. 1 Mechanisms of action for pathogen inactivation by S-303/GSH. Amustaline is a modular compound with three components: an acridine anchor, an effector and a linker. The anchor selectively targets nucleic acids where it intercalates and reversibly binds to the helical regions of the molecule. The effector then irreversibly reacts with guanine bases creating adducts and cross-links, thereby preventing nucleic acid replication or transcription. The linker is hydrolysed to release S-300, a non-reactive degradant resulting from the reaction. [Colour figure can be viewed at wileyonlinelibrary.com]

the viral levels tested with the maximum loads reported in blood donors using molecular testing.

A systematic analysis of the S-303/GSH system effect on the amplification of different size amplicons has not been performed yet; however, it is expected that the system may inhibit signal amplification by polymerase chain reaction approaches, presumably through the formation of adducts and cross-links.

The study utilizes the same protocol as previously published to demonstrate the efficacy of inactivation of DENV [36] and ZIKV in RBCs [30]. We report the inactivation of high levels of CHIKV in RBCs using S-303/GSH.

Material and methods

We used a protocol previously published [30, 36, 39, 40] with the same experimental design (Fig. 2) as previously used to demonstrate the inactivation of DENV and ZIKV in RBCs using S-303/GSH [30, 36].

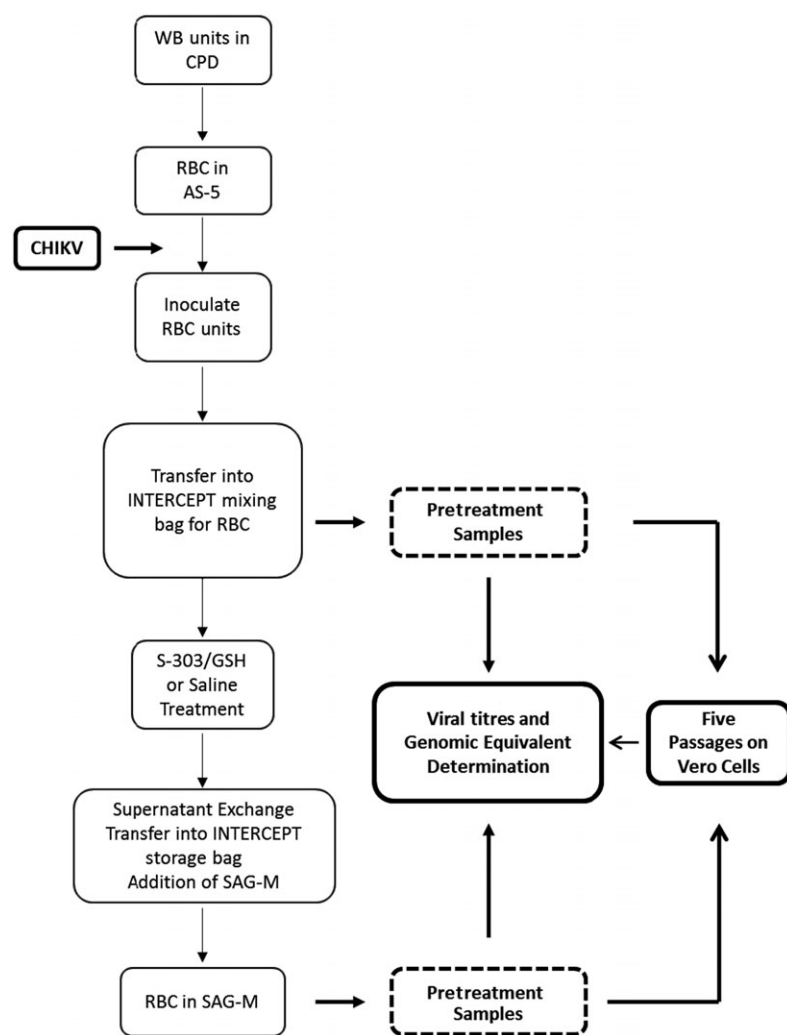


Fig. 2 Schematic flow diagram of the experimental design. Saline, or a GSH solution in saline, was transferred into the INTERCEPT Blood System for red-blood-cell mixing bag. Leucoreduced RBCs manufactured with AS-5 were inoculated with CHIKV and immediately transferred into the mixing bag. Samples were collected from the mixing bag (pretreatment samples). Saline or an S-303 solution in saline was transferred into the mixing bag, and the content from the mixing bag was then transferred into the incubation bag and stored for 20 h at room temperature. After incubation, treated RBCs were centrifuged at 4100 *g* for 6 min at 21°C. The supernatant was exchanged for SAG-M additive solution, and the resulting RBCs were transferred to the final storage container. Post-treatment samples were then collected from the storage bag. Pre- and post-treatment samples (including post-inactivation test samples and non-inactivated control samples) were then characterized for viral titres and genomic equivalent determination and passaged five times on Vero cells.

Blood products

Blood products purchased from Bonfils Blood Center (Denver, CO, USA), a non-endemic area for CHIKV, were screened using the CHIKjj *Detect*TM IgG and IgM ELISA kits (InBios International, Inc., Seattle WA, USA) to ensure the lack of antibodies against CHIKV. Whole blood (CPD) components were processed by standard procedures to produce AS-5 leucoreduced RBCs at Cerus Corporation (Concord, CA, USA) and shipped to *Institut Louis Malardé* (ILM; Tahiti, French Polynesia).

Virus

The CHIKV strain PF14-270514-51 (GenBank accession no. KJ939333) previously isolated from a French Polynesian patient was propagated in Vero cells [30, 36, 39, 40]. Four CHIKV concentrates were produced using Centricon Plus-70 centrifugal filter devices (Millipore, Billerica, MA)

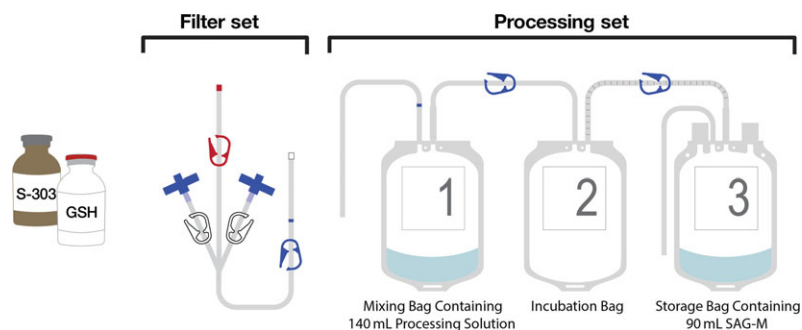


Fig. 3 INTERCEPT Blood System for red-blood-cells disposable kit and processing steps. Each kit includes a filter set with tubing and two 0.2 μm filters with capped Luer lock fittings and a processing set with the following sequentially integrated containers: one mixing bag, containing 140 mL of processing solution, one incubation bag, and one final storage bag containing 90 mL of saline adenine glucose-mannitol (SAG-M) solution. Processing includes the following steps: after sterile connection of the processing set and filter set is performed, the input RBC component is sterile connected to the filter set, glutathione is reconstituted and added to the mixing bag through one of the 0.2 μm filter using a syringe, input RBCs are transferred to mixing bag, amustaline is dissolved and added to mixing bag through the remaining 0.2 μm filter using a syringe, RBCs are transferred to the incubation bag and held for incubation at 18–25°C for 18–24 h. After the incubation is complete, the incubation bag is centrifuged at 4100 g for 6 min at 21°C, and the supernatant is expressed into the mixing bag, which is separated and discarded. Additive solution is transferred into the incubation bag and the resulting RBCs are transferred to the storage bag as the final product. [Colour figure can be viewed at wileyonlinelibrary.com]

as previously described [30, 36, 39, 40] and stored at –80°C with foetal bovine serum (FBS, Life technologies, Carlsbad, CA, USA) added at a 1:5 dilution.

Spiking of RBC concentrates and inactivation process

Four full-size RBC units were spiked each with 1.5 mL of concentrated CHIKV stock using a 5 mL syringe without plunger to act as a funnel for introduction of the virus through the luer lock adapter connected to a blind lead from each RBC unit (Fig. 2). A 600 mM GSH sodium salt solution and a 6 mM S-303 solution were prepared using a 20 mL syringe connected to a luer lock spike with 0.2 μm hydrophobic air filter (Quickpin; Grifols, Los Angeles, CA, USA) to reconstitute 1 vial of 3600 mg GSH sodium salt and 1 vial of 57 mg of S-303 per unit with 0.9% sodium chloride solution. Test units 1, 2 and 3 were treated with S-303/GSH (Fig. 3), while Unit 4 was ‘mock-treated’ with an equal volume of saline in lieu of S-303/GSH.

As previously described, two control samples were removed from each untreated test unit (UT) after the addition of GSH at a final 20 mM concentration, but prior to addition of S-303 [30]. For the mock-treated unit, two analogous control samples were removed after the addition of saline. The first untreated control samples from both test and mock units were frozen immediately after collection (UT0). The second untreated control samples from both test and mock units were incubated at room temperature for 20 h (UT20) alongside the treated test units. For treatment of test units, S-303 was added at a

final 200 μM concentration and the units incubated at room temperature for 20 h. The mock-treatment unit was similarly incubated at room temperature for 20 h. After centrifugation, an exchange step was performed on the treated test units and mock-treated unit; the supernatant was exchanged for SAG-M additive solution (90 mL), and the resulting RBCs were transferred to the storage bag as the final product. Post-inactivation test samples (TT20 h) were collected from treated units 1, 2 and 3, and also the mock-treated Unit 4 and were immediately frozen.

Detection of replicative CHIKV and CHIKV infectivity titration

Detection of replicative CHIKV and viral titrations were performed as previously described [30]. For the detection of replicative CHIKV, 200 μL of all pre-inactivation, post-inactivation, non-inactivated and mock-treated RBC samples diluted 1:40 in culture medium was inoculated in triplicate on Vero cells plated at approximately 3×10^5 cells/mL in 24-well plates. To avoid cross-contamination, samples were tested in separate plates. After 30 min of incubation, inocula were removed and cells were rinsed twice with culture medium to remove any residual viral particles and nucleic acids. Inoculated cells were incubated at 37°C with 5% CO_2 during 5 days. Five consecutive passages of 4–5 days each were performed. For CHIKV titration, triplicate 10-fold dilutions of each RBC sample were inoculated on Vero cells in 96-well plates. For both assays, the presence of infectious CHIKV was detected by indirect immunofluorescence assay (IFA) using anti-alphavirus mouse antibodies 3582 (Santa Cruz

Biotechnology, Dallas, TX, USA) diluted 1:100 in phosphate-buffered saline. Viral titres were expressed as 50% tissue culture infectious dose (TCID₅₀/mL) using the method of Reed and Muench [41].

CHIKV RNA quantification

RNA extractions were performed from 200 µL of all RBC samples and cell supernatants using the QIAcube extraction system (Qiagen, Hilden Germany), and real-time reverse transcription polymerase chain reaction (RT-PCR) was performed in a Bio-Rad CFX96 thermocycler using previously described primers F-CHIK 5'-AAGC-TYCGCGTCCTTTACCAAG-3' and R-CHIK 5'-CCAATTGTCCYGGTCTTCCT-3', and probe P-CHIK 5'-CCAATGTCYTCMGCCTGGACACCTTT-3' [42, 43]. The amplified region was 208 bp long. To estimate the copy number of CHIKV RNA in the samples, a CHIKV RNA synthetic transcript of known concentration was serially diluted and included within the RT-PCR run to generate a standard curve as previously described [30]. Results were expressed in genomic equivalents (GEq/mL).

Results

Detection of residual replicative CHIKV and CHIKV infectivity titration

For test units 1–3, the average CHIKV infectious titre was $5.81 \pm 0.18 \log_{10}$ TCID₅₀/mL in pre-inactivation control samples (UT0) and $5.99 \pm 0.12 \log_{10}$ TCID₅₀/mL in non-inactivated control samples (UT20 h) (Table 1). Immediately after inactivation, no replicative CHIKV was detected in any of the post-inactivation test samples (TT20 h) for units 1–3. Serial passage cultures of pre-inactivation (UT0) and non-inactivated (UT20 h) control samples for units 1–3 as well as each of Mock Unit 4 pre-treatment (UT0), non-treated (UT20 h) and mock-treated (TT20 h) samples contained replicative viruses during successive passages, as demonstrated with IFA (Table 1). For Unit 4, the reduction observed for the mock-treated sample (reduction from 5.76 to 4.50 \log_{10} TCID₅₀/mL) is consistent with the dilution of the virus during the supernatant exchange for saline adenine glucose-mannitol (SAG-M) solution.

Table 1 Detection of replicative CHIKV and CHIKV infectious titres (\log_{10} TCID₅₀/mL) in RBCs before and after PI treatment

Samples			Initial viral titres (\log_{10} TCID ₅₀ /mL)	First passage	Second passage	Third passage	Fourth passage	Fifth passage	Log reduction
Unit 1									
Pre-inactivation	(UT0)	Control	5.68	+ ^b	+	+	+	+	>5.68
Non-inactivated	(UT20 h)	Control	5.85	+	+	+	+	+	
Post-inactivation	(TT20 h)	Test	–	– ^c	–	–	–	–	
Unit 2									
Pre-inactivation	(UT0)	Control	5.74	+	+	+	+	+	>5.74
Non-inactivated	(UT20 h)	Control	6.02	+	+	+	+	+	
Post-inactivation	(TT20 h)	Test	–	–	–	–	–	–	
Unit 3									
Pre-inactivation	(UT0)	Control	6.02	+	+	+	+	+	>6.02
Non-inactivated	(UT20 h)	Control	6.09	+	+	+	+	+	
Post-inactivation	(TT20 h)	Test	–	–	–	–	–	–	
Units 1–3 Average ± SD									
Pre-inactivation	(UT0)	Control	5.81 ± 0.18	NA	NA	NA	NA	NA	>5.81
Non-inactivated	(UT20 h)	Control	5.99 ± 0.12	NA	NA	NA	NA	NA	
Post-inactivation	(TT20 h)	Test	NA	NA	NA	NA	NA	NA	
Unit 4 (mock-treated) ^a									
Pretreatment	(UT0)	Control	5.76	+	+	+	+	+	
Non-treated	(UT20 h)	Control	5.77	+	+	+	+	+	
Mock treatment	(TT20 h)	Test	4.50	+	+	+	+	+	

NA, Non-applicable.

^aSaline used in lieu of S-303 and GSH during treatment.

^bPositive immunofluorescence.

^cNegative immunofluorescence.

Table 2 CHIKV RNA loads (\log_{10} GEq/mL) in RBCs before and after PI treatment

Samples			Initial viral titres (\log_{10} GEq/mL)	First passage	Second passage	Third passage	Fourth passage	Fifth passage	Log reduction
Unit 1									
Pre-inactivation	(UT0)	Control	10-33	10-51	10-27	10-57	9-8	10-44	>10-33
Non-inactivated	(UT20 h)	Control	9-07	10-46	9-89	9-81	10-21	10-22	
Post-inactivation	(TT20 h)	Test	7-25	ND ^b	ND	ND	ND	ND	
Unit 2									
Pre-inactivation	(UT0)	Control	10-54	10-68	10-55	10-63	10-05	10-35	>10-54
Non-inactivated	(UT20 h)	Control	10-55	10-34	10-46	10-00	10-31	10-46	
Post-inactivation	(TT20 h)	Test	7-61	ND	ND	ND	ND	ND	
Unit 3									
Pre-inactivation	(UT0)	Control	10-61	10-74	10-46	10-92	10-14	10-77	>10-61
Non-inactivated	(UT20 h)	Control	10-45	10-69	10-76	10-32	10-73	10-52	
Post-inactivation	(TT20 h)	Test	7-37	ND	ND	ND	ND	ND	
Units 1–3 Average \pm SD									
Pre-inactivation	(UT0)	Control	10-49 \pm 0-15	10-64 \pm 0-12	10-43 \pm 0-14	10-71 \pm 0-19	10-00 \pm 0-18	10-52 \pm 0-22	>10-49
Non-inactivated	(UT20 h)	Control	10-02 \pm 0-83	10-50 \pm 0-18	10-37 \pm 0-44	10-04 \pm 0-26	10-42 \pm 0-28	10-40 \pm 0-16	
Post-inactivation	(TT20 h)	Test	7-41 \pm 0-18	ND	ND	ND	ND	ND	
Unit 4 (mock-treated) ^a									
Pretreatment	(UT0)	Control	10-62	10-61	10-64	10-75	10-28	10-67	
Non-treated	(UT20 h)	Control	10-36	10-59	10-52	10-23	10-55	10-53	
Mock treatment ^a	(TT20 h)	Test	9-19	10-52	10-43	10-77	10-41	10-41	

^aSaline used in lieu of S-303 and GSH during treatment.

^bCHIKV RNA not detected.

CHIKV RNA quantification

For test units 1–3, viral RNA loads averaged 10.49 ± 0.15 \log_{10} GEq/mL in pre-inactivation control samples (UT0) and 10.02 ± 0.83 \log_{10} GEq/mL in non-inactivated control samples (UT20 h) (Table 2). Immediately after treatment, CHIKV RNA loads averaged 7.41 ± 0.18 \log_{10} GEq/mL in the post-inactivation test samples (TT20 h) for test units 1–3. The loss of 2–3 log of quantitative RT-PCR signal may be partially attributed to the efficient modification of the amplified region by the S-303/GSH system. In addition, the exchange step performed at the end of the treatment may also contribute, by about 1 log, to the reduction in some of the RNA loads, as shown for the mock-treated sample. The detection of CHIKV RNA immediately following treatment is expected due to the presence of nucleic acid sequences from inactivated input virus that is unable to replicate, but that could still be amplified. Treatment with nucleic acid-targeting approaches, even though effective in eliminating or reducing infectivity, has been shown to only partially inhibit the amplification of amplicons commensurate to the ability to inactivate [37, 38].

This is supported by the lack of detection of infectious CHIKV after the first inoculation of treated test samples (units 1–3; TT20 h) onto Vero cells. Additionally, all subsequent passages resulted in no detectable CHIKV RNA,

suggesting that the RNA measured after the first inoculation was from residual inactivated CHIKV input that was not able to replicate. In contrast, both pre-inactivation (UT0) and non-inactivated (UT20 h) control samples from test units 1–3 demonstrated consistent viral genomic titres across serial passages with mean values of 10.46 ± 0.30 and 10.35 ± 0.29 \log_{10} GEq/mL, respectively. Similarly, all samples in the mock-treated Unit 4 demonstrated high titres of CHIKV RNA, ranging from 10.48 ± 0.14 to 10.59 ± 0.15 \log_{10} GEq/mL on average for UT0, UT20 h and TT20 h samples at all passages.

Discussion

The strategies to mitigate TTIs include pre-donation screening of blood donors, post-donation symptom reporting, importation of blood products from non-endemic areas, nucleic acid testing (NAT) of blood donations and PI treatment.

The rate of asymptomatic CHIKV infections is generally lower (~3–25%) [25, 26] than for other arboviruses causing TTIs (DENV, ZIKV and WNV) [44], even though in some locations, asymptomatic infections up to 80% have been reported [45]. While only symptomatic blood donors can be deferred based on clinical presentation, pre-symptomatic donors, in addition to infected blood donors who remain asymptomatic, may be allowed to donate blood

while viremic, posing a risk to the safety of the blood supply [27].

Suspension of blood collection in epidemic regions and importation of blood components from non-endemic areas is not always feasible for all components, though this was the approach adopted during the 2005–2006 La Reunion Island CHIKV outbreak where an estimated 244 000 cases (35% of the population) were reported [6]. Blood collection was suspended on January 2006, and RBCs and plasma were imported from metropolitan France. The INTERCEPT™ Blood System was implemented for preparation of apheresis platelets [46] and approved by the *Agence Française de Sécurité Sanitaire des Produits de Santé* (AFSSAPS, French Agency of Medical Safety of Health Products) in 2005. Retrospective NAT testing implemented from January to May 2006 demonstrated that 0.4% of apheresis donors tested positive for CHIKV RNA [47] and estimated that 0.7% of apheresis donations may have been contaminated during the outbreak [47]. The risk of blood product contamination was estimated at 132/100 000 donations (1500/100 000 at the peak of the outbreak) [48]. Similar findings were reported during the 2007 CHIKV outbreak in Italy [8]. Blood collection was suspended, PI of platelets was implemented, and the estimated risk for viremic donations was 1.05/100 000 at the peak of the outbreak [49].

Research and laboratory developed NAT assays demonstrated a high frequency of CHIKV RNA-positive blood donors: 0.54% in Puerto Rico in 2014–2015 [50], 1.9% from September to November in Puerto Rico in 2014 (2.1% at the peak of the outbreak) [27] and 0.4% in the French Caribbean (1–2% during the peak of the outbreak) in 2014–2015 [51]. Mathematical models have been developed to estimate the risk of emerging infectious disease transmission through transfusion [52]; in Thailand in 2009, the mean risk and the maximum estimated risk for viremic blood donations in the absence of mitigation strategy were 0.9 and 4.8%, respectively [53].

These data led AABB to classify CHIKV as an ‘agent with sufficient scientific/epidemiologic evidence of risk regarding blood safety that might support elevation to a higher priority in the future’ [54, 55].

In tropical and subtropical areas, arboviruses co-circulate [44, 56–58]. Co-infections with DENV, CHIKV and ZIKV have been reported [59, 60]. Therefore, a need exists for strategies that can help prevent the transmission of all these pathogens. PI technologies offer the potential to inactivate DENV, ZIKV and CHIKV along with other pathogens in a single proactive procedure regardless of the circulating agent responsible for a given outbreak. In addition, in the early stage of an outbreak, the aetiological agent may not be identified for several weeks. This was exemplified by the lapse

between the recognition of clinical infections and the identification of the responsible agent during the ZIKV outbreak in Brazil [61]. In such a context, the implementation of mitigation strategies based on detection by NAT is inherently retroactive and delayed, because of potential needs for agent identification, test development and regulatory approval, while a proactive strategy such as PI treatment can be effective [62]. Multiplex assays that detect DENV, CHIKV and ZIKV are available for clinical diagnosis [63, 64] but not for blood donor screening. Single-plex ZIKV NAT assays are currently used in the USA under an investigational new drug application [65], and DENV NAT assays are available in Europe, but there is no licensed CHIKV NAT assay for blood donor screening.

Chikungunya virus can be effectively inactivated using amotosalen/UVA with ≥ 6.4 log reduction attained in platelet components and ≥ 7.6 log reduction attained in plasma [31]. Other technologies have demonstrated various levels of reduction including: riboflavin with 1.4–3.1 log reduction in platelets [32] and 2–2.2 log reduction in plasma [32], as well as methylene blue with ≥ 5.38 in plasma [33] and ultraviolet C with ≥ 6.34 log reduction in platelets [35].

In this new study, we demonstrate that the mean inactivation of CHIKV in RBCs was $>5.81 \pm 0.18 \log_{10}$ 50% TCID₅₀ with a mean viral RNA load of $10.49 \pm 0.15 \log_{10}$ GEq/mL, resulting in inactivation of the virus to the limit of detection immediately after completion of the PI process. In addition, the level of inactivation was higher than the highest CHIKV RNA loads detected in asymptomatic blood donors (10^9 copies/mL) [27].

Even though the addition of the viral inoculate in whole blood, followed by manufacturing of RBC, would more closely simulate the natural introduction of the infection in the blood system, we chose to spike the virus in the RBC, because any additional manipulations during manufacturing would result in reduction in the viral titre unrelated to the treatment itself. For instance, assuming a complete distribution of the virus in the whole blood with typical haematocrit (35–45%) and volume (500 mL), approximately 3/5 of the virus would be removed with the expression of the plasma portion, decreasing the titre. We aimed in providing as high a challenge to the inactivation system as possible. In these experiments, the final titre is limited by the ability to generate stock concentrates, currently achievable at 7–8 log/mL. The robust efficacy of the system at inactivating high viral titres in such challenging conditions brings an added level of confidence that the system designed to be used for the treatment of RBC concentrates is performing optimally for its intended use.

The level of CHIKV inactivation ($>10.49 \pm 0.15 \log_{10}$ GEq/mL) in RBCs using the S-303/GSH treatment meets

the criteria of the FDA, which recommends that pathogen reduction technologies achieve a 6 to 10 log₁₀ GEq/mL pathogen load reduction [66].

Of note, during the CHIKV outbreak in the French Caribbean in 2014–2015, 10 recipients received INTERCEPT-treated platelets from CHIKV RNA-positive blood donations with no clinical evidence of CHIKV transmission [51].

PI using S-303/GSH is a chemical treatment, and the process only requires a biological containment hood and does not require instrumentation beyond what is required to generate a transfusable red cell unit in additive solution. Additionally, the chemical treatment is achieved at room temperature using reagents that are stable at room temperature. This is of particular interest in remote areas or resource limited countries where reagent transportation under refrigerated conditions is challenging.

This study, along with previous findings, demonstrates the ability of the S-303/GSH system to inactivate CHIKV

in addition to DENV [36] and ZIKV [30] in RBCs. As the licensed amotosalen/UVA system can achieve robust inactivation of these pathogens in plasma [31, 39, 40] and platelet [31, 67, 68] components, continued development and regulatory approval of the S-303/GSH system may provide an opportunity for robust inactivation of high levels of CHIKV, DENV and ZIKV in all blood components.

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Conflict of interest

Andrew Laughhunn, Felicia Santa Maria, Marion C. Lanteri and Adonis Stassinopoulos are employees of Cerus Corporation. Didier Musso and Maite Aubry declare no conflict of interest regarding this manuscript.

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