

Case Report

A blood donor with bacteraemia

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A 56-year-old woman with a history of well-controlled insulin-dependent diabetes mellitus and hypertension donated platelets in July, 2004. She had donated platelets approximately 50 times in the previous 5 and a half years. We did routine testing for bacterial contamination, and cultured *Streptococcus bovis* from the units she donated, 11.4 hours after incubation. We contacted the woman to discuss this result, and she reported no additional medical history. She had never had a colonoscopy, and we encouraged her to see her doctor for follow-up. We destroyed the units from her donation, and told her that she could not donate until she was cleared of not having an obvious source of *S bovis* bacteraemia. Her doctor ordered two sets of blood cultures to look for persistent bacteraemia. These cultures were negative. The woman then had a colonoscopy that showed a friable mass, 3 cm diameter, in the sigmoid colon. Sigmoidectomy was done in September, 2004, for moderately differentiated adenocarcinoma. The woman tolerated surgery well, and had no complications.

The major cause of platelet contamination is usually normal skin flora,¹ which can be introduced at the time of blood collection. Donor bacteraemia is less likely, though it can occur after dental manipulation such as tooth extraction, tooth brushing, or use of gum irrigation devices.¹ *S bovis* is not typical skin flora or a ubiquitous environmental agent. *S bovis* bacteraemia has been found in association with gastrointestinal neoplasia (ranging from colonic polyps to cancer), extracolonic malignancies, liver disease, endocarditis, cholangitis, meningitis, and diabetes mellitus.²⁻⁴ The American Society of Hematology and American Association of Blood Banks has published guidelines on the regulation of bacterial contamination of blood components,¹ recently updated to include measures to detect and limit bacterial contamination in all platelet components.⁵ Bacterial sepsis related to transfusion is the second most frequent transfusion-related cause of fatalities in the USA.⁵

Specific identification of bacterial organisms found in testing of platelet donations can have value to the donor, in addition to the obvious benefit for any potential recipient or for quality control. Bacterial culture in this apparently healthy blood donor led to a timely diagnosis of malignancy, and a good clinical outcome.

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References

- 1 Hillyer CD, Josephson CD, Blajchman MA, et al. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. *Hematology (Am Soc Hematol Educ Program)* 2003: 575-89
- 2 Gold JS, Bayar S, Salem RR. Association of *Streptococcus bovis* bacteremia with colonic neoplasia and extracolonic malignancy. *Arch Surg* 2004; 139: 760-65.
- 3 Lee RA, Woo PCY, To APC, et al. Geographical difference of disease association in *Streptococcus bovis* bacteraemia. *J Med Microbiol* 2003; 52: 903-08.
- 4 Jean SS, Teng LJ, Hsueh PR, et al. Bacteremic *Streptococcus bovis* infections at a university hospital, 1992-2001. *J Formos Med Assoc* 2004; 103: 118-23.
- 5 Centers for Disease Control and Prevention (CDC). Fatal bacterial infections associated with platelet transfusions—United States, 2004. *MMWR Morb Mortal Wkly Rep* 2005; 54: 168-70.

PT → SARS



医薬品
 医薬部外品 研究報告 調査報告書
 化粧品

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2005 年 7 月 28 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	SARS-coronavirus (SARS-CoV) and the safety of a solvent/detergent (S/D) treated immunoglobulin preparation H. F. Rabenau, L. Biesert, T. Schmidt, G. Bauer, J. Cinatl, H. W. Doerr Biologicals, 2005; 33: 95-99	公表国 英国	
販売名 (企業名)						
研究報告の概要	SARS コロナウイルス (SARS-CoV) は新たに出現したウイルスであり、2002 年 11 月から 2003 年 9 月までの期間に 8000 人以上が感染し、800 人近くを死に至らしめた極めて病原性の高いウイルスである。大半が呼吸飛沫を介したヒトからヒトへの伝播であるが、それ以外の伝播経路も否定できない。SARS-CoV ウイルス血症は高力価になることはないと考えられているが、輸血または FFP などの血液製剤や血症分画製剤を介した伝播は否定できない。全ての血症分画製剤の製造工程は、定められたウイルス不活化/除去工程に従うものでなければならない。有機溶媒と界面活性剤の組み合わせ (S/D 処理) により、製剤の有効性に影響を及ぼすことなく輸血により媒介されるウイルスの大半が不活化されることがわかっている。S/D 処理は、HIV, HBV, HCV, および CMV などのエンベロプを不可逆的に破壊する。本試験では、免疫グロブリン製剤 (OCTAGAM, Octapharma Pharmazeutika Produktionsges. m. b. H., オーストラリア, ウィーン) の製造工程における SARS-CoV 不活化能について評価した。実験では不活化時間を 95% 以上短縮し、かつ S/D 濃度を製造工程で使用する濃度の 75% として使用した。その結果、検体中の SARS-CoV は、S/D 処理後 1 分以内で検出限界以下まで完全に不活化されることが示された。したがって、現行の S/D 処理で実施されている標準的な S/D 処理の効果は本実験よりも高く、仮に本実験よりもウイルス負荷が高かった場合でも OCTAGAM の製造工程において現行で実施されている標準 S/D 処理能は十分に高いと考えられる。					使用上の注意記載状況・ その他参考事項等
	報告企業の意見	今後の対応				
SARS-CoV はエンベロプを持つ RNA ウイルスであり、その感染性はエンベロプの完全性に左右される。血漿分画製剤の製造工程ではエンベロプが破壊されるため、ウイルスが不活化される。本論文は、免疫グロブリン製剤の製造工程に用いられている S/D 処理により SARS-CoV が完全に不活化されることを確認したものである。	現時点で弊社が新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。					

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SARS-coronavirus (SARS-CoV) and the safety of a solvent/detergent (S/D) treated immunoglobulin preparation

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Abstract

SARS-coronavirus (SARS-CoV) is a newly emerged, highly pathogenic agent that caused over 8000 human infections with nearly 800 deaths between November 2002 and September 2003. While direct person-to-person transmission via respiratory droplets accounted for most cases, other modes have not been ruled out. SARS-CoV viraemia does not seem to reach high titres, however, it has to be excluded that virus transmission may occur via blood transfusion or application of therapeutic plasma products, e.g. fresh-frozen plasma or single components derived thereof. Manufacturing processes of all plasma derivatives are required to comprise dedicated virus inactivation/removal steps. Treatment with a mixture of solvent and detergent (SD) has successfully been applied to inactivate the most members of the transfusion-relevant viruses without affecting therapeutic properties of the products. The SD treatment irreversibly disrupts the lipid envelope of viruses such as HIV, HBV, HCV, HGV and CMV. In this study we evaluated the manufacturing process of an immunoglobulin preparation (OCTAGAM, manufactured by Octapharma Pharmazeutika Produktionsges.m.b.H., Vienna, Austria) for its capacity to inactivate the SARS-CoV. Our results demonstrate that SARS-CoV was completely inactivated below the limit of detection. This was found to occur within 1 min of SD treatment.

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1. Introduction

The Severe Acute Respiratory Syndrome (SARS) is a novel infectious disease that first occurred in China in November 2002, but was not known to the public before February 2003. SARS is caused by a newly emerged virus belonging to the coronaviridae family, provisionally termed SARS-coronavirus (SARS-CoV) [8]. Available sequence data indicate that SARS-CoV is clearly different from all previously known coronaviruses [20]. A new, fourth genetic lineage has been proposed for SARS-CoV [15].

Although human-to-human transmission of SARS-CoV is less efficient than for other air-borne viruses such as influenza A viruses, the recent SARS epidemic was characterised by several explosive outbreaks [4,24]. Most infections resulted from direct transmission via respiratory droplets during close personal contact and adequate protective measures were shown to be effective [22,25]. However, there are a number of instances when transmission occurred through other modes not yet well defined. In central China, around 50% of probable SARS patients did not have an apparent history of close personal contact with another victim [14]. At least a portion of these cases may have arisen from modes of transmission different from respiratory droplets. Besides virus shedding into stool samples, SARS-CoV viraemia occurs, but does not seem to reach high titres (≈ 200 copies/ml) [16,21]. During the SARS-CoV-epidemic,

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concerns were raised about the possibility of SARS-CoV transmission by transfusion of blood components or therapeutic plasma products. Manufacturing processes of all plasma derivatives are required to comprise dedicated virus inactivation/removal steps for enveloped viruses [5]. Treatment with a mixture of solvent and detergent (SD) is used by many manufacturers worldwide and has successfully been applied to clotting factor and immunoglobulin preparations [2,11,17]. The SD treatment causes enveloped viruses to be irreversibly destroyed. These include the human immunodeficiency virus types 1 + 2 (HIV-1, HIV-2), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis G virus (HGV) and human cytomegalovirus (CMV) [17].

On the basis of existing validation data for enveloped viruses, it can be anticipated that the inactivation/removal steps incorporated into manufacturing processes for plasma-derived medicinal products will also be effective for SARS-CoV. To confirm this assumption, verification studies with SARS-CoV were performed for the SD treatment conditions used in the manufacturing of an immunoglobulin concentrate intended for intravenous application (OCTAGAM, manufactured by Octapharma Pharmazeutika Produktionsges. m.b.H., Vienna, Austria). In full-scale manufacturing, SD process conditions include treatment with 0.3% (w/w) tri(*n*-butyl)phosphate (TNBP) and 1.0% (w/w) Octoxynol (trade name: Triton X-100) at 6.0 ± 0.5 °C and at pH 5.3 ± 0.2 for a minimum of 4 h. In compliance with the CPMP [5,6], the robustness of each virus inactivation method has to be demonstrated experimentally. Therefore, the inactivation of SARS-CoV by SD treatment was investigated in the laboratory scale at lowered concentration of solvent and detergent (75% of standard SD concentration) and at a shortened process time (30 min).

2. Materials and methods

2.1. Viruses and cells

The SARS-CoV isolate FFM-1 [7] was obtained from the sputum of a patient hospitalised with a diagnosis of probable SARS in the Isolation Unit of Frankfurt University Hospital, Germany. Virus stocks were stored at -80 °C. The maintenance medium consisted of minimum essential medium (MEM) without foetal calf serum (FCS) and was supplemented with 100 IU/ml of penicillin and 100 µg/ml of streptomycin. Virus was propagated and titrated on confluent Vero cells and titres were determined as 50% tissue culture infective doses (TCID₅₀/ml) in 96-well microtitre plates with 8 replicates per dilution (10-fold) and 50 µl inoculum per well [12,23]. The initial titre of the undiluted virus stock was 1.12×10^7 TCID₅₀/ml ($=7.05 \pm 0.35 \log_{10}$ TCID₅₀/ml [hereafter referred to as virus stock control 0 min]) and

2×10^7 TCID₅₀/ml ($=7.30 \pm 0.44 \log_{10}$ TCID₅₀/ml [virus stock control 30 min]).

In accordance with WHO recommendations, all work involving infectious SARS-CoV was performed under bio-safety level (BSL)-3 conditions in a BSL-3 facility.

2.2. Study design

The validation experiments were performed according to current CPMP guidelines and the recommendations of the Paul-Ehrlich-Institut in Germany [3,5,6]. The aim of the study was to evaluate the total capacity of the SD process for inactivating SARS-CoV and to define the minimal incubation time to inactivate the viruses below the detection limit. The exact incubation times with SD were 1, 3, 5, 10, 20 and 30 min (Fig. 1). Furthermore, the purpose of this validation study was to investigate the impact of a significantly lowered concentration of TNBP and Octoxynol (75% of standard SD concentration, i.e. 0.23% (w/w) TNBP/0.75% (w/w) Octoxynol) on virus inactivation. SD treatment was performed at 6.0 ± 0.5 °C and at pH 5.3 ± 0.2 for the above mentioned incubation periods in two independent tests.

A down-scaled version of the manufacturing process examined was established and the equivalence of the down-scale and manufacturing-scale intermediate was demonstrated, e.g. by measurement of their protein concentrations.

For the purification of IgG from cryopoor plasma, the Cohn–Oncley method is applied. This process involves

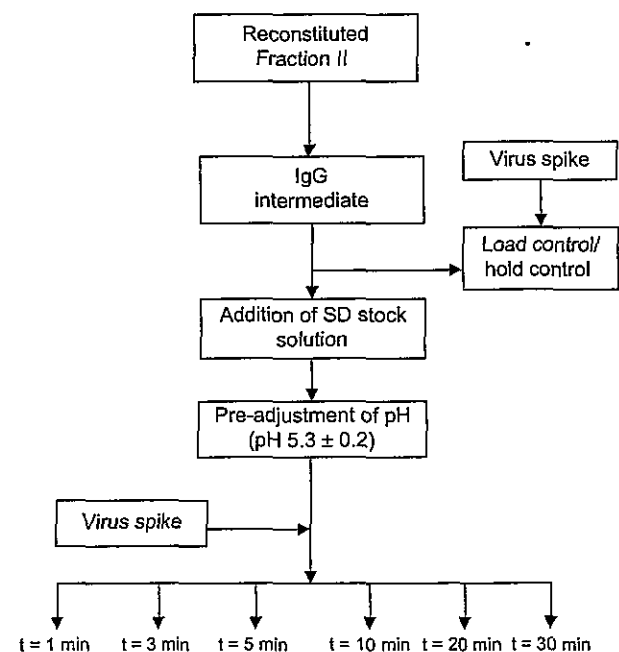


Fig. 1. Impact of a SD process on inactivation of SARS-CoV in an immunoglobulin preparation (OCTAGAM).

the sequential purification of protein fractions by adding various concentrations of ethanol with concurrent adjustments in pH, ionic strength and temperature. The precipitation and separation of fraction I + III from fraction II is considered the most viral safety relevant step for the non-lipid enveloped viruses. Starting materials in this study were process intermediates (resuspended fraction II) obtained from the manufacturing scale, which were spiked 1 in 20 with virus. An aliquot of the starting material without SD was individually spiked also 1 in 20 with virus and titrated to confirm the amount of virus added. This spiked intermediate (without SD) was titrated at the beginning of the process (virus-spiked intermediate control 0 min) and at the end of the process (virus-spiked intermediate control 30 min). This separate spiking was required, because, in the presence of SD reagents, the initial titre of any virus-spiked process intermediate would not represent the quantity of virus actually inoculated. Infectivity was determined by endpoint titration on Vero cells. The SD treatment was terminated in the laboratory studies by diluting 1:250 with cell culture medium. In order to prove that the inactivation by SD was terminated by our dilution procedure, an aliquot (100 µl) of the SD containing intermediate was prediluted 1:237.5 with cell culture medium. This solution was then spiked with virus (1 in 20), resulting in a final dilution factor of 1:250 with respect to SD (termination control). Specific non-spiked process intermediates were obtained for control procedures and tested for their potential cytotoxicity (see below) for the Vero cells and for their potential interference with the detection of low virus titres. All controls were serially 10-fold diluted and eight cultures (inoculum size 0.05 ml) of each dilution were established.

From the samples taken after 1, 3, 5, 10 or 20 min of SD treatment, 48 × 1 ml of the 1:250 dilution (corresponds to 192 µl of undiluted sample) was used as inoculum, but for 30 min the inoculum volume was increased to 720 × 1 ml (corresponds to 2.88 ml of undiluted sample). The cell cultures were maintained in a CO₂ incubator at 37 °C. Vero cultures were examined microscopically and evaluated for virus-induced (or cytotoxic) cell changes. The virus titres were calculated according to the method of Spearman and Kärber [12,23]. The virus titre for samples where no virus replication is observed, can be determined by using the Poisson distribution at 95% upper confidence limits. For the conversion to TCID₅₀/ml, the concentration of virus particles is divided by ln 2. Therefore, if virus replication was below the limit of detection, a theoretical, though very low content of viruses had to be accepted (1.37 log₁₀ TCID₅₀/ml for 1, 3, 5, 10, 20 min and 0.18 log₁₀ TCID₅₀/ml for 30 min process time). The virus reduction factor, which quantitatively determines the capacity of the process step to inactivate viruses, was calculated from the ratio of the virus titre detectable in

spiked OCTAGAM intermediate without SD and the virus titre potentially present after the SD step.

3. Results

For the study, appropriate in-process samples were collected from OCTAGAM full-scale production. Before pre-adjustment of the pH, this process intermediate was supplemented with SD and finally spiked with SARS-CoV at a ratio of 1 in 20. This starting material (with only 0.23% [w/w] TNBP and 0.75% [w/w] Octoxynol) was incubated at 6.0 ± 0.5 °C for 30 min. To determine the kinetics of virus inactivation, test samples were taken at predefined intervals during the process (1, 3, 5, 10, 20 and 30 min). The SD treatment was terminated by a 1:250 dilution with cell culture medium. The diluted test samples were screened with susceptible cells (Vero) for SARS-CoV infectivity by bulk analysis.

The SARS-CoV titre detected in the starting material prior to SD treatment was 5.93 ± 0.25 log₁₀ TCID₅₀/ml. The results of Fig. 2 and Table 1 demonstrate that SARS-CoV was completely inactivated below the limit of detection within 1 min of SD treatment. Depending on the sample volume tested the detection limit varied between 1.37 log₁₀ TCID₅₀/ml and 0.18 log₁₀ TCID₅₀/ml. In both independently performed tests similar results were obtained. Taking into consideration the various handling procedures during the laboratory experiment (e.g. spike with virus, careful mixing of the virus-spiked intermediate, sterile sampling of an aliquot, dilution with cell culture medium for terminating the inactivation process), less than 1 min was required. The resulting log reduction factor is ≥ 5.75 ± 0.25 log₁₀ TCID₅₀/ml.

Both virus stock controls and virus-spiked intermediate controls showed the expected values and demonstrated the validity of the study (Table 1). This means that the differences of the virus titre at the beginning and at the end of the incubation period was < 0.5 log₁₀

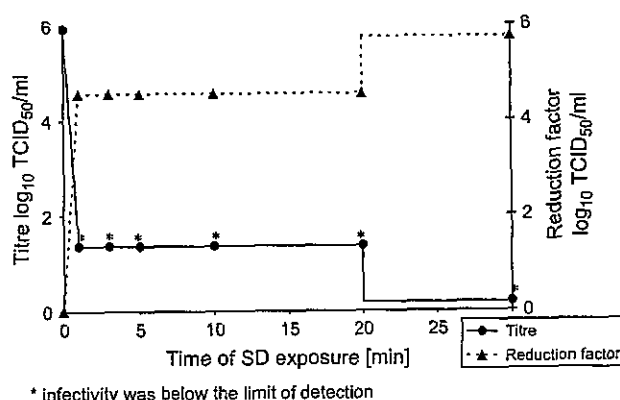


Fig. 2. SARS-CoV inactivation by SD treatment – influence of incubation time.

Table 1
SARS-CoV inactivation by SD treatment

Sample description	Dilution of sample/culture volume/test sample volume	Positive cultures	Virus titre \log_{10} TCID ₅₀ /ml	Reduction factor \log_{10}
Virus stock control 0 min	—	—	7.05 ± 0.35	—
Virus stock control 30 min	—	—	7.30 ± 0.44	—
Virus-spiked intermediate control 0 min	—	—	5.93 ± 0.25	—
Virus-spiked intermediate control 30 min	—	—	6.30 ± 0.44	—
Termination control	—	—	5.93 ± 0.25	—
1 min SD exposure	1:250/2 × 24 × 1 ml/0.192 ml	0/48	≤ 1.37	≥ 4.56 ± 0.25
3 min SD exposure	1:250/2 × 24 × 1 ml/0.192 ml	0/48	≤ 1.37	≥ 4.56 ± 0.25
5 min SD exposure	1:250/2 × 24 × 1 ml/0.192 ml	0/48	≤ 1.37	≥ 4.56 ± 0.25
10 min SD exposure	1:250/2 × 24 × 1 ml/0.192 ml	0/48	≤ 1.37	≥ 4.56 ± 0.25
20 min SD exposure	1:250/2 × 24 × 1 ml/0.192 ml	0/48	≤ 1.37	≥ 4.56 ± 0.25
30 min SD exposure	1:250/30 × 24 × 1 ml/2.88 ml	0/720	≤ 0.18	≥ 5.75 ± 0.25

Virus stock control: virus titre of the undiluted virus stock at 0 min and 30 min process time. Virus-spiked intermediate control: titre of a 1 in 20 virus-spiked intermediate (without SD) at 0 min (represents the initial virus titre for calculating the reduction factors) and 30 min process time. Termination control: virus spiked 1 in 20 into process intermediate with 1:250 diluted SD.

TCID₅₀/ml. Furthermore, the termination control confirmed that the 1:250 dilutions were more than sufficient to terminate the inactivation of SARS-CoV by SD (titre difference < 0.5 \log_{10} TCID₅₀/ml). In a cytotoxicity and interference pre-study, this dilution was found to be non-cytotoxic and did not interfere with the SARS-CoV/Vero cells system (titre difference < 0.5 \log_{10} TCID₅₀/ml).

4. Discussion

The SARS outbreak seems to have been halted for the present time, indicating that the stringent control measures taken to prevent person-to-person transmission were effective. Nevertheless, it remains important to assess the possible risk for other modes of spread, for example via blood transfusion or application of therapeutic plasma products such as immunoglobulins. In general, the level and duration of viraemia and viral loads in plasma pools are critical parameters for risk assessment from emerging viruses. The risk of SARS-CoV transmission via blood or therapeutic plasma products seems remote, particularly because SARS-CoV viraemia does not seem to reach long-lasting high titres [16,21]. In order to reduce the potential risk of SARS-CoV transmission by transfusion of cellular blood components, screening of blood donors by nucleic acid techniques (NAT), e.g. PCR, might be useful [21]. Furthermore, it is valuable to obtain information about the anti-SARS-CoV efficacy of virus inactivation steps like the SD procedure, which is widely used by many companies world-wide in the manufacture of therapeutic plasma-derived products including immunoglobulins. The acceptance of such pharmaceutical products does not rely on their therapeutic benefit alone, but to a large extent on

their documented viral safety [13]. The SD method irreversibly disrupts the lipid coat of enveloped viruses and its associated binding sites under mild process conditions. The efficacy of the SD method has been extensively validated with human and model viruses, as well as by animal studies under both standard and robustness conditions of the product-specific manufacturing processes [1,9,17]. Due to the non-selective mode of action, the SD method is capable of inactivating not only the viruses of most concern, e.g. HIV, HBV and HCV, but also those viruses not tested for during routine screening at the blood collection centres, e.g. human T-lymphotropic virus (HTLV)-1 + 2, HGV and CMV.

The safety margins of plasma-derived medicinal products are validated utilising down-scaled models of their respective manufacturing processes in order to investigate the inactivation capacity of a broad range of physicochemically diverse model viruses. In the case of SARS-CoV, several facts argued against its relevance in terms of plasma product safety. Firstly, as already mentioned, the reported SARS-CoV titres in plasma are low. Secondly, the extensive number of validation studies using other enveloped viruses indicates that any other enveloped virus, such as SARS-CoV, would become properly inactivated as well. On the other hand it could be shown that SARS-CoV has some differences in behaviour towards physicochemically noxa in comparison to other known human coronaviruses [18], while it is easily destroyed by common disinfectants [19]. Thus, it is prudent to perform validation studies in order to confirm the safety hypothesis.

In our investigation, the SD treatment of OCTAGAM process intermediates resulted in a very rapid inactivation to below the detection limit of the SARS-CoV. Compared to routine manufacturing of OCTAGAM, the

conditions under which SD treatment was performed were challenged significantly. The inactivation time was reduced by more than 95%, whereas the concentration of SD reagents was decreased to only 75% of production conditions. Despite the worsened process conditions and the improved level of detection, infectious SARS-CoV could not be found after 1 min of SD treatment, demonstrating the robustness of virus inactivation by this technology.

Taken into account the starting virus titre, the reduction factor was $\geq 5.75 \pm 0.25 \log_{10} \text{TCID}_{50}/\text{ml}$. Our current investigation confirmed the predicted behaviour of SARS-CoV, when treated with the SD under the described conditions, and corroborated earlier findings [2,10]. This is not surprising, as the SD method confirmed to be an effective technology used for the inactivation of enveloped viruses [2].

In conclusion, the results obtained in our investigation demonstrated that the process conditions specified for the SD treatment of OCTAGAM are very sufficient to inactivate enveloped viruses such as SARS-CoV to below the limit of detection. The safety margin is very high, as the detection limit was already reached within 1 min of SD exposure even at a significantly reduced concentration of TNBP and Octoxynol. Furthermore, with such a convincing inactivation capacity and kinetic at extreme process conditions, the efficacy of the standard SD treatment used for OCTAGAM to inactivate SARS-CoV must be even higher than the one demonstrated by our study, even if the virus load were higher than the one simulated in this study.

References

- [1] Biesert L, Gärtner T, Lemon S, Horowitz B, Suhartono S, Wang L, et al. Octaplas: eine virusinaktivierte Alternative zu gefrorenem Frischplasma. *Infusther Transfusmed* 1995;22 (Suppl. 1):2–4.
- [2] Biesert L. Virus validation studies of immunoglobulin preparations. *Clin Exp Rheumatol* 1996;14(Suppl. 15):47–52.
- [3] Bundesgesundheitsamt und Paul-Ehrlich-Institut, Bundesamt für Sera und Impfstoffe. Bekanntmachung über die Zulassung von Arzneimitteln. Anforderungen an Virusvalidierungsstudien zum Nachweis der Virussicherheit von Arzneimitteln aus menschlichem Blut oder Plasma vom 20. Dezember 1993/21. Januar 1994; *BAnz.* vom 04.05.1995;4742–4.
- [4] Centers for Disease Control and Prevention (CDC). Update: outbreak of severe acute respiratory syndrome – worldwide, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:241–6, 248.
- [5] Committee for Proprietary Medicinal Products (CPMP). Note for guidance on plasma derived medicinal products. The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit 1996; *CPMP/BWP/269/95*; 1–17.
- [6] Committee for Proprietary Medicinal Products (CPMP). Note for guidance on virus validation studies; the design, contribution and interpretation of studies validating the inactivation and removal of viruses. The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit 1996; *CPMP/BWP/268/95*; 1–13.
- [7] Drosten C, Günther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003; 348:1967–76.
- [8] Drosten C, Preiser W, Günther S, Schmitz H, Doerr HW. Severe acute respiratory syndrome: identification of the etiological agent. *Trends Mol Med* 2003;9:325–7.
- [9] Horowitz B, Bonomo R, Prince AM, Sing NC, Brotman B, Shulman RW. Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma. *Blood* 1992;79:826–31.
- [10] Horowitz B, Lazo A, Grossberg H, et al. Virus inactivation by solvent/detergent treatment and the manufacture of SD-plasma. *Vox Sang* 1998;74(Suppl. 1):203.
- [11] Horowitz B, Wiebe ME, Lippin A, Stryker MH. Inactivation of viruses in labile blood derivatives. I. Disruption of lipid-enveloped viruses by tri(*n*-butyl)phosphate detergent combination. *Transfusion* 1985;25:516–22.
- [12] Kärber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* 1931;152:380.
- [13] Kreil T, Berting A, Kistner O, Kindermann J. West Nile virus and the safety of plasma derivatives: verification of high safety margins, and the validity of predictions based on model virus data. *Transfusion* 2003;43:1023–8.
- [14] Liang W, Zhu Z, Guo J, Liu Z, He X, Zhou W, et al. for the Beijing Joint SARS Expert Group (2003). Severe acute respiratory syndrome, Beijing, 2003. *Emerg Infect Dis* 2004;10:25–31.
- [15] Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, et al. The genome sequence of the SARS-associated coronavirus. *Science* 2003;300:1399–404.
- [16] Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 2003;361:1767–72.
- [17] Piet MPJ, Chin S, Prince AM, Brotman B, Cundell AM, Horowitz B. The use of tri(*n*-butyl)phosphate detergent mixtures to inactivate hepatitis viruses and human immunodeficiency virus in plasma and plasma's subsequent fractionation. *Transfusion* 1990;30:591–8.
- [18] Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS-associated Coronavirus (SARS-CoV). *J Med Microbiol Immunol* 2005;194:1–6.
- [19] Rabenau HF, Kampf G, Bauer G, Doerr HW. Inactivation of SARS-coronavirus (SARS-CoV). *Hospital Infection* 2005, in press.
- [20] Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 2003;300:1394–9.
- [21] Schmidt M, Brixner V, Ruster B, Hourfar MK, Drosten C, Preiser W, et al. NAT screening of blood donors for severe acute respiratory syndrome coronavirus can potentially prevent transfusion associated transmissions. *Transfusion* 2004;44:470–5.
- [22] Seto WH, Tsang D, Yung RW, Ching TY, Ng TK, Ho M, et al. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). *Lancet* 2003;361:1519–20.
- [23] Spearman C. The method of "right or wrong cases" (constant stimuli) without Gauss's formulae. *Br J Psychol* 1908;2:227.
- [24] WHO environmental health team. Report on Amoy Gardens. World Health Organization Regional Office for the Western Pacific; 2003. Available at: <<http://www.info.gov.hk/info/ap/who-amoye.pdf>> [accessed on 2 December 2003].
- [25] Wu J, Xu F, Zhou W, Feikin DR, Lin CY, He X, et al. Risk factors for SARS among persons without known contact with SARS patients, Beijing, China. *Emerg Infect Dis* 2004; 10:210–6.