Inactivation of Zika virus by solvent/detergent treatment of human plasma and other plasma-derived products and pasteurization of human serum albumin

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BACKGROUND: In 2016 the World Health Organization declared the mosquito-borne Zika virus (ZIKV) a "public health emergency of international concern." ZIKV is a blood-borne pathogen, which therefore causes concerns regarding the safety of human plasma–derived products due to potential contamination of the blood supply. This study investigated the effectiveness of viral inactivation steps used during the routine manufacturing of various plasma-derived products to reduce ZIKV infectivity.

STUDY DESIGN AND METHODS: Human plasma and intermediates from the production of various plasma-derived products were spiked with ZIKV and subjected to virus inactivation using the identical techniques (either solvent/detergent [S/D] treatment or pasteurization) and conditions used for the actual production of the respective products. Samples were taken and the viral loads measured before and after inactivation.

RESULTS: After S/D treatment of spiked intermediates of the plasma-derived products Octaplas(LG), Octagam, and Octanate, the viral loads were below the limit of detection in all cases. The mean log reduction factor (LRF) was at least 6.78 log for Octaplas(LG), at least 7.00 log for Octagam, and at least 6.18 log for Octanate after 60, 240, and 480 minutes of S/D treatment, respectively. For 25% human serum albumin (HSA), the mean LRF for ZIKV was at least 7.48 log after pasteurization at 60°C for 120 minutes.

CONCLUSION: These results demonstrate that the commonly used virus inactivation processes utilized during the production of human plasma and plasma-derived products, namely, S/D treatment or pasteurization, are effective for inactivation of ZIKV.

The Zika virus (ZIKV) is a mosquito-borne virus (arbovirus) first identified in Uganda in 1947.[1,2] It belongs to the *Flaviviridae* family (which also includes the West Nile virus [WNV] and Dengue virus) and the *Flavivirus* genus and consists of a positive-sense, single-stranded RNA.[3] The enveloped virions are approximately 40 nm in diameter and composed of an icosahedral nucleocapsid.[3] The historical endemic areas of ZIKV are the tropical regions of Africa and Asia;

ABBREVIATIONS: LRF = log reduction factor; LVP = large-volume plating; MPN = most probable number; NCPV = National Collection of Pathogenic Viruses; PHE = Public Health England; $TCID_{50}$ = 50% tissue culture infectious dose; TnBP = tri-n-butyl phosphate; ZIKV = Zika virus.

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however, outbreaks have been recorded since 2007 in the Pacific area and, since 2015, the spread of ZIKV in the Americas has gained public attention.[4,5] To date, no vaccines or medications are available to prevent or treat ZIKV infections.[6] The most common, usually mild, symptoms of ZIKV disease are fever, rash, joint pain, and conjunctivitis; however, the majority of people infected by ZIKV are asymptomatic.[2,7]

The evolving outbreak of ZIKV infections in the Americas and the Pacific region coincided with an increase in cases of microcephaly and other adverse outcomes during pregnancy, as well as Guillain-Barré syndrome in adults. The Centers for Disease Control and Prevention concluded that ZIKV infection is responsible for the adverse outcomes during pregnancy and is linked to various problems in infants, including eye defects, hearing loss, and impaired growth.[8]

While most infections are suspected to result from direct bites from infected *Aedes aegypti* mosquitoes, further infection routes via sexual contact and via blood or nonvirally inactivated blood products have been described.[2,9,10] The potential for ZIKV transmission via blood transfusion was demonstrated during an outbreak in French Polynesia between November 2013 and February 2014, where approximately 3% of blood donors tested positive for ZIKV.[11] In Brazil, suspected cases of ZIKV transmission through blood transfusion are under investigation.[12,13] Thus, it is important to consider the potential risk of ZIKV transmission in the production of human plasma and plasma-derived products.[11,14,15]

Several guidelines exist to ensure the safety of human plasma and derived products for use in patients, including effective donor selection.[14,16,17] Overall pathogen safety of plasma-derived products is ensured by the use of several well-investigated dedicated process steps during manufacturing and purification, such as solvent/detergent (S/D) treatment, nanofiltration, or pasteurization. In addition, process steps such as cold ethanol fractionation and low pH treatment contribute significantly to the pathogen safety of plasma derivatives.

S/D treatment is the gold standard procedure to inactivate enveloped viruses, such as human immunodeficiency virus types 1 + 2 (HIV-1, HIV-2); hepatitis B virus (HBV); human cytomegalovirus; and viruses of the *Flaviviridae* family such as the WNV, hepatitis C virus (HCV), hepatitis G virus, or bovine viral diarrhea virus[18-20] in plasma-derived products.[21,22] The efficacy, robustness, and reliability of S/D virus inactivation has been demonstrated for enveloped model viruses such as bovine viral diarrhea virus, sindbis virus, pseudorabies virus, or vesicular stomatitis virus with different combinations of S/D reagents, concentrations, and exposure times. Based on these experiences, lipid solvents and detergents used in S/D treatment are expected to inactivate ZIKV.

Another important safety step in the manufacture of plasma-derived products is pasteurization. Pasteurization (i.e., heat treatment at 60°C) effectively eliminates all enveloped and most nonenveloped viruses. The effectiveness of this process depends, however, on the protein concentration of the plasma solution and concentration of stabilizers.[23-26]

On the basis of existing data for enveloped viruses, the inactivation and/or removal steps incorporated into manufacturing processes for human plasma and plasma-derived medicinal products may be effective against ZIKV. To verify this assumption, viral safety studies with ZIKV were performed on the S/D treatment steps used in the production of a human plasma for transfusion (Octaplas(LG)/Octaplas, named Octaplas(LG)), an intravenous immunoglobulin preparation (Octagam 5%/10%), and a Factor VIII and von Willebrand Factor concentrate (Octanate). Furthermore, the effect of pasteurization, as used in production of an albumin concentrate (25% human serum albumin [HSA]; concentrate from human plasma; trade names Albumin, Albuminativ, Albunorm, and plasma protein fraction), was evaluated.

MATERIALS AND METHODS

Viruses and cells

Two ZIKV isolates were used in these studies. The ZIKV isolate H/PF/2013 (clinical isolate, French Polynesia 2013) was provided by M. Eickmann (Philipps-Universität Marburg) with permission from the European Virus Archive (EVAg), Aix Marseille Université, on behalf of Emergence des Pathologies Virales (EPV).

An additional ZIKV strain MP1751 (ZIKV 1308258v) was purchased from Public Health England (PHE). This strain was deposited with the National Collection of Pathogenic Viruses (NCPV). NCPV were not initially given any origin or strain information, but partial sequencing of the *Envelope* gene shows 98% identity to the strain MR766 (DQ859059).[27] Further investigation with the depositor's records revealed that it is most likely to be Strain MP1751, isolated in Uganda, in November 1962.[28] The passage history before deposit with NCPV included up to four passages between 1962 and 1972. This was followed by one passage in Vero cells in 2011.[29]

The ZIKV isolates were propagated and titrated on Vero cells (an established kidney cell line from African green monkey, ECACC Code 84113001). The cells were maintained in RPMI 1640 with L-glutamine, supplemented with 5% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. These cultures were maintained for 7 days before microscopic evaluation. The titer of the virus stock H/PF/2013 was approximately 7.2 log 50% tissue culture infectious dose

($TCID_{50}$/mL). The titer of the virus isolate purchased from PHE was approximately 8.1 log $TCID_{50}$/mL.

Infectivity assays

For infection, Vero cells were seeded in 96-well culture plates at 1×10^4 cells/mL and cultivated at 37°C, 5% CO_2, and in saturated humidity. To determine the titers of both ZIKV isolates, serial threefold dilutions were made. A quantity of 0.1 mL of each dilution was inoculated into 0.12-mL cultures of Vero cells that had been established 1 day prior (eight replicates each). For comparison, Vero cells were taken from the identical passage and grown on a separate 96-well plate in the absence of test samples. After being incubated at 37°C for 7 days, the wells were evaluated microscopically for virus-induced cell changes and scored either positive or negative. To independently demonstrate the reliability and robustness of the assays, a control of ZIKV reference stock with a known titer was included in each assay.

Calculation of viral titer

Viral titers were determined by the $TCID_{50}$ assay based on endpoint dilution. The assay was performed in 96-well plates with susceptible cells seeded 1 day before titration. Threefold serial dilutions were prepared and eight replicates per dilution tested. The tested sample volume per replicate was 100 μL. The titer was estimated using the Spearman-Karber method.[30,31]

The applicability of the Spearman-Karber method is given when dilutions with 100 and 0% positive cultures are included in the titration or can be estimated. In the case that only a few positive cultures were found, and the Spearman-Karber method was not applicable, the most probable number (MPN) method was used.[32-34] The MPN method estimates the virus concentration and can be transferred to $TCID_{50}$.[31,35]

The large-volume plating (LVP) assay was used to improve the limit of detection by increasing the tested sample volume. In a prestudy, the sample dilution that is not cytotoxic and does not interfere with the virus and cell test system was determined. A defined volume of this dilution was tested. Ninety-six–well plates were used and 100 μL of the sample dilution per well was tested. The LVP assay was used for process samples, which are expected to contain none or only few infectious viruses. Virus titers for samples without positive cultures were determined according to the Poisson distribution at 95% confidence interval (CI). If positive cultures were found in the LVP assay, the MPN method was used to estimate the virus titer. Calculations of the virus reduction factor and 95% CI were undertaken using the methodology outlined in European regulatory guidelines.[36]

Process intermediates

Studies were performed using in-process material collected from commercial scale batches.

Study design

Samples of Octaplas(LG), 5%/10% Octagam, and Octanate were treated with S/D at standard process conditions. Samples of 25% HSA were treated by pasteurization under standard process conditions. Validation experiments were performed according to current Committee for Proprietary Medicinal Products guidelines[37] and the recommendations of the Paul-Ehrlich-Institut.[38] The aim of the study was to evaluate the total capacity of the S/D and pasteurization processes to inactivate ZIKV and to define the minimal incubation time needed to inactivate the viruses to below the detection limit. A downscaled version of the manufacturing process was established and followed strictly during the infectivity studies. Tests for both the spiked samples and the controls were performed in duplicates (Test 1 and Test 2).

S/D treatment of spiked Octaplas(LG) process intermediates

In-process intermediates were spiked with ZIKV isolate H/PF/2013 at a ratio of 1:10. S/D treatment was performed with 1% (wt/wt) TnBP (tri-*n*-butyl phosphate) and 1% (wt/wt) octoxynol-9 at 30.0 ± 1.0°C and at pH 6.9 to 7.4 for 60 minutes. To determine the kinetics of virus inactivation, test samples were taken at 5, 15, 30, and 60 minutes. The S/D treatment was terminated by C-18 resin processing to remove the S/D reagents, followed by a 1:50 dilution with cell culture medium. Prediluted aliquots with in-process material without S/D were used as a control for initial viral load and in-process material with S/D followed by C-18 resin processing served as controls for the termination procedure and spike recovery. Controls and samples were serially diluted threefold and screened with Vero cells for ZIKV infectivity by endpoint titration. Additionally, the S/D samples at 30 and 60 minutes were tested with the LVP assay. Two volumes, 48 and 480 mL, of the diluted test samples, respectively, were tested to account for different detection limits.

S/D treatment of spiked 5%/10% Octagam process intermediates

In-process intermediates were spiked with ZIKV isolate H/PF/2013 in a ratio of 1:10. S/D treatment was performed with 0.3% (wt/wt) TnBP and 1% (wt/wt) octoxynol-9 at 6.0 ± 2.0°C for 240 minutes. Test samples were taken at 5, 15, 30, 120, and 240 minutes. The S/D treatment was terminated by C-18 resin processing to remove the S/D reagents followed by a 1:9 dilution with cell culture medium. Prediluted aliquots with in-process material without S/D and in-process material with S/D followed by

C-18 resin processing served as experimental controls. Additionally, pH-adjusted controls were prepared to determine the initial viral load. Controls and samples were diluted as above and screened with Vero cells for ZIKV infectivity by endpoint titration. S/D samples after 30 and 240 minutes were tested with the LVP assay. Forty-eight and 192 mL of the diluted test samples, respectively, were tested to account for different detection limits.

S/D treatment of spiked Octanate process intermediates

In-process intermediates were spiked with ZIKV strain MP1751 (PHE 1308258v) at a ratio of 1:10. S/D treatment was performed with 0.3% (wt/wt) TnBP and 1.0% (wt/wt) polysorbate 80 at 25.5 ± 0.5°C and at pH 7.1 ± 0.1 for 5, 15, 30, 60, 120, 240, and 480 minutes. S/D treatment was terminated by dilution of the test samples with cell culture medium (1:200). Prediluted S/D containing in-process material served as a control for the termination procedure and in-process material without S/D was used for viral load and hold controls. The hold control was stored at process conditions over 480 minutes. Controls and samples were diluted as described above. The S/D treated samples after 60 min were additionally screened for infectivity by LVP. After 60 and 120 minutes of S/D treatment, 9.6 mL of the diluted test samples were tested. S/D treatment after 240 and 480 minutes was investigated using 48 and 288 mL of the diluted sample, respectively, to account for different detection limits.

Pasteurization of spiked 25% HSA process intermediates

In-process intermediates were spiked with ZIKV strain MP1751 (PHE 1308258v) at a ratio of 1:11. Pasteurization was performed at 60.0 ± 0.5°C and at neutral pH for 120 minutes. Samples were taken at 0, 10, 30, 60, 90, and 120 minutes. Pasteurization was terminated by transferring the test samples into an icewater bath for at least 2 minutes followed by a 1:9 dilution with cell culture medium. Prediluted aliquots with in-process material served as controls for the viral load. Controls and samples were diluted as described and screened with Vero cells for ZIKV infectivity by endpoint titration. After 120 minutes, the pasteurization sample was also tested with the LVP assay. A quantity of 192 mL of the diluted test sample was tested.

RESULTS

ZIKV inactivation during S/D treatment of spiked Octaplas(LG) process intermediates

The mean initial viral load was 8.44 log $TCID_{50}$. After 60 minutes of S/D treatment the viral load was below the limit of detection, with a mean log reduction factor (LRF) of at least 6.78 log. For the endpoint dilution assay, the detection limit was calculated to be not more than 2.43 log $TCID_{50}$/mL (Detection Limit 1). Detection Limit 2 for the sample after 30 minutes was calculated to be not more than 0.65 log $TCID_{50}$/mL and Detection Limit 3 after 60 minutes was not more than −0.35 log $TCID_{50}$/mL. Based on endpoint titration, no infectious ZIKV was detected after S/D treatment. Even 5 minutes of exposure to S/D was sufficient to destroy ZIKV to below the limit of detection (Detection Limit 1). Despite the significant increase in volume tested for the samples after 30 and 60 minutes, ZIKV infectivity remained below the limit of detection (Detection Limits 2 and 3, respectively). The analysis of the hold controls (8.26 log [Test 1] and 7.90 log [Test 2]) showed no virus inactivation during the process time. The virus inactivation kinetics of ZIKV (isolate H/PF/2013) for Octaplas(LG) are shown in Fig. 1A. The viral loads and LRFs between both tests were similar and are shown in Table 1. The mean viral loads of the controls are shown in Fig. 2A.

ZIKV inactivation during S/D treatment of spiked 5%/10% Octagam process intermediates

The mean initial viral load was 8.33 log $TCID_{50}$. After 240 minutes of S/D treatment, the viral load was below the limit of detection, with a mean LRF of at least 7.00 log. Viral load was already below the detection limit within 30 minutes (LRFs of $\geq 6.46 \pm 0.23$ log [Test 1] and $\geq 6.34 \pm 0.21$ log [Test 2]), whereas the process time is at least 240 minutes in the production unit. The pH-adjusted stability controls, the termination control, and the spike recovery controls showed no significant or only mild reduction of viral infectivity ($\Delta = 0.7$-0.1 log) meaning that the product matrix, S/D removal, and C-18 resin had no impact on the viral load. However, analyses of stability controls at process pH 5.3 ± 0.2 without addition of S/D reagents showed a pronounced decrease of viral load over time (Fig. 2B). This effect was even observed at an intermediate pH 6.0 ± 0.2. Thus, inactivation of ZIKV during S/D treatment at process pH is a combined effect of S/D treatment and, to a minor extent, low pH exposure.

In conclusion, ZIKV was inactivated in the Octagam S/D process step by at least 7.00 ± 0.16 log (mean values of Test 1 and Test 2 after 4 hr).[39] The virus inactivation kinetics of ZIKV (isolate H/PF/2013) for 5%/10% Octagam are shown in Fig. 1B. The viral loads and LRFs between both tests were similar and are shown in Table 1. The mean viral loads of the controls are shown in Fig. 2B.

ZIKV inactivation during S/D treatment of spiked Octanate process intermediates

The mean initial viral load was 8.68 log $TCID_{50}$. After 480 minutes of S/D treatment, the viral load was below the limit of detection, with a mean LRF of at least 6.18 log. For endpoint dilution assays, the detection limit was

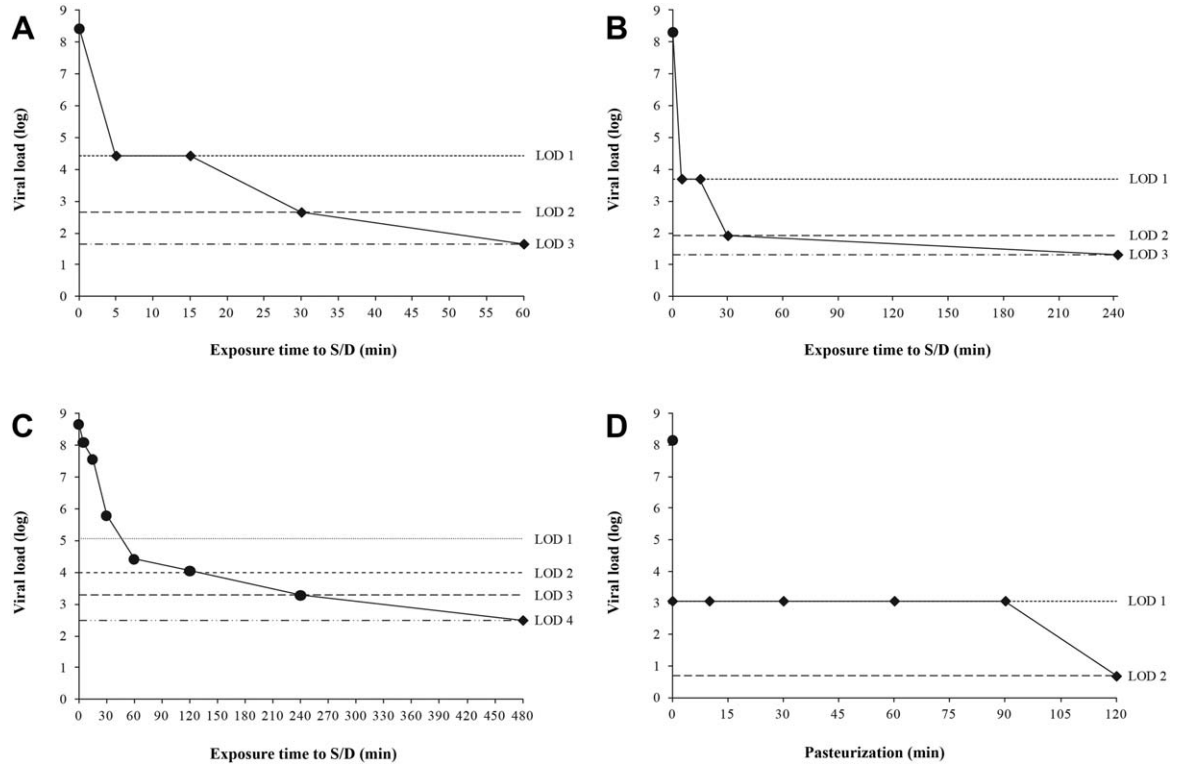

Fig. 1. Virus inactivation kinetics after S/D treatment of octaplasLG (A), Octagam (B), and Octanate (C) and pasteurization of 25% HSA (D). Each point on the graphs represents a mean of two separate tests. LOD = limit of detection. (●) Virus load not below the detection limit; (◆) virus load below the detection limit.

TABLE 1. Impact of S/D treatment on ZIKV loads

	Viral load (log) of spiked process intermediates					
	octaplasLG		Octagam		Octanate	
Duration of S/D treatment (min)	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
0	8.50	8.38	8.38	8.27	8.75	8.60
5	≤4.44	≤4.44	≤3.70	≤3.71	8.32	7.87
15	≤4.44	≤4.44	≤3.70	≤3.71	7.63	7.49
30	≤2.66*	≤2.66*	≤1.92*	≤1.93*	5.38	6.19
60	≤1.66*	≤1.66*	ND	ND	4.47*	4.36*
120	ND	ND	≤3.70	≤3.71	3.98*	4.11*
240	ND	ND	≤1.32*	≤1.33*	3.28*	≤3.28*
480	ND	ND	ND	ND	≤2.50*	≤2.50*
Reduction factor (based on last time point)	≥6.84*	≥6.72*	≥7.06*	≥6.94*	≥6.25*	≥6.10*

* LVP; LRF based on final time point.
ND = not done.

calculated to be not more than 3.03 log $TCID_{50}$/mL (Detection Limit 1). Detection Limit 2 for the samples after 60 and 120 minutes was not more than 1.96 log $TCID_{50}$/mL. Detection Limit 3 for the time point after 240 minutes was calculated to be not more than 1.26 log $TCID_{50}$/mL, and Detection Limit 4 after 480 minutes was not more than 0.48 log $TCID_{50}$/mL.

Residual infectivity after 240 minutes was found in Test 1, whereas no residual infectivity was found during Test 2. No residual infectivity was found after 480 minutes of S/D treatment in both tests (Detection Limit 4). The viral loads of the hold controls were 8.63 log (Test 1) and 8.70 log (Test 2) and showed no virus inactivation during the process time. The viral loads of the termination controls were 8.31 log (Test 1) and 8.92 log (Test 2; Table 1). The virus inactivation kinetics of ZIKV strain MP1751 (PHE 1308258v) for Octanate are shown in Fig. 1C. The viral loads and LRFs between both tests were similar and are shown in Table 1. The mean viral loads of the controls are shown in Fig. 2C.

ZIKV inactivation during pasteurization of spiked 25% HSA process intermediates

The mean initial viral load was 8.17 log $TCID_{50}$. After pasteurization at 60°C for 120 minutes, the viral load was below the limit of detection, with a mean LRF of at least 7.48 log. For the endpoint dilution assay, the detection limit was calculated to be not more than 1.69 log $TCID_{50}$/mL (Detection Limit 1) and Detection Limit 2 for the sample after 120 minutes was calculated to be not more than −0.69 log $TCID_{50}$/mL (Table 2). Based on endpoint titration, no infectious ZIKV was detected at the beginning of pasteurization (0 min, after heat-up phase); that is, ZIKV was inactivated to below the limit of detection (Detection Limit 1). Despite the significant increase in volume tested for the sample after 120 minutes, ZIKV infectivity still remained below the limit of detection (Detection Limit 2). The analysis of the hold controls (8.17 log [Test 1] and 8.28 log [Test 2]) showed no virus inactivation during the process time. The virus inactivation kinetics of ZIKV strain MP1751 (PHE 1308258v) for 25% HSA are shown in Fig. 1D. The viral loads and LRFs of both tests were similar and are shown in Table 2.

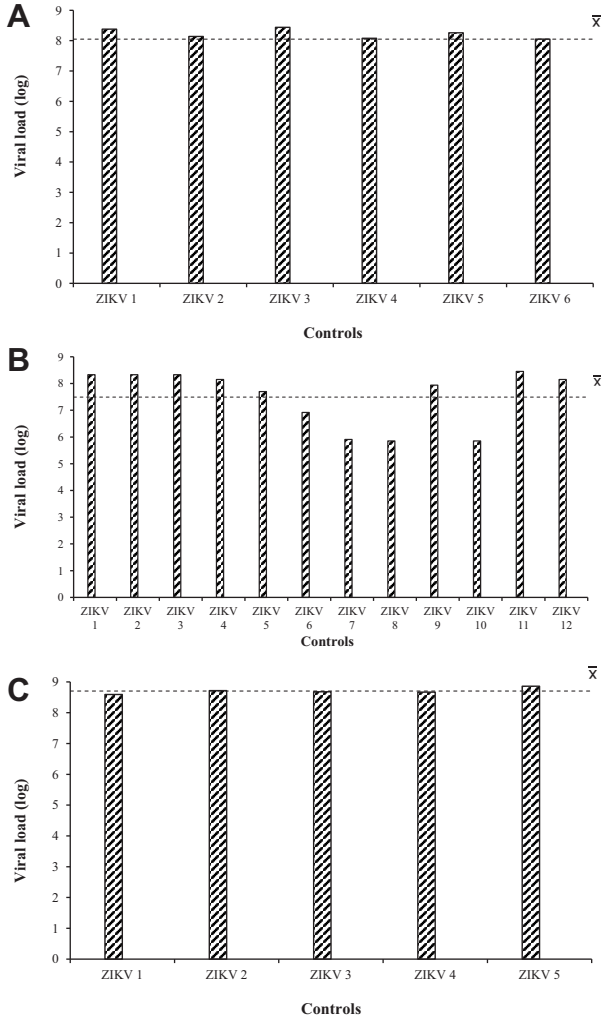

Fig. 2.

DISCUSSION

On February 1, 2016, the World Health Organization declared ZIKV a public health emergency of international concern.[40] ZIKV poses a significant problem to public health in affected countries, even though most ZIKV-infected people show no symptoms.[11,41,42] It is therefore important to assess the risk for all possible modes of spread, including blood transfusion or use of therapeutic plasma products, such as human plasma, immunoglobulins, HSA, or coagulation factors.[43-45]

It is valuable to assess the anti-ZIKV efficacy of virus inactivation steps, such as S/D treatment, that are widely

Fig. 2. Virus loads of the controls of octaplasLG (A), Octagam (B), and Octanate (C). (A) ZIKV 1 = virus spike control (at the beginning of the test); ZIKV 2 = virus spike control (after 60 min of storage at 30.0 ± 1.0°C); ZIKV 3 = positive control or load control (at the beginning of the test); ZIKV 4 = positive control or bench top control (after 60 min of storage at 30.0 ± 1.0°C); ZIKV 5 = termination control; ZIKV 6 = spike recovery control. Bars represent the mean values of control samples of Test 1 and Test 2. $\bar{x}$ represents the mean value of all control samples (bars). (B) ZIKV 1 = virus spike control (at the beginning of the test); ZIKV 2 = virus spike control (after 240 min of storage at 6.0 ± 2.0°C); ZIKV 3 = positive control or load control (adjusted to pH 7.0 ± 0.2 at the beginning of the test); ZIKV 4 = positive control (adjusted to pH 7.0 ± 0.2 after 30 min of storage at 6.0 ± 2.0°C); ZIKV 5 = positive control or bench top control (adjusted to pH 7.0 ± 0.2 after 240 min of storage at 6.0 ± 2.0°C); ZIKV 6 = Stability Control 1 (adjusted to pH 5.3 ± 0.2 at the beginning of the test); ZIKV 7 = Stability Control 1 (adjusted to pH 5.3 ± 0.2 after 30 min of storage at 6.0 ± 2.0°C); ZIKV 8 = Stability Control 1 (adjusted to pH 5.3 ± 0.2 after 240 min of storage at 6.0 ± 2.0°C); ZIKV 9 = Stability Control 2 (pH 6.0 ± 0.2 at the beginning of the test); ZIKV 10 = Stability Control 2 (pH 6.0 ± 0.2 after 30 min of storage at 6.0 ± 2.0°C); ZIKV 11 = termination control (adjusted to pH 7.0 ± 0.2); ZIKV 12 = spike recovery control (adjusted to pH 7.0 ± 0.2). Bars represent the mean values of control samples of Test 1 and Test 2. $\bar{x}$ represents the mean value of all control samples (bars). (C) ZIKV 1 = virus spike control (at the beginning of the test); ZIKV 2 = virus spike control (after 480 min of storage at 25.5 ± 0.5°C); ZIKV 3 = positive control or load control (at the beginning of the test); ZIKV 4 = positive control or bench top control (after 480 min of storage at 25.5 ± 0.5°C); ZIKV 5 = termination control. Bars represent the mean values of control samples of Test 1 and Test 2. $\bar{x}$ represents the mean value of all control samples (bars).

TABLE 2. Impact of pasteurization on ZIKV load in spiked 25% HSA process intermediates

	Viral load (log)	
Duration of pasteurization (min)	Test 1	Test 2
Load control	8.11	8.22
0 (heat-up phase)	≤3.07	≤3.07
10	≤3.07	≤3.07
30	≤3.07	≤3.07
60	≤3.07	≤3.07
90	≤3.07	≤3.07
120	≤0.69*	≤0.69*
Reduction factor	≥7.42*	≥7.53*

* LVP; LRF based on final time point.

used in the manufacture of therapeutic plasma or plasma-derived products. The S/D method is an effective and robust step in the inactivation of enveloped viruses, irreversibly disrupting the lipid coat and associated binding sites under mild process conditions.[18,46] The efficacy of the S/D method has been validated extensively using a broad range of models with human viruses and in animal studies under robust conditions.[18]

Due to the nonselective mode of action, the S/D method is capable of inactivating not only the viruses of most concern, that is, HIV, HBV, and HCV, but also viruses not tested for during routine screening at blood collection centers, such as human T-lymphotropic virus-1 and -2, hepatitis G virus, human cytomegalovirus, or WNV.[18,47,48] In this investigation, the S/D treatment protocols used in the production of Octaplas(LG), Octagam, and Octanate resulted in successful inactivation of the enveloped ZIKV to below the limit of detection. The effectiveness of ZIKV inactivation during the S/D treatment depended on the source of S/D solution: the combination of TnBP and polysorbate 80 required up to 480 minutes of incubation for inactivation of ZIKV, compared with only 60 to 240 minutes for the combination of TnBP and octoxynol-9.

These Octagam results also demonstrated a reduction of ZIVK infectivity at lower pH (Fig. 2B). It is known that other flaviviruses (e.g., WNV) deploy the pH shift in endosomes as a trigger signal for endosomal escape via their E2 envelope protein. Inactivation of flaviviruses at values below pH 6 have been studied and showed that conformational changes occurred in a pH-dependent manner, with an upper threshold at pH 7.0 and maximum conversion occurring at pH 6.4 and below.[49]

Pasteurization or heat treatment is a widely used viral inactivation method effective against both lipid and non-enveloped viruses.[50] This study showed that the heat-sensitive ZIKV was rapidly inactivated by pasteurization at 60°C for 120 minutes. Even at the beginning of the heating phase, no ZIKV could be detected by endpoint titration. It has been previously shown that ZIKV can be inactivated after heating for 30 minutes at 58°C or for 15 minutes at 60°C.[51] Other viruses within the *Flaviviridae* family, such as HCV, WNV, and yellow fever virus, have also been reported to be heat sensitive and inactivated by pasteurization. HCV can be inactivated after 40 minutes at 56°C.[52] Yellow fever virus is routinely inactivated by heating to 56°C for 30 minutes and WNV has been shown to be inactivated under the same conditions.[52,53]

Another widely used step in the manufacture of plasma-derived products is the removal of viruses by nanofiltration. Both enveloped and nonenveloped viruses can be removed without denaturing plasma proteins or affecting the quality of the derived products by extreme pH levels or temperature. Virus elimination by nanofiltration via a mechanism based on size exclusion, that is, with pore sizes of 20 nm, is capable of removing viruses above this cutoff value, including ZIKV, whose enveloped virions have a diameter of approximately 40 nm.[3,54] In conclusion, this study demonstrates that the S/D treatment or pasteurization conditions commonly used in the production of selected plasma and plasma-derived products are more than sufficient to inactivate the enveloped ZIKV to below the limit of detection.

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CONFLICT OF INTEREST

DK, SM, AP, KUR, AV, and TS are employees of Octapharma.

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