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研究報告の概要	使用上の注意記載状況・その他参考事項等
ヒトパルボウイルス B19（B19V）の感染性アッセイが可能となる以前は，モデルウイルスと呼ばれる動物パルボウイルス(例：マウスパルボウイルス)が，血漿タンパクの製造工程におけるウイルス不活化の確認に使用されており，一般的にパルボウイルスは熱不活化に耐性があると考えられていた。しかし，最近の知見より，B19V は動物パルボウイルスよりも熱に弱いことが明らかになってきた。 本文献は，数種の血液凝固因子製剤において STIM-4 蒸気加熱処理装置を用いた不活化処理を行い，B19V とモデルウイルスとして用いられていたマウス微小ウイルス（MMV）間での不活化効果の比較を行っている。 血液凝固因子製剤の中間体の種類に関わらず，試験に用いた B19V（遺伝子 1 型，2 型）はいずれも動物パルボウイルスと比較して，STIM-4 蒸気加熱処理工程によって効果的に不活化された（Log 減少ファクター，3.5～4.8）。これより，蒸気加熱処理による B19V の効果的な不活化が示唆され，B19V に対する STIM-4 蒸気過熱処理を行った血液凝固因子製剤の安全性が高まると考えられた。	BYL-2008-0320

報告企業の意見	今後の対応
加熱処理によるウイルス不活化の程度は各製剤によって左右されるため，製剤毎に確認する必要があると考える。 弊社のポリグロビン N の製造に使用されているプール血漿においては，B19V に対する NAT を実施し，10E5 IU/mL 以上が確認された場合は，そのプール血漿を製造工程から除去している。感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	今後とも利用可能な B19V の検出方法の改善に関する情報収集に努める。

⑦

Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates

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BACKGROUND: To enhance the viral safety margins, nanofiltration has been widely integrated into the manufacturing process of plasma-derived medicinal products. Removal of smaller agents such as parvovirus B19 (B19V) by filtration, however, is typically less efficient. Because recent investigations have demonstrated that B19V may be more heat sensitive than animal parvoviruses, the potential B19V inactivation by a proprietary vapor heating procedure (STIM-4) as incorporated into the manufacturing processes of several nanofiltered coagulation factor concentrates was investigated.

STUDY DESIGN AND METHODS: An infectivity assay based on quantitative reverse transcription–polymerase chain reaction (TaqMan, Applied Biosystems) detection of B19V mRNA after inoculation of a permissive cell line (UT7 Epo S1 cells) was used to investigate the virus inactivation capacity of the STIM-4 vapor heat treatment as used during the manufacture of nanofiltered second-generation Factor VIII inhibitor-bypassing activity (FEIBA), F IX complex, and FVII products.

RESULTS: In contrast to animal parvoviruses, both B19V genotypes investigated, that is, 1 and 2, were shown to be surprisingly effectively inactivated by the STIM-4 vapor heat treatment process, with mean log reduction factors of 3.5 to 4.8, irrespective of the product intermediate tested.

CONCLUSION: The newly demonstrated effective inactivation of B19V by vapor heating, in contrast to the earlier used animal parvoviruses, results in significant B19V safety margins for STIM-4–treated coagulation factor concentrates.

To further enhance the safety margins of plasma-derived medicinal products against any residual virus safety concerns, manufacturers have continuously sought to implement dedicated virus reduction steps into the manufacturing processes of these products. Once robustly established and widely available, nanofiltration has frequently been considered an option for this purpose (for review see Burnouf and Radosevich[1]).

In selecting the appropriate pore sizes of these filters, commercially available between 15 and 75 nm, a delicate balance needs to be struck between maintaining an appropriate yield of the respective product intermediate while effectively removing viruses. Especially for larger-molecular-weight protein preparations the removal of smaller viruses has thus been difficult,[2] unless virus antibodies present in the intermediate increased the effective filtration size of a virus by formation of virus–antibody complexes,[3,4] or specific product formulations contributed to virus removal by inducing virus aggregation.[5] Particularly parvovirus B19 (B19V), currently the only known parvovirus associated with significant pathogenicity for humans, can thus often not be efficiently removed from larger-molecular-weight biologic entities of medicinal importance by these procedures.

B19V contaminates human blood or plasma donations, at reported frequencies of 1 in 800-5950[6,7] and levels

ABBREVIATIONS: B19V = parvovirus B19; FEIBA = Factor VIII inhibitor-bypassing activity; LRF(s) = log reduction factor(s); MMV = mice minute virus; NF/VH = nanofiltered and vapor heat treated.

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of viremia up to 10^{12} genome equivalents (geq) per mL.[6,8] Polymerase chain reaction (PCR) testing of the plasma supply has thus become state-of-the-art, and use of the technology has reduced the mean B19V load of plasma manufacturing pools by many orders of magnitude. Given the wide prevalence of B19V, however, supply considerations have prevented eliminating the virus from plasma by PCR testing, and thus virus reduction during the manufacturing process remains the critical safeguard of final product safety also in this instance.

Before an infectivity assay for B19V itself was available, animal parvoviruses, for example, porcine, murine, bovine, etc., parvoviruses, were used as so-called "model viruses"[9] in studies validating the virus reduction capacity of the manufacturing processes of plasma proteins. Based on the very high physicochemical resistance of these animal parvovirus models, virus inactivation procedures incorporated into these processes were considered less effective against parvoviruses.[10] More recently, however, initial data obtained with a novel infectivity assay for B19V itself indicated that the actual virus of concern for humans is much more heat sensitive than the animal parvoviruses used for earlier validation studies.[11-13]

Adding to the complexity, several more recently discovered human parvoviruses, for example, V9[14] and A6,[15] have now been reclassified to taxonomically represent B19V genotypes rather than distinct parvovirus species.[16]. Little is known, however, about the biologic properties of these newer B19Vs, for example, the sensitivity of these to inactivation.[17]

In this study the B19V reduction capacity of a proprietary and dedicated virus inactivation step was investigated, that is, the STIM-4 vapor heat treatment, in direct comparison to mice minute virus (MMV), an earlier used animal parvovirus model. The procedure was investigated with intermediates of several different coagulation factor concentrates that had been upgraded with respect to virus safety margins by implementation of nanofiltration during their manufacture, that is, Factor (F)VIII inhibitor-bypassing activity–nanofiltered and vapor heat treated (FEIBA NF/VH), F IX complex NF/VH, and FVII NF/VH.

MATERIALS AND METHODS

Viruses, cells, and infectivity assay

As a source of B19V, highly viremic plasma donations (990237, Genotype 1, 11.8 log geq/mL; IM81, Genotype 2, 11.4 log IU/mL) as identified by the routine plasma screening procedure of Baxter Bioscience were used. B19V were titrated on UT7 Epo S1 cells (provided by Dr Kevin E. Brown, Virus Reference Department, Center for Infections, Health Protection Agency, London, UK; with permission from Dr Kazuo Sugamura, Department of Microbiology and Immunology, Tohoku University, Graduate School of Medicine, Tohoku, Japan), essentially as earlier described.[18] Briefly, mRNA of infected cells was isolated and quantified by reverse transcription (RT)-PCR with the following procedure. Initially serial 10-fold dilutions of B19V samples of known PCR titer were incubated with UT-7 cells, and the B19V mRNA analyzed by RT-PCR. A regression line of the samples' known PCR titers versus the number of RT-PCR cycles required to obtain a positive signal for the same sample was then plotted to form a calibration curve. With this calibration curve, the PCR titer of any unknown sample was back-calculated from the mRNA RT-PCR titer obtained after incubation with susceptible cells. Typically, several 10-fold dilutions of unknown samples were analyzed by RT-PCR, to ensure that one or several of the results would lie on the linear part of the calibration curve. Whenever more than one result fitted onto the calibration curve, means were calculated for the PCR titer. The limit of detection was 3.7 log per mL,[18] and standard errors of means for multiple measurements were always not more than 0.5 log.

MMV, strain prototype (ATCC VR-1346, American Type Culture Collection, Rockville, MD) was propagated and titrated on A9 cells (ATCC CCL-1.4). Samples containing MMV were titrated by $TCID_{50}$ assay, that is, eightfold replicates of serial half-log sample dilutions were incubated with cells for 7 days before evaluation for a cytopathic effect. MMV concentrations were calculated according to the Poisson distribution and expressed as log $TCID_{50}$ per mL.

RT-PCR

For detection of B19V Genotype 1, primers sets for two mRNA splicing variants (splicing at nucleotide 1910 or 2030, Accession Number M13178[19]) were used (PA3 or PA4, respectively): PA3—primers PA3F (positions 365-386), PA3R (positions 1957-1978), and the fluorescent probe PA3P (5′-6-FAM-TTTGTGAGCTAACTAACAGATGCCCTCC ACCCAGAC-TAMRA-3′); and PA4—primers PA4F (positions 367-389), PA4R (2080-2102), and the fluorescent probe PA4P (5′-6-VIC-TGAGCTAACTAACAGGCGCCTGG AACA-TAMRA-3′).

For detection of B19V Genotype 2 (Accession Number AY903437[17]), the primer set G2 was used, G2-F (positions 369-391), G2-R (positions 1962-1983), and the fluorescent probe G2-P (5′-6-FAM-TTTGCCTGCTAATTAACAGATGCC CTCCACCCAGAC-3′).

Downscaled manufacturing processes for plasma derivatives

Downscaled versions of the manufacturing processes examined were established and the equivalence of critical product and process parameters to the respective manufacturing-scale processes established. Temperature is a critical process measure for virus inactivation and was

therefore monitored throughout all the processes investigated. Starting materials were process intermediates obtained from the manufacturing scale, which were spiked 1 in 10 with virus stock suspensions. Immediately after spiking, samples were drawn and titrated to confirm the amount of virus added. Further samples were collected and titrated at predetermined points throughout and at the end of the inactivation processes. MMV-spiked samples were directly titrated on A9 cells, whereas B19V-spiked samples were titrated on UT7 cells followed by mRNA isolation as described earlier.[18] Specific unspiked process intermediates were obtained from control procedures and tested for their potential cytotoxicity for the indicator cell line and for their potential interference with the detection of low virus titers. Virus reduction factors for the manufacturing processes examined were calculated in accordance with Committee for Proprietary Medicinal Products guidance.[9]

During their manufacture, the investigated products (all from Baxter BioScience, Zurich, Switzerland), that is, FEIBA NF, F IX complex NF (PPK NF), or together with FVII NF (prothrombin complex NF, PKT NF), are subjected to the STIM-4 vapor heating process. Specifically, a lyophilized intermediate of 7 to 8 percent residual moisture is heat treated for a minimum of 500 minutes at 60 ± 0.5°C, followed by heating to 80 ± 0.5°C, and then heating at 80 ± 0.5°C for 60 minutes. The downscaled versions of these processes were performed at the lower limits of these temperature and incubation time specifications or just below those specified for the manufacturing-scale process. To provide further assurance regarding the robustness of the virus inactivation by these processes, separate runs were performed at the upper and lower limits of the residual moisture content specified for manufacture; or runs were performed within these specifications.

Determination of the residual moisture was performed by the Karl Fischer method for non–virus-spiked control samples. The residual moisture content for all samples, including those containing virus, was confirmed by NIRVIS spectroscopy (System NIRVIS, Büchi Ltd, Flawil, Switzerland).

Product intermediates of 14.4 to 33 g per L protein concentration, 5 to 6 g per L salt concentration, and pH 7.0 to 8.0 were spiked with virus, lyophilized, and then heat-treated according to the procedure described above. Specific product measures, e.g., FEIBA (clotting assay), FII activity (clotting assay), FX activity (chromogenic assay), FVII activity (chromogenic assay), F IX activity (chromogenic assay), and protein concentration were determined for the downscale intermediate before and after the vapor heating process. The results were compared with the respective values for intermediates from the manufacturing scale to confirm equivalence of the different scale processes.

RESULTS

Vapor heating of FEIBA NF/VH

FEIBA intermediate was spiked with either B19V or MMV for downscaled vapor heating experiments. For B19V, two different primer sets (PA3, PA4) specifically designed to detect two different B19V VP1/VP2 splicing variants[20] were used for RT-PCR analysis (TaqMan, Applied Biosystems, Foster City, CA).

As can be seen in Table 1, significant inactivation of B19V was observed already after the 60°C heating phase of the process (experimentally conducted at 59.5 ± 0.5°C, i.e., worst case with respect to virus inactivation) with individual log reduction factors (LRFs) of 3.9 to 4.5. At completion of the 80°C heating phase of the process

TABLE 1. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of FEIBA NF/VH process intermediates*

Percent residual moisture content:	MMV†		B19V‡					
	7	8	7-8					
Primer pairs:			Titration 1		Titration 2		Titration 3	
			PA3	PA4	PA3	PA4	PA3	PA4
Virus stock suspension	8.3	8.2	11.2	11.2	11.8	11.8	ND	ND
Spiked process intermediate§	7.2	7.2	10.3	10.4	10.6	10.4	10.6	10.4
Spiked and lyophilized intermediate	6.6	6.7	9.7	9.9	10.0	9.9	9.8	9.8
Heated at 59.5°C ± 0.5°C, 180 ± 5 min	ND	ND	7.8	7.7	7.7	7.6	ND	ND
Heated at 59.5°C ± 0.5°C, 505 ± 5 min	6.7	6.7	6.2	5.9	6.6	6.5	6.5	6.5
Reduction factor (after 60°C phase)	0.5	0.5	4.1	4.5	4.0	3.9	4.1	3.9
Heated at 79.5°C ± 0.5°C, 55 ± 5 min§	6.3	6.3	5.7	5.6	5.5	5.4	5.7	5.8
Reduction factor	0.9	0.9	4.6	4.8	5.1	5.0	4.9	4.6
Mean reduction factor	0.9				4.8			

* For the detection of spliced B19V mRNA two different primer sets, i.e., PA3 and PA4, were used.
† MMV titers are expressed as [log($TCID_{50}$/mL)].
‡ B19V titers are expressed as [log geq/mL].
§ Titers at this sampling stage were used to calculate the virus reduction factor after the entire heating phases.
ND = not determined.

(experimentally conducted at 79.5 ± 0.5°C), some residual B19V infectivity was still detectable, although LRFs of 4.6 to 5.1 were obtained, with a mean LRF of 4.8. Because use of primer sets PA3 and PA4 resulted in fully equivalent results, only one of the primer sets (PA3) was used for the detection of B19V Genotype 1 mRNA in further experiments.

In contrast to the effective inactivation of B19V by the vapor heating process, the inactivation observed for the animal parvovirus MMV was insignificant, with a mean LRF of 0.9.

Vapor heating of F IX complex NF/VH

To investigate the vapor heating process of F IX complex, the respective intermediate was spiked with either B19V or MMV. Because residual moisture during the vapor heating process might be considered a critical parameter for the effectiveness of virus inactivation, separate vapor heating runs were performed at the upper and lower limit of the specified residual moisture content of the manufacturing process, that is, 7 and 8 percent (wt/wt), to investigate the robustness of virus inactivation by the process. For B19V-spiked runs, some residual infectivity was still detected after the entire heating process, but the results obtained demonstrate comparable reduction factors for runs at either 7 or 8 percent residual moisture content with a mean LRF of 4.6 (Table 2). A substantial inactivation of B19V was observed already after the 60°C heating phase of the process (investigated at 59.5 ± 0.5°C) with individual LRFs of 3.7 to 4.2. As the reduction factors obtained between the individual titrations at 7 and 8 percent residual moisture content were comparable, consequently, the following vapor heating experiments were performed at between 7 and 8 percent residual moisture content, that is, within the specifications of the large-scale process.

MMV, again in sharp contrast to the effective inactivation of B19V by the vapor heating process, was not significantly inactivated even at the end of the entire heating process, with a mean LRF of 0.9. As seen with B19V before, there were again no differences between MMV inactivation results for individual heating runs conducted at 7 and 8 percent residual moisture.

Vapor heating of FVII NF/VH

F IX complex and FVII are separately produced and are individual products. Both components can, however, also be combined to the prothrombin complex total product. Because FVII is the second component of prothrombin complex total, the B19V and MMV inactivation by STIM-4 vapor heating was also investigated.

At the end of the entire heating phase, effective inactivation of B19V was observed, with a mean LRF of greater than 4.0 (Table 3). Also, substantial inactivation of B19V was found already after the 60°C heating phase (investigated at 59.5 ± 0.5°C) of the process (LRFs of 4.0 and 4.5), confirming earlier findings for the other prothrombin complex total compound. Again in sharp contrast to effective B19V inactivation, the parvovirus model MMV was inactivated only ineffectively, with a mean LRF of 1.7.

STIM-4 inactivation of B19V Genotype 1 versus Genotype 2: FEIBA, for example

To understand any potentially different thermosensitivity of the recently classified B19V Genotype 2, versus the earlier investigated B19V Genotype 1, FEIBA intermediate was now spiked with B19V Genotype 2 and treated as described earlier (see "Vapor heating of FEIBA NF/VH"). mRNAs isolated after culture with UT-7 cells were analyzed by TaqMan RT-PCR with either, as before, Genotype 1 primer sets (PA3), or now also specific Genotype 2 (G2) primer sets.

TABLE 2. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of F IX complex NF/VH intermediate

	MMV*		B19V†			
Percent residual moisture content:	7	8	7		8	
			Titration 1	Titration 2	Titration 1	Titration 2
Virus stock suspension:	7.4	7.5	ND	ND	ND	ND
Spiked process intermediate‡	6.5	6.7	10.7	10.6	11.0	11.0
Spiked and lyophilized intermediate	6.5	6.4	10.8	10.5	10.1	10.4
Heated at 59.5°C ± 0.5°C, 495 ± 5 min	6.1	6.2	6.9	6.9	7.1	6.8
Reduction factor (after 60°C phase)	0.4	0.5	3.8	3.7	3.9	4.2
Heated at 79.5°C ± 0.5°C, 55 ± 5 min‡	5.8	5.6	6.4	6.6	5.8	6.3
Reduction factor	0.7	1.0	4.3	4.1	5.2	4.7
Mean reduction factor (log)	0.9		4.6			

* MMV titers are expressed as [log($TCID_{50}$/mL)].
† B19V titers are expressed as [log geq/mL].
‡ Titers at this sampling stage were used to calculate the virus reduction factor after the entire heating phases.
ND = not determined.

TABLE 3. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of FVII NF/VH intermediate

Percent residual moisture content:	MMV*		B19V†			
	7	8	7-8			
			Run 1		Run 2	
			Titration 1	Titration 2	Titration 1	Titration 2
Virus stock suspension:	7.7	8.1	12.3	ND	ND	ND
Spiked process intermediate‡	6.5	7.2	11.0	10.6	10.8	10.8
Spiked and lyophilized intermediate	6.9	7.0	ND	9.9	9.9	10.1
Heated at 59.5°C ± 0.5°C, 525 ± 5 min	5.3	6.1	6.5	6.6	positive§	positive§
Reduction factor (after 60°C phase)	1.2	1.1	4.5	4.0	ND	ND
Heated at 79.5°C ± 0.5°C, 55 ± 5 min‡	5.2	5.2	<6.8	<6.8	<6.8	<6.8
Reduction factor	1.3	2.0	>4.1	>3.8	>4.0	>4.0
Mean reduction factor	1.7		> 4.0			

* MMV titers are expressed as [log($TCID_{50}$/mL)].
† B19V titers are expressed as [log genome equivalents/mL].
‡ Titers at this sampling stage were used to calculate the virus reduction factor.
§ These samples were tested positive; a titer could, however, not be calculated, because the PCR cycle numbers necessary to obtain a positive fluorescence signal were outside the range covered by the mean regression line.
ND = not determined.

TABLE 4. B19V Genotype 2 inactivation by vapor heat treatment during the manufacture of FEIBA NF/VH*

Percent residual moisture content:	B19V GT 2			
	7-8			
	Run 1	Run 2	Run 1	Run 2
	PA3	PA3	G2	G2
Reduction factor†	>3	4	>3	>4
Mean reduction factor	3.5			

* The TaqMan RT-PCR was performed with either Genotype 1 (PA3)- or Genotype 2 (G2)-specific primer sets.
† Reduction factor determined after the entire vapor heating procedure. The goodness-of-fit values of the standard regression lines were less than optimal resulting in a high standard deviation at lower virus titers. Therefore, reduction factors were determined by the difference in integer log sample dilutions between the spiked intermediate and the sample after completion of vapor heating.

As can be seen, use of both the two primer sets (PA3/PA4) designed for two Genotype 1 splicing variants revealed highly comparable results (Table 1). In addition insignificant differences between B19V Genotype 1 and Genotype 2 occurred, with both viruses effectively inactivated by the STIM-4 vapor heating process (Tables 1 and 4). Specifically, here for B19V Genotype 2, effective inactivation was observed at the end of the heating process, with calculated LRFs between greater than 3 and greater than 4, that is, a mean LRF of 3.5. Using the Genotype 1 (PA3)- or the Genotype 2 (G2)-specific primer sets, highly comparable inactivation results were obtained.

DISCUSSION

Since its discovery in 1975,[21] B19V has been associated with an ever-broadening panel of diseases. While initially only known as the causative agent of an erythematous childhood disease (fifth disease), more recently the virus is appreciated as the causative agent of more severe diseases such as, for example, hydrops fetalis,[22] arthritis,[23] hepatitis,[22] and possibly a significant number of myocarditis cases.[24]

The introduction of B19V PCR testing of plasma for fractionation, as initially defined under the Plasma Protein Therapeutics Association's voluntary standards,[25] has reduced plasma pool loads of the virus by several orders of magnitude[26] and correspondingly enhanced the B19V safety margins of plasma products. In support of the notion, while episodes of B19V transmissions have historically occurred,[27] such reports have not been received for the implicated products after the introduction of B19V PCR testing. There is, however, still a residual concern around the potential B19V contamination of plasma.

The final safeguard of product safety, that is, the virus reduction that occurs during the manufacturing process, has thus been of particular interest, also with respect to B19V. Unfortunately though, the lack of a widely available B19V infectivity assay has forced studies aimed at validating the B19V reduction capacity of manufacturing processes to be conducted with animal parvoviruses as "model viruses." Where these viruses are particularly resistant to physicochemical inactivation, the results obtained were often less reassuring.

There was consequently significant interest in a suitable B19V assay to investigate the virus of concern itself, and development efforts were lately rewarded. Initial use of the newly available approaches revealed that B19V itself was significantly more susceptible to inactivation by, for example, pasteurization,[11] low pH,[28] and dry heat,[13] than the earlier used animal parvovirus models. Also, however, research conducted with these assays has indicated that B19V has unique properties in terms of heat sensitivity

and its inactivation is particularly dependent on the composition of the matrices during (liquid) heating.[29]

The proprietary STIM-4 vapor heating process is a heat treatment step conducted at lyophilized product of 7 to 8 percent residual moisture that has been incorporated in the manufacturing process of several coagulation factor concentrates, in addition to a 35-nm nanofiltration step for FEIBA NF/VH, F IX complex NF/VH, and FVII NF/VH. Here we describe the efficient inactivation of B19V, in marked contrast to an animal parvovirus model, that is, MMV, by this vapor heat treatment.

The results of the two-phase vapor heat treatment demonstrate that B19V is effectively inactivated by this process step, whereas MMV is only marginally reduced. By use of both the two primer sets (PA3/PA4) designed to detect two Genotype 1 VP1/VP2 splicing variants highly comparable results were obtained (Table 1), indicating that the splicing variants in infected UT7-Epo S1 cells occur in rather similar concentrations.

After the entire heating process for B19V Genotype 1 mean log reduction factors of 4.8, 4.6, and more than 4.0 were obtained with highly comparable results for the panel of coagulation factor intermediates investigated, that is, FEIBA, F IX complex and FVII (see Tables 1-3). These findings support the robustness of the STIM-4 vapor heat treatment in inactivating B19V. Moreover, the significant inactivation of B19V already after the first heating phase at 60°C for the coagulation factors investigated (see Tables 1-3) provides further reassurance. In addition, varying the residual moisture content during the heat treatment, that is, to the lower (7%) and the upper limit (8%) specified for the manufacturing process, still resulted in highly comparable inactivation of B19V (Table 2).

The discovery of additional human erythrovirus genotypes, that is, Genotypes 1, 2, and 3,[16] and also novel parvoviruses,[30,31] has raised new questions about their biologic properties, their pathogenic potential and also their relevance to the viral safety of plasma-derived products. B19V Genotype 2 has been detected in human blood at high titers, and recently this genotype has also been found in a few lots of plasma-derived coagulation factor concentrates; all of them, however, were cocontaminated with Genotype 1 DNA.[32] These findings indicate that Genotype 2 has established a moderate prevalence in the population and therefore investigations regarding the inactivation of this B19V genotype may also be desirable. Recent studies that investigated the inactivation capacity of liquid heating and low pH[17] incubation for B19V Genotypes 1 and 2 in parallel suggested comparable inactivation capacity and kinetics and thus maybe also comparable physicochemical properties for the virus particles of both genotypes.[17]

In this report, the physicochemical stability of virus particles of Genotype 1 and 2 were compared during the vapor heat treatment process step described. The results demonstrate that both B19V genotypes are inactivated with comparable mean LRFs of 4.8 and 3.5 (Tables 1 and 4). These data indicate that B19V Genotype 1 and 2 particles have very similar physicochemical properties and thus data obtained in studies with Genotype 1 should also be indicative for Genotype 2 behavior. This argument is strengthened by very recent findings that anti-B19V–positive plasma samples or intravenous immune globulin product were able to neutralize B19V Genotype 1 and Genotype 2.[18] Furthermore, studies by Ekman and colleagues[33] suggest that all three B19V genotypes are similar variants of the same species and constitute a single serotype. Not surprising in this context, the pathogenic potential of different B19V genotypes also seems to be comparable.[14]

Altogether the results of this study demonstrate that the STIM-4 vapor heat treatment is a highly effective and robust virus inactivation step for the relevant parvovirus B19, both Genotype 1 and Genotype 2. Specifically, the STIM-4 vapor heat treatment process substantially contributes to the safety margins of the plasma-derived products FEIBA NF/VH, F IX complex NF/VH, and FVII NF/VH. As suggested by a recent article,[29] however, the inactivation capacity of heat treatment may significantly depend on the specific matrix investigated, and thus B19V inactivation needs to be confirmed for every specific product and process individually.

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別紙様式第2-1

No. 12

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 5. 26	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)		Pettersson L, Boman J, Juto P, Evander M, Ahlm C. Emerg Infect Dis. 2008 May;14(5):808-10.	スウェーデン

研究報告の概要

○スウェーデンのPuumalaウイルス感染アウトブレイク
ハンタウイルスの一種Puumalaウイルスは、スウェーデン、フィンランド、ノルウェー、ロシア、中央ヨーロッパの一部に土着しており、自然宿主であるハタネズミの排泄物からヒトに感染する。スウェーデンにおけるPuumalaウイルスの予期せぬ大規模アウトブレイクにより、2007年のVästerbotten地方の流行性腎症患者の数は100,000人当り313人に至った。齧歯類の増加の他、気候温暖化および地表を覆う積雪の減少により、ウイルスを媒介するハタネズミの活動が活発だったことが、当該アウトブレイクの一因であろうと考えられる。

報告企業の意見	今後の対応
スウェーデンにおいてPuumalaウイルスの大規模アウトブレイクが発生し、2007年Västerbotten地方の流行性腎症患者の数は100,000人当り313人に至ったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

二

Outbreak of Puumala Virus Infection, Sweden

Lisa Pettersson,* Jens Boman,*† Per Juto,* Magnus Evander,* and Clas Ahlm*

An unexpected and large outbreak of Puumala virus infection in Sweden resulted in 313 nephropathia epidemica patients/100,000 persons in Västerbotten County during 2007. An increase in the rodent population, milder weather, and less snow cover probably contributed to the outbreak.

Members of the genus *Hantavirus* (family *Bunyaviridae*) are rodent-borne pathogens, and virus is transmitted to humans by inhalation of infected rodent excreta (*1*). In Sweden, Finland, Norway, Russia, and parts of central Europe, Puumala virus (PUUV) is endemic in bank voles (*Myodes glareolus*). PUUV infection in humans cause nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS). In Sweden ≈90% of all NE cases are found in the 4 northernmost counties. Västerbotten County (Figure 1) has the highest incidence of human hantavirus infection in Sweden and probably one of the highest worldwide. Historically, the incidence rate is 20 per 100,000 persons per year (*2*), but the true incidence is considered to be 7–8 times higher (*3*).

There is a 3–4-year periodicity in the number of NE cases that is linked to the bank vole population dynamics in northern Sweden (*2*). After inhaling infectious aerosols originating from rodent saliva, urine, or feces, the patient has a 1–5-week incubation period before onset of disease symptoms. The most common NE symptoms are fever, headache, nausea, abdominal and back pain, vomiting, myalgia, and visual disturbance. One third of the patients have mostly mild hemorrhagic manifestations (*4*,*5*). Renal failure is typical with initial oliguria during the acute phase and polyuria in the convalescence phase. Dialysis is sometimes needed and <0.5% of NE cases are fatal. There is no effective treatment or available vaccine.

The Study

The local University Hospital of Umeå is the reference center for diagnosis of NE serving the 4 northernmost counties of Sweden, and many patients with NE are hospitalized here. In 2007, a sudden and large outbreak of hantavirus infections occurred in northern Sweden. The outbreak peaked in January 2007 (Figure 1) with many NE patients who had a considerable effect on public health services. The NE outbreak continued in the following months, but with fewer cases than in early 2007 (Figure 1).

For NE diagnosis, we used an immunofluorescence assay to detect PUUV-reactive immunoglobulin (Ig) M and IgG antibodies in serum of all patients with clinically suspected NE (*6*). A real-time reverse transcription–PCR (*6*) was used to obtain an amplification product from 1 patient sample. This product was sequenced and the S-segment sequence obtained (GenBank accession no. EU177630) was highly homologous to those of other rodent PUUV isolates from the area.

NE is a reportable disease under the Swedish Communicable Diseases Act. The outbreak peaked during the first 3 months of 2007; 972 cases were recorded in Sweden and 474 cases in Västerbotten County. NE patients mostly showed classic HFRS symptoms and mild to severe disease requiring hospitalization and occasionally intensive care. Accordingly, as many as 30% of the patients whose conditions had been diagnosed as NE were hospitalized, and 2 known deaths (case-fatality rate 0.25%) in the 2 northernmost counties in Sweden were recorded during the first 3 months of 2007. No patient had to continue dialysis after the acute phase of the disease.

We detected PUUV RNA in the milk of 2 breastfeeding women with a diagnosis of NE. Their children did not show any clinical symptoms of NE. However, we did not have access to samples to analyze whether the children had asymptomatic infections. Three pregnant women also had received a diagnosis of NE, but no clinical evidence of transmission from mother to child was reported. Analyses of the placentas did not detect any PUUV RNA. Only maternal IgG antibodies to PUUV were found in blood from umbilical cords. One woman miscarried after 12 weeks of pregnancy 3 weeks before showing symptoms of clinical NE, and death of the fetus may have been caused by viremia during the incubation period. During the peak of the outbreak (December 2006–March 2007), 488 cases occurred in Västerbotten County, and, as expected, more men (58%, 281/488) than women (42%, 207/488) had NE; most cases (72%) were among persons 35–74 years of age (Table).

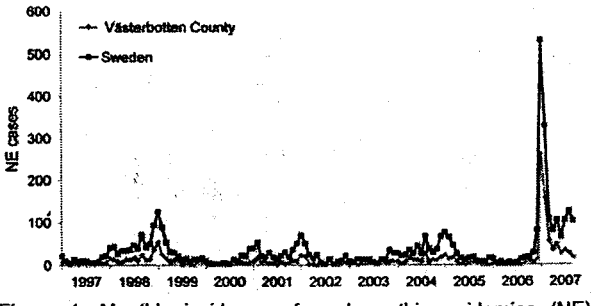

Figure 1. Monthly incidence of nephropathia epidemica (NE) in Sweden and Västerbotten County, Sweden, January 1997–September 2007.

*Umeå University, Umeå, Sweden; and †County Council of Västerbotten, Umeå, Sweden

The incidence of NE in Västerbotten County was 313 diagnosed cases/100,000 persons in 2007 compared with 73/100,000 in 1999, 38/100,000 in 2002, and 61/100,000 in 2005 (Figure 1). The number of NE cases usually depends on the size of the vole population, which peaks every third to fourth year (*2,7*). An increase in the bank vole population was reported in northern Sweden in the fall of 2006, with a trap index of 7.64. This index is similar to those of 2 NE peaks in the fall of 1998–1999 and 2004–2005 when trap indices were ≈8 (*8*). Trapping indices represents the number of voles captured per 100 trapping nights, a reflection of the relative population size on each sampling occasion (*9*). Thus, the bank vole population was high, but not more than in previous peak years and could not explain the high number of NE cases in 2007.

We considered other possible factors influencing hantavirus transmission to humans. One factor is increased exposure of humans to infected rodent excreta. We had received several reports from inhabitants in areas where bank voles normally live that more bank voles were found in traps inside houses than usual. When we investigated the weather conditions during this period, December 2006 was exceptional with respect to the mild weather with no or little snow and hard ice cover in the coastal area of northern Sweden. In Västerbotten County, the average temperature in December was 6.0°C–9.0°C warmer than normal (normally the average temperature in Västerbotten County varies by –4°C along the coast and –13°C in the mountains) The average temperature in Sweden was 4.5°C–9.5°C warmer than normal in December 2006 (Figure 2). The snow cover during winter is important for bank vole survival because bank voles have access to food below the snow and hide from predators and the cold (*10*). During 2 previous NE peak periods (2001–2002 and 2004–2005), the ground was already covered with snow in early winter (Figure 2). For these reasons, during December 2006, when the ground had no snow cover for 25 of 31 days (Figure 2), bank voles may have sought refuge in barns and houses and other buildings, thereby increasing the exposure for the human population at risk. A concurrent epizootic may have occurred among bank voles, which resulted in larger numbers of infectious animals, as shown in previous rodent studies (*11,12*). However, we did not have access to rodents during this period and this hypothesis needs to be studied.

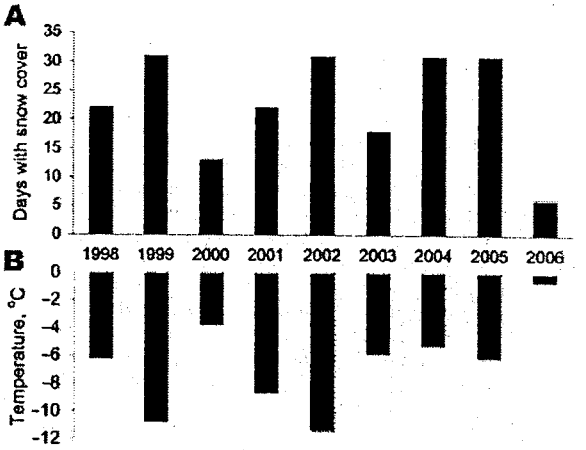

Figure 2. Climate conditions, December 1998–2006, in the nephropathia epidemica outbreak area of Västerbotten County, Sweden. A) Number of days with a snow cover. B) Average temperature. Snow cover was defined as a snow depth >0 cm. Measurements were made in locations ≈30 km from the coast. Data were obtained from the Swedish Meteorological and Hydrological Institute.

Table. Nephropathia epidemica cases in Västerbotten County, Sweden, December 1, 2006–March 31, 2007

Age group, y	No. (%) cases
<1–4	1 (0.20)
5–14	16 (3.3)
15–24	34 (7.0)
25–34	48 (9.8)
35–44	82 (17)
45–54	89 (18)
55–64	103 (21)
65–74	78 (16)
75–84	32 (6.6)
85–94	5 (1.0)
Total	488 (100)

Conclusions

This report shows how a zoonotic disease can suddenly result in an unexpected and large human outbreak. Presently, the numbers of NE cases in northern Sweden are still unusually high. Data indicate that the bank vole population during the fall of 2007 increased to an even higher level and a new outbreak is forecasted (*8*). However, the size of the rodent population is not the only factor that determines the size of a hantavirus epidemic. As shown in this report, climate factors may have contributed to the recent large outbreak in northern Sweden.

This study was supported by the county councils of northern Sweden and the medical faculty of Umeå University.

Dr Pettersson is a clinical virologist working at the Umeå University Hospital. Her major research interest is the biology and epidemiology of hantaviruses.

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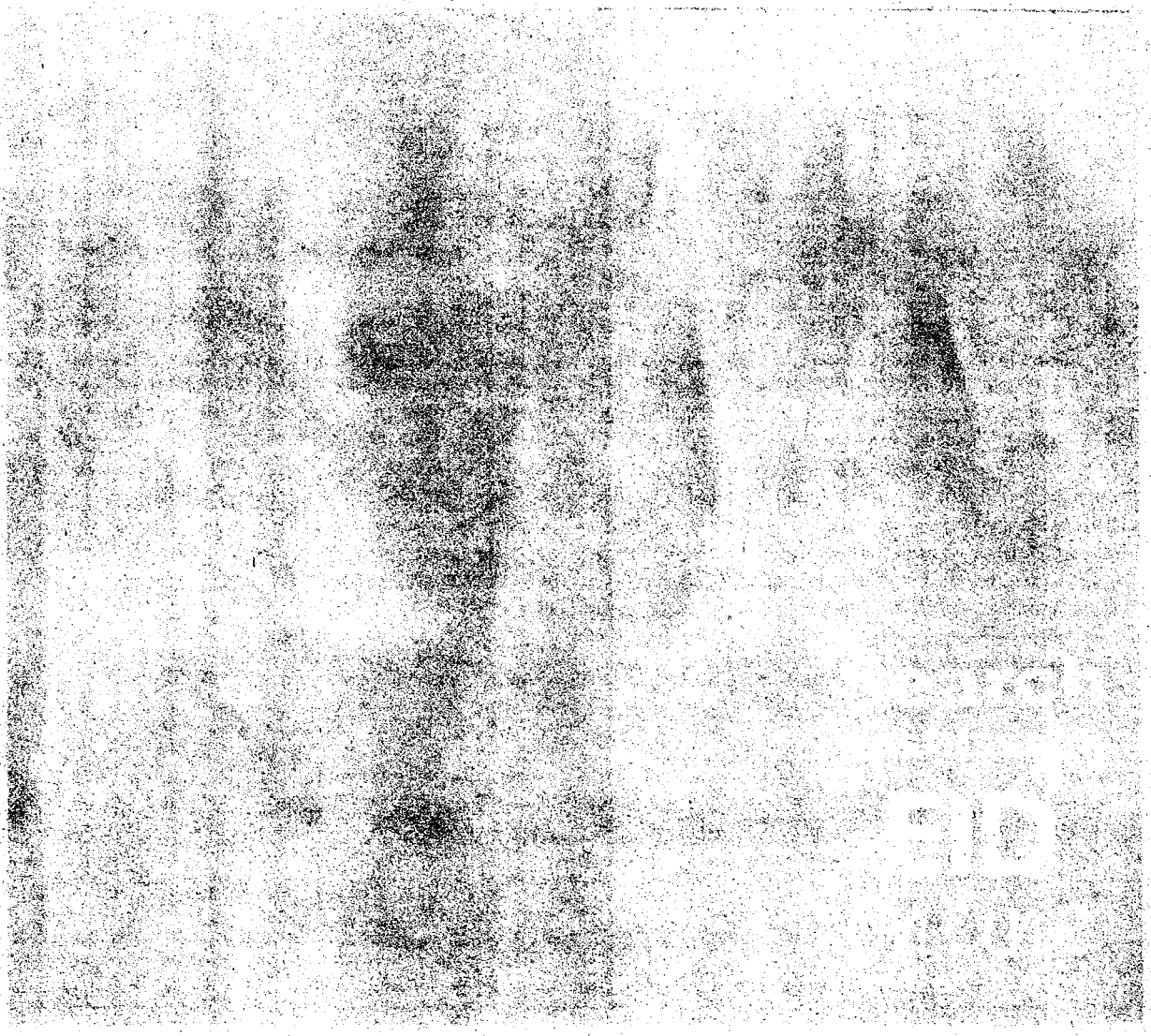

別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.boston.com/news/local/articles/2008/05/13/1_dies_1_ill_after_receiving_kidneys?mode=PF	公表国 米国	
販売名（企業名）	－					

研究報告の概要

ボストン病院で一人の臓器提供者から腎臓移植を受けた 70 歳の女性が死亡し、57 歳の男性が重態となった。
臓器提供者は回復不能な脳障害をこうむった 49 歳のホームレスの男性で、エイズウイルス、B 型肝炎、C 型肝炎などについては検査されていたが、2005 年にマサチューセッツとロードアイランドで 3 人の移植患者が死亡した際と同じ病原体である「リンパ球性脈絡髄膜炎ウイルス (LCMV)」に感染していた。
この LCMV は、一般的にげっ歯類によって伝播し、健康な人々ではインフルエンザ様の症状で特に問題はない。
臓器移植の際には、臓器の組織損傷を防ぐため、エイズウイルス、肝炎、ヘルペスのような容易に検査できる病原体は検査されるが、あまり一般的ではなく、時間のかかる LCMV のような病原体は検査されない。
移植を受けた女性は、家に帰ってすぐ具合が悪くになり (発熱、下痢などの、腎に特有でない症状)、外科手術のおよそ 2 週間後に再入院したが、さらに悪化し死亡した。
もう一例の男性は外科手術の 2.5 週間後に発熱で再入院しており、集中治療室で抗ウイルス薬を投与されている。
CDC において、死亡した臓器提供者と移植を受けた患者 2 人の検体が検査したところ、LCMV 陽性であることがわかり、臓器提供者からの伝播であることが確認された。

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
リンパ球性脈絡髄膜炎ウイルスは、エンベロープを有するアレナウイルス科の RNA ウイルスで、直径 60～280nm の多形性であるので、ウイルス除去膜処理、加熱処理などにより、物理的除去又は不活化されると考えられる。	今後とも、リンパ球性脈絡髄膜炎ウイルスに関する情報に留意していく。

12

boston.com

1 dies, 1 ill after receiving kidneys

The Boston Globe

Donor infected with hard-to-find virus

By Stephen Smith, Globe Staff | May 13, 2008

A 70-year-old woman has died, and a 57-year-old man is critically ill in a Boston hospital after each received a kidney from a donor infected with a hard-to-detect virus, health authorities said yesterday.

The donor, a 49-year-old homeless man who suffered irreversible brain damage after cardiac arrest, carried a germ called lymphocytic choriomeningitis virus, or LCMV, the same infection that killed three transplant patients from Massachusetts and Rhode Island in 2005. The virus, most often transmitted by rodents, is usually unnoticed by healthy people who suffer no more than flulike symptoms.

Knowing that organs perish quickly, doctors test donors for what is easily analyzed, such as the AIDS virus, hepatitis, and a common herpes germ. But the lack of quick tests for less common conditions prevents screening for diseases such as the lymphocytic choriomeningitis virus.

Because the demand for organs always far exceeds the supply, recipients will accept organs even from high-risk donors such as the homeless. Waiting too long for a new kidney, liver, or heart can prove riskier.

"People are literally dying for organs," said Dr. Alfred DeMaria, top disease tracker at the Massachusetts Department of Public Health. "The list of potential things you can test for is enormous. But balancing that against the risk of not getting the organs, you have to make some decisions about what's feasible and what's not feasible to test for."

The homeless donor died in mid-March. After his family authorized the removal of viable organs, doctors took his kidneys. He had been tested for the AIDS virus, the liver diseases hepatitis B and C, and other diseases regularly checked by the New England Organ Bank, the region's organ procurement agency. There was no evidence of worrisome infections.

Still, his status as a man who had lived on the street, potentially exposed to a host of dangerous germs, led transplant surgeons to brand him as a high-risk donor.

Transplant surgeons at the hospitals with the two potential recipients - the woman was at Boston Medical Center, the man at Beth Israel Deaconess Medical Center - alerted the patients that the donor was regarded as high risk. The surgeons and patients decided to proceed.

"We all know that as much as we explain to the patients and inform them, they're relying on us and our medical judgment about whether this is a safe transplant," said Dr. Douglas W. Hanto, chief of the Division of Transplantation at Beth Israel Deaconess. "We feel a tremendous sense of responsibility to the patient and their family and feel terrible that this patient has had this infection and a bad outcome.

"But, on the other hand, we see patients who die every day on dialysis" awaiting a kidney transplant, he said.

The 57-year-old man transplanted at Hanto's hospital had lingered four years on the waiting list for a kidney. According to the United Network for Organ Sharing, an independent agency that sets organ procurement policies, 80,130 patients in the United States currently need a kidney.

It was the woman transplanted at Boston Medical who got sicker sooner after returning home. Like the donor and the other recipient, the woman was not identified by health authorities, who cited patient confidentiality laws.

The woman returned to Boston Medical about two weeks after her surgery, said Dr. Greg Grillone, the hospital's interim chief medical officer. She had a fever, diarrhea, "but oddly, symptoms not specific to the kidney," Grillone said.

Her condition kept deteriorating and, in mid-April, the woman died. Doctors at the hospital were stumped. There was no obvious cause of her precipitous demise.

But it turned out that one of the surgeons involved in the case, Dr. Amitabh Gautam, had been connected to

the 2005 Rhode Island and Massachusetts transplant cases.

He became suspicious that the Boston Medical patient had the same virus and alerted the federal Centers for Disease Control and Prevention. The virus has been known to have spread via transplant only two other times, in Wisconsin and Australia.

"Interestingly, what happened was this doctor had seen this before and thought, 'OK, this is a long shot, but I have seen it before and it can happen,' " Grillone said.

"If you take your car to the auto dealer with some very, very rare problem and you're lucky enough to get the mechanic who saw that same problem three years ago in the same make or model of the car, he might think: 'Oh, I saw this same problem three years ago. it might be the same problem," he said.

The man who had received his kidney at Beth Israel Deaconess returned with a fever 2 1/2 weeks after the surgery. On April 18, the doctors there got word that the Boston Medical patient had died. A transplant specialist at Beth Israel Deaconess also speculated that the virus might be at fault.

Samples from the deceased donor and the two patients were rushed to the CDC in Atlanta. All three tested positive for the virus, and investigators said all evidence points to the donor. The 57-year-old recipient remains in intensive care and is receiving the only drug known to possibly treat the virus.

"I don't believe this ever put the general public at risk," said Dr. Anita Barry, who leads the Boston Public Health Commission's investigation of the infections. "You have to be very, very unlucky to get LCMV from a transplant."

The virus is not transmitted casually from person-to-person; in addition to transplants, the only identified human transmissions have been from mother to fetus. Most people who are exposed catch it from the droppings of rodents, including wild animals and pets.

Because the virus causes few health problems in those who contract it, there has been little incentive to develop a rapid test.

The only tests currently available take time and are not widely available, said Dr. Eileen Farnon, a CDC medical epidemiologist.

"If you had a few days or a week for testing you could do that," Farnon said. "But in general that's not how the organ transplantation business works."

Stephen Smith can be reached at stsmith@globe.com. ■

医薬品　研究報告　調査報告書

<table>
<tr><td colspan="2">識別番号・報告回数</td><td></td><td>報告日</td><td>第一報入手日
2008年5月7日</td><td>新医薬品等の区分
該当なし</td><td rowspan="3">総合機構処理欄</td></tr>
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公表状況</td><td rowspan="2">Infect Genet Evol. 2008 Mar 4. [Epub ahead of print]</td><td>公表国
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<tr><td colspan="2">販売名（企業名）</td><td>別紙のとおり</td><td></td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：コスタリカにおいて、高病原性で新しい血清型に分類されるレプトスピラ株がヒトから分離された。

コスタリカにおいて、地域で流行しているレプトスピラの血清型を同定するため、通院している患者からレプトスピラを分離・解析した。レプトスピラ症の症状を呈して入院していた患者から分離された MAVJ401 株は、ウサギ抗血清パネルで Javanica 血清群型の血清型に対して著しく凝集価が上昇したが、標準的な Cross Agglutinin Absorption Test では血清学的にユニークであった。そのため MAVJ401 株は、Javanica 血清群型の新しい血清型（Arenal と命名）であると推測された。また、MAVJ401 株は、遺伝子学的解析によりラテンアメリカ諸国で多く発生している種である Leptospira santarosai に分類された。同じ地区の重症患者から分離された株も Arenal と同一の血清型であったことから、これが外来の血清型ではなく、この地域に流行する新規の高病原性の血清型であると考えられた。この新しい血清型に分類されるレプトスピラ株は、地域の公衆衛生と家畜衛生に脅威をもたらすおそれがある。</td><td>使用上の注意記載状況・
その他参考事項等

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<tr><td colspan="3">報告企業の意見</td><td colspan="3">今後の対応</td><td></td></tr>
<tr><td colspan="3">別紙のとおり</td><td colspan="3">今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。</td><td></td></tr>
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別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	レプトスピラ症は、病原性レプトスピラ感染に起因する人獣共通の細菌(スピロヘータ)感染症である。レプトスピラは通常、長さ 6～20μm、直径 0.1μm のらせん状の細菌で、病原性レプトスピラと非病原性レプトスピラに大別される。病原性レプトスピラは、げっ歯類をはじめ多くの野生動物や家畜(ウシ、ウマ、ブタ、ヒツジなど)、ペット(イヌ、ネコなど)の腎臓に保菌され、尿中に排出される。ヒトは、保菌動物の尿で汚染された水や土壌から経皮的あるいは経口的に感染する。レプトスピラ症は急性熱性疾患であり、感冒様症状のみで軽快する軽症型から、黄疸、出血、腎障害を伴う重症型(ワイル病)まで多彩な症状を示す。レプトスピラは現在、13 の遺伝種からなり、さらに免疫学的性状により 250 以上の血清型に分類されている。日本におけるレプトスピラ症の患者数は近年激減したが、南西諸島・本土南部地域では他の地域に比べて多く散発している。また世界的に見ると、特に東南アジアや中南米などの亜熱帯、熱帯地域で患者発生が多い。レプトスピラは感染初期にヒトの血液や尿から直接観察される場合があることから、本剤の原料への混入を完全に否定できないと考え、本報告を行った。 仮に、製造原料にレプトスピラが混入していたとしても、弊所で製造している全ての血漿分画製剤の製造工程には、約 0.2μm の「無菌ろ過工程」および、レプトスピラよりも小さいウイルスの除去を目的とした平均孔径 19nm 以下の「ウイルス除去膜ろ過工程」が導入されており、これらの工程により除去されるものと考えられる。更に、これまでに当該製剤によるレプトスピラ感染の報告例は無い。 以上の点から、当該製剤はレプトスピラ感染に対する安全性を確保していると考える。しかし、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない

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Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica

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ABSTRACT

Leptospirosis is a worldwide distributed zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*. The basic taxon of *Leptospira* is the serovar. Currently, nearly 300 serovars have been identified. Leptospirosis is particularly prevalent in warm and humid tropical regions where conditions for transmission and survival of pathogenic leptospires in the environment are optimal. Leptospirosis probably constitutes a serious veterinary and public health problem in Central America but solid figures are missing. To determine distribution of leptospirosis in Costa Rica and to identify locally circulating pathogenic serovars, we performed a sentinel-based study, isolating and characterizing leptospires from patients attending hospitals. Strain MAVJ 401 was isolated from a hospitalized patient in the Alajuela province. The isolate produced agglutination titers notably with reference rabbit antisera against serovars of serogroup Javanica but appeared serologically unique in the standard Cross Agglutinin Absorption Test. Therefore, MAVJ 401 was considered to represent a new serovar, designated Arenal, of the serogroup Javanica. Genotypic analysis revealed that strain MAVJ 401 belongs to *Leptospira santarosai*, a species that almost exclusively occurs in Latin America. This is not a unique finding of an exotic serovar. Recent isolates from severely ill patients in the same region appeared to be identical to Arenal.

We have identified a novel highly virulent serovar from a patient in Costa Rica that is common in this area, thus posing a threat for the local public and veterinary health.

1. Introduction

Leptospirosis is a worldwide zoonosis, transmitted to humans through contaminated water or direct exposure to the urine of infected animals. Human infection may be acquired through occupational, recreational, or avocational exposures. Direct contact with infected animals accounts for most infections in farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers and other occupations which require contact with animals. Indirect contact is important for sewer workers, miners, soldiers, septic tank cleaners, fish farmers, gamekeepers, canal workers, rice field workers, taro farmers, banana farmers and sugar cane cutters (Levett, 2001).

The clinical spectrum of the disease ranges from mild influenza-like to severe forms such as the Weil's syndrome, characterized by hepato-renal dysfunctions and a bleeding tendency and Acute Respiratory Distress Syndrome (ARDS) with mortality rates exceeding 50% (Levett, 2001; McBride et al., 2005).

Development of a subclinical infection or clinical disease might depend on both host and causative agent related factors such as immunological competence, age, physical condition and virulence and size of the inoculum, respectively. Animals with subclinical infections as well as those that recover from the clinical disease become a potential source of infection for other susceptible hosts, because they continue to excrete leptospires for a prolonged period of time (Faine, 1982; Faine et al., 1999).

The causative agents of leptospirosis belong to the genus *Leptospira*, which contains both saprophytic and pathogenic species (Levett, 2001). The isolation and identification of an infecting *Leptospira* strain is cumbersome and time consuming. Isolation is difficult due to the slow growth rate, notably when combined with a concomitant contamination with faster growing microorganisms, and stringent and fastidious in vitro culture requirements of these bacteria (Faine, 1994; Faine et al., 1999). The

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initial identification of a *Leptospira* is morphological, by dark field microscopy observation. Definitive identification of the isolates requires the use of serological and molecular techniques (Dikken and Kmety, 1978; Brenner et al., 1999; Levett, 2003). In the conventional classification system, all pathogenic leptospires belong to the species *Leptospira interrogans* sensu lato (Dikken and Kmety, 1978; Faine and Stallman, 1982). Based on serological criteria, strains of *Leptospira* are differentiated into serovars, which represent the basic taxon (ICSB Sub-committee on the taxonomy of Leptospira, 1987; Kmety and Dikken, 1993). Serovars that are antigenically related are placed into serogroups. Serogroups do not have an official taxonomic status, but are of clinical and epidemiological importance (Levett, 2003). The list is updated periodically and more than 250 pathogenic serovars arranged in 26 serogroups are currently known. The recent genotypical classification system is based on DNA homology. In this system, leptospires are placed into 17 *Leptospira* species of a pathogenic, saprophytic and doubtful nature (Yasuda et al., 1987; Perolat et al., 1998; Brenner et al., 1999; Levett et al., 2006). There is a poor correlation between the serological and genotypic classification systems (Brenner et al., 1999; Yasuda et al., 1987).

The species *Leptospira santarosai* contains 61 serovars of multiple serogroups (Brenner et al., 1999). The type strain of *L. santarosai*, serovar Shermani strain 1342 K was isolated from a spiny rat (*Proechymis semispinousus*) in the Panama Canal Zone (Yasuda et al., 1987). Several additional reports confirmed that *L. santarosai* is pathogenic for humans and domestic animals (Brenner et al., 1999; Hsieh and Pan, 2004; Rossetti et al., 2005).

In this paper, we describe a new leptospiral serovar belonging to the species *L. santarosai* isolated from the blood of a severely ill leptospirosis patient.

2. Materials and methods

2.1. Case description

A 45-year-old man was hospitalized in Ciudad Quesada San Carlos Hospital, Costa Rica, with a 3–4 day history of fever, headache and myalgia. The patient is a biologist employed by a Costa Rican fish farm. At the day of admission his temperature was 39°. He had tachycardia and his blood pressure was 120/60 mmHg. Clinical examination showed a conscious man, with bilateral headache, sore throat, provoked myalgia of the legs, hepatalgia, hepatomegaly, and conjunctivitis. There were no signs of rash, meningeal irritation and cervical rigidity. Laboratory tests revealed increased SGOT:79.8 U/L (normal range (nr) 12.0–46.0), 76.2 U/L (nr 3–50), creatine phosphokinase: 915 U/L (nr 24–195), direct bilirubin: 0.53 mg/dL (nr 0.0–0.2), total bilirubin: 1.49 mg/dL (nr 0.0–1.0), associated with hyperglycemia: 143 mg/dL, alkaline phosphatase: 202 U/L (value is within normal range, nl), albumin: 3.3 g/dL (nl), and protein levels: 5.92 g/dL (nl), ureic nitrogen: 8.62 mg/dL (nl), creatinine: 1.26 mg/dL (nl). The leukocyte count was 8, $2 \times 10^3/\mu L$ with 80% polymorph nuclear forms. Thrombocytopenia: $145 \times 10^3/\mu L$ (last control: $99 \times 10^3/\mu L$) was also observed. Results of urinalysis were normal. Malaria blood smears, blood cultures and serology for dengue were negative.

The patient received a 7-day treatment with penicillin, 2 million units 4 times a day, which resulted in a resolution of symptoms. Oral treatment with penicillin was continued for 6 more days.

Leptospirosis was confirmed by seroconversion in the Microscopic Agglutination Test (MAT) with a titer of 1:100 with serovar Canicola in the second sample. Also the rapid screening test Lepto dipstick (Gussenhoven et al., 1997) gave a positive outcome (data not shown).

2.2. Bacterial culture

Culturing was performed in Ellinghausen and McCullough modified Johnson and Harris (EMJH) culture medium (DifcoTM). Aliquots of 0.1 and 0.01 mL of heparin anticoagulanted whole blood were inoculated into 6 mL EMJH culture medium. Incubation was at 30 °C and cultures were inspected by darkfield microscopy for growth of leptospires at regular intervals. Isolates were subcultured and maintained in EMJH medium and in Fletcher medium supplemented with 5 fluoro-uracil (200 μg/mL) as a selective inhibitor for contaminating microorganisms (Faine and Stallman, 1982; Faine et al., 1999; Hartskeerl et al., 2006).

2.3. Microscopic agglutination test

The microscopic agglutination test (MAT) was performed as per standard procedure (Comisión Cientifica Permanente sobre Leptospirosis de la AAVL, 1994) starting with a serum dilution of 1:20 up to 1:20480. The highest dilution of serum showing 50% reduction in free-moving leptospires under dark field microscope was considered the end-titre. Rabbit anti-*Leptospira* sera were prepared following the standard procedure (ICSB Sub-committee on the taxonomy of *Leptospira*, 1984).

2.4. Serological typing: MAT with group sera and monoclonal antibodies

To identify the isolate up to serogroup level, MAT was performed following standard procedure using a panel of 38 anti-*Leptospira* rabbit antibodies (Dikken and Kmety, 1978; Hartskeerl et al., 2006). Isolates were further typed at the serovar level by performing MAT with panels of monoclonal antibodies (mAbs) that characteristically agglutinate serovars from the serogroups Icterohaemorrhagiae and Sarmin (F12C3, F20C3, F20C4, F52C1, F52C2, F70C4, F70C7, F70C13, F70C14, F70C20, F70C24, F70C26, F82C1, F82C2, F82C7, F82C8, F89C3, and F89C12) as described by Korver et al. (1988) and from serogroup Javanica (F12C3, F20C3, F20C4, F70C20, F98C4, F98C5, F98C8, F98C12, F98C17, F98C19 and F98C20) with cross-agglutinations of serovars of the closely related serogroups Sarmin and Celledoni (Alex et al., 1993).

2.5. Cross Agglutinin Absorption Test

The Cross Agglutinin Absorption Test (CAAT), the standard assay for serological classification of *Leptospira* serovars was carried out by staff of INCIENSA as described elsewhere (Dikken and Kmety, 1978; Kmety and Dikken, 1993; Hartskeerl et al., 2006, ICSB Sub-committee on the taxonomy of *Leptospira*, 1984). Staff of the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, The Netherlands confirmed the CAAT results.

2.6. Genetic characterization

Strains and isolates were grown at 30 °C in EMJH medium and harvested by centrifugation during the late logarithmic phase. DNA was isolated as described by Boom et al. (1990). PCR was performed on the DNA extracts using the primer set G1/G2 that specifically amplifies a 285 bp fragment of the *secY* gene from all pathogenic species except *L. kirschneri* (Gravekamp et al., 1993; Oliviera et al., 2003). PCR conditions and controls were as previously described (Gravekamp et al., 1993; Bal et al., 1994). PCR products were analyzed by electrophoresis in 1.5% agarose gels, stained with ethidium bromide using standard procedures and subsequently judged by eye under UV illumination.

For sequencing, DNA concentration of PCR products was adjusted in the range of 10–20 ng per reaction and applied to

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the sequence reaction using the BigDye® Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems, United Kingdom) and subsequently analyzed on an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, United Kingdom). DNA sequence clustal alignments were done using the LaserGene software package (DNASTAR). Species determination was done on basis of highest sequence identity of PCR products from *Leptospira* reference strains (Gravekamp et al., 1993; Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007).

3. Results

3.1. Isolation

The culture with 0.1 mL blood inoculation became positive after two weeks. The isolate was named strain MAVJ 401. Under the darkfield microscope, strain MAVJ 401 showed typical *Leptospira* motility and morphology. The strain grew well in EMJH and Fletcher medium at 30 °C.

3.2. Serological characterization

When testing the strain against a panel of 38 rabbit anti-*Leptospira* sera to determine potential serogroups, highest agglutination titers were found against serogroup Sarmin serovar Weaveri and serogroup Javanica serovar Poi. Low cross-agglutinating titers were also produced with members of the serogroups Icterohaemorrhagiae and Celledoni. No agglutinations were found with reference sera from intermediate and saprophytic reference strains, suggesting a pathogenic status of the isolate.

Subsequent testing with the panel of mAbs against serovars of the Icterohaemorrhagiae and Sarmin groups only revealed a titer 1:320 against one of the 18 mAbs in the panel. No match was found with the agglutination pattern of any of the serovars in these two serogroups (results not shown). The agglutination pattern obtained with the mAbs against serovars of the Javanica group was most similar with that of serovar Javanica, strain Veldrat Batavia 46 (Table 1). No match was found with serovars of the closely related serogroup Celledoni and, again, serogroup Sarmin.

Cross-agglutinations and CAAT were performed to confirm the presumptive results obtained via mAbs typing.

Cross-agglutination experiments were executed between strain MAVJ 401 and antiserum against all serovars from the groups Javanica, Sarmin and Celledoni and vice versa. No significant cross-agglutinations (>10% compared to the homologous agglutination) were observed with sera from the serogroups Celledoni and Sarmin and vice versa, serum against MAVJ 401, virtually excluding that MAVJ 401 belongs to these serogroups. A significant cross-agglutination titer in both cross-agglutination experiments was only found against serogroup Javanica serovar Fluminense strain Aa3. Surpisingly only low cross-agglutination titers were found against serovar Javanica strain Veldrat Batavia 46.

Table 1
Comparison of agglutination titers of strain MAVJ 401 and the reference serovar Javanica, strain Veldrat Batavia 46 with mAbs against serogroup Javanica

mAb	Reciprocal titers against strain MAVJ 401	Reciprocal titers against strain Veldrat Batavia 46
F12C3	–	–
F20C3	–	–
F20C4	320	320
F70C20	–	–
F98C4	–	–
F98C5	–	–
F98C8	5120	5120
F98C12	20480	5120
F98C17	–	–
F98C19	10240	10240
F98C20	–	≤80

(–) No agglutination.
Up to a 4-fold titer difference is acceptable in mAbs typing.

Table 2
Cross-agglutinations and CAAT between MAVJ 401 and reference strains

Serum	Strain	Cross agglutination (%)[a]	CAAT, residual titer (%)[b]
Aa3	MAVJ 401	50	50
MAVJ 401	Aa3	12.5	100
Sofia 874	MAVJ 401	12.5	50
MAVJ 401	Sofia 874	0.2	ND
Cox	MAVJ 401	6.25	50
MAVJ 401	Cox	0.4	ND
Veldrat Batavia 46	MAVJ 401	1.5	100
MAVJ 401	Veldrat Batavia 46	0.2	ND
Sorex Jalná	MAVJ 401	100	100
MAVJ 401	Sorex Jalná	0.2	ND
L 82	MAVJ 401	12.5	100
MAVJ 401	L 82	0.8	ND
MMD 3	MAVJ 401	50	100
MAVJ 401	MMD 3	6.25	ND
Rr 5	MAVJ 401	25	50
MAVJ 401	Rr5	6.25	ND
CZ 390	MAVJ 401	25	100
MAVJ 401	CZ 390	1.5	ND

[a] (Heterologous titer: homologous titer) × 100%; >10% is significant.
[b] (Homologous titer after absorption: homologous titer before absorption) × 100%; <10% indicates similarity of the serovars.

CAAT was performed in duplicate and independently by two persons to assure reproducibility. The following reference strains were included in the test, Javanica group; serovar Fluminense strain Aa3, serovar Sofia strain Sofia 874, serovar Coxi strain Cox, serovar Javanica strain Veldrat Batavia 46, serovar Sorexjalna strain Sorex Jalná, serovar Zhengkang strain L 82 and serogroup Sarmin; serovar Machiguenga strain MMD 3, serovar Rio strain Rr 5 and serovar Weaveri strain CZ 390.

According to the definition of the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospira* (1984, 1987), strain MAVJ 401 was not serologically identical to any of these strains (Table 2) and therefore MAVJ 401 represents a new serovar, designated Arenal. Based on the initial serological reactions it is proposed that this serovar is placed within the pathogenic serogroup Javanica.

3.3. Species determination

Consistent with its pathogenic status, DNA from MAVJ 401 was amplified by primer pair G1/G2 (Gravekamp et al., 1993). To determine the species of MAVJ 401, the sequence of its G1/G2 amplicon was compared with 65 other sequences (Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007). The sequence of the amplicon showed highest percentage identity with a number of strains from *L. santarosai*, i.e. 97.1% with serogroup Sejroe; serovar Caribe strain TRVL 61866 and serovar Gorgas strain 1413 U, serogroup Mini; serovar Georgia strain LT 117 and Tabaquite strain TRVL 3214, serogroup Pyrogenes; serovar Princestown strain TRVL 112499, serogroup Javanica; serovar Vargonis strain 24, serogroup Sarmin; serovar Weaveri strain CZ 390 and 96.7% identity with serogroup Pomona, serovar Tropica strain CZ 299.

Percentages sequence identity outside *L. santarosai* ranged from 71.3% (*L. meyeri*, serovar Semaranga strain Veldrat Semarang 173) to 94.7% (*L. weilli* serovar Mengrun strain A 102 and *L. weilii*, serovar Coxi strain Cox). Taking the highest percentage of identity with eight strains of *L. santarosai*, we believe that MAVJ 401 belongs to this species.

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

4. Discussion

We describe the isolation and characterization of a novel *Leptospira* serovar isolated from a Costa Rican patient. The patient was admitted to the hospital with signs and symptoms compatible with leptospirosis and standard antibiotic treatment with penicillin was effective. Leptospirosis was serologically confirmed. It likely concerns here an occupational disease as the patient worked on a fish farm where he obviously acquired the infection via fish ponds contaminated with urine of carrier animals.

The morphology and motility of the bacterium under darkfield microscopy is consistent for the genus *Leptospira*. Serologically, the isolate showed titers notably against members of the serogroups Javanica and Sarmin. Cross-agglutination titers were also found in the serogroups Icterohaemorrhagiae and Celledoni. This likely represents intra-serogroup cross-agglutinations because serogroups Javanica and Celledoni on one hand and Javanica, Sarmin and Icterohaemorrhagiae on the other hand form 'serogroup complexes' comprising antigenic related serovars (Hartskeerl et al., 2006). Because of this overlapping antigenic relationship between these groups and the fact that highest agglutinating titers were produced with serovars of serogroup Javanica we suggest to place MAVJ 401 into this serogroup.

We found contrasting data by mAbs typing and the CAAT. mAbs typing generated a pattern that was highly similar to that of the reference serovar Javanica strain Veldrat Batavia 46 of the Javanica group. However, cross-agglutination and CAAT revealed only little similarity with this serovar. Moreover, CAAT, which is the standard method to determine the serovar as basic taxon, revealed that this isolate is unique. The serovar status is mainly, if not exclusively, based on the composition and structure of the highly antigenic LPS (Faine et al., 1999). A likely explanation of the discrepancy in typing with monoclonal and polyclonal sera is that panels of agglutinating mAbs are directed to a limited number of epitopes while polyclonal hyperimmune sera cover the full spectrum of epitopes. Apparently, it is possible that a set of mAbs recognizes a limited number of common epitopes on furthermore different LPS in distinct serovars within a serogroup. As shown in this study, incorrect mAbs-based identification can be avoided by determining cross agglutination with polyclonal hyperimmune serum against the presumably corresponding reference strain.

We designated the isolate serovar Arenal after the volcano in the Costa Rica near the residence of the patient in the province Alajuela.

DNA sequence analysis indicated that serovar Arenal most likely belongs to species *L. santarosai*, which is distributed almost exclusively in Latin America (Chappel et al., 1998).

Serovar Arenal likely is not an exotic serovar and might be common in and around the Alajuela province of Costa Rica. Recently, two out of 21 isolates obtained from Costa Rica were identified as serovar Arenal implying that 13.6% (3/22) of the isolates consisted of Arenal. The two additional Arenal isolates, preliminary coded as isolate 7 and 11, were cultured from severely ill patients living in the Puntarenas province that flanks Alajuela. Molecular analysis of MAVJ 401/isolate 7 by Multilocus Sequence Typing showed that it formed a distinct branch that was positioned closely to, but apart from the clade of *L. santarosai* (Ahmed et al., 2006). This supports the unique character of this novel serovar, also on genotypical grounds.

The infection source of isolate 11 is unknown. Infection with isolate 7 was very likely acquired via contact with cattle. The environment of the fish farm of MAVJ 401 makes it possible that the ponds have been contaminated with urine of infected cattle. It is therefore tempting to speculate that cattle form the infection reservoir of this novel serovar. However, further research on potential infection sources in the region will be needed to confirm or refute this.

L. santarosai, serovar Arenal, type strain MAVJ 401 has been deposited under this designation in the culture collections of the National Reference Center for Leptospirosis, Costa Rican Institute for Research in Nutrition and Health, Tres Ríos, Costa Rica and the WHO/FAO/OIE and National Collaborating Centre for Reference & Research on Leptospirosis, Royal Tropical Institute, Amsterdam, Netherlands. The novel serovar designation of strain MAVJ 401 has been ratified by the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospiraceae*.

Acknowledgments

The authors wish to thank Mr. J.I. Chaverri and Dr. M. Liem for their technical assistance. The work was supported by EU-INCO grant ICA4-CT-2001-10086. N.A. would like to thank CDFD for core grant support.

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別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 200 年 4 月 10 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Portsmouth woman's death under investigation dailypress.com, April 11, 2008	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
異型クロイツフェルト・ヤコブ病(vCJD)に関連すると疑われる脳変性疾患を呈した米国の女性の症例が報告された。しかし，感染症，脳内酸素欠乏，肝不全，腎不全，毒物暴露，代謝疾患，脳腫瘍，頭蓋内圧の上昇，栄養不足など多数の原因が，本症例の脳疾患に関連していると考えられており，原因究明には更なる調査が必要である。MRI 又は脳スキャンの結果が，アトランタの疾病対策センターに送付され，バージニア大学及び National Prion Disease Pathology Surveillance Center (NPDPSC) で更に調査されることになっているが，結果が出るまでには数ヶ月間を要すると考えられている。	BYL-2008-0316

報告企業の意見	今後の対応
弊社の血漿分画製剤は米国の血漿を使用しているが，現在までに報告されている米国での vCJD 3 例は，米国以外の国で暴露された患者に限定されている。また，弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されており，弊社製剤による vCJD 感染リスクは極めて低いと考えられる。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

14

dailypress.com/news/local/southofjames/dp-local_deathprobe_0411apr11,0,2417128.story

dailypress.com

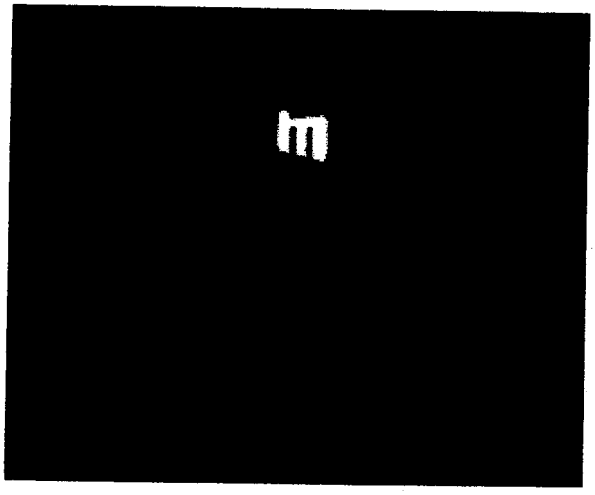
Portsmouth woman's death under investigation

By VERONICA GORLEY CHUFO

247-4741

April 11, 2008

RICHMOND



The illness and Wednesday death of a Portsmouth woman spurred a Virginia Department of Health investigation Thursday.

The woman suffered from encephalopathy, a degenerative brain disease. Her illness has been linked in news reports to variant Creutzfeldt-Jakob Disease — the human form of mad cow disease.

It's a very rare condition related to the consumption of beef infected with bovine spongiform encephalopathy. It's always fatal, the health department said in a news release.

The woman's name was not released by the health department but news reports have identified her as Aretha Vinson.

The illness could have been caused by a number of things, State Health Commissioner Karen Remley said in the release.

"Infections, lack of oxygen to the brain, liver failure, kidney failure, toxic exposures, metabolic diseases, brain tumors, increased intracranial pressure and poor nutrition are all related to encephalopathy," Remley said. "Further testing is the only way to know what caused this illness."

An MRI, or brain scan, was sent to the Centers for Disease Control and Prevention in Atlanta. Additional tests will be handled by the University of Virginia and the National Prion Disease Pathology Surveillance Center in Cleveland. Results are expected to take several months.

At least 200 cases of variant Creutzfeldt-Jakob Disease have been reported worldwide since 1996. Three cases have been reported in U.S. residents, and they were all exposed outside the country, Remley said. It's not spread casually from person to person.

For more information, visit cdc.gov, cjdfoundation.org or vdh.virginia.gov.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-2

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 4 月 14 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Prion Filter for Donated Blood Medgadget LLC, April 9, 2008	公表国 カナダ	
販売名（企業名）						

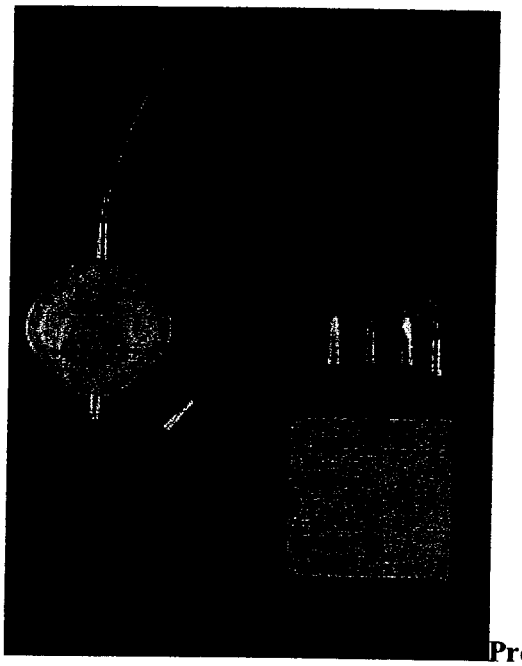
研究報告の概要	使用上の注意記載状況・その他参考事項等
カナダ，ケベック州の ProMetic Life Science 社が血液中の異型クロイツフェルト・ヤコブ病(vCJD)プリオンを除去するフィルターを開発した。 ProMetic Life Science 社の開発チームは，血液中のプリオンと親和性が高いペプチドをスクリーニングした後，市販のポリメタクリル酸樹脂にペプチドを固定し，これを膜状にしたものを幾層にも重ねて本フィルターを開発した。本使い捨てフィルターにより，1時間足らずで 1 ユニットの汚染血液からプリオンを除去することが可能であり，又，この工程によって血液自体の変性はおきない。さらに，プリオン感染ハムスターのフィルター処理した血液をプリオン非感染ハムスターに投与したところ，疾患は発現しなかった。	BYL-2008-0317

報告企業の意見	今後の対応
現在までに，血液中における vCJD 検査は可能となっていない。本フィルターの使用により，輸血による vCJD 感染に対する安全性は高まると考えられる。弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されている。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

(57)

Print

Prion Filter for Donated Blood



ProMetic Life Sciences, a company out of Mont-Royal, Quebec, has developed a blood filter touted to remove prions responsible for variant Creutzfeldt-Jakob disease (vCJD). Considering that currently there is no available test for vCJD in donated blood, filtering may soothe the nerves of potential transfusion recipients.

> The team took five years to create the hand-sized filter, screening millions of small peptides to find one that had the strongest affinity for the prions found in contaminated blood. They stuck the best peptide onto commercial polymethacrylate resins, and then sandwiched these in alternating layers with a membrane
>
> In tests, the disposable filter can clean the prions out of a single pack of contaminated blood in less than an hour. No prions remain in the cleaned blood, which is otherwise unchanged by the process. Tests with prion-infected hamsters showed that their filtered blood could be injected into disease-free hamsters with no ill effects.
>
> The team hope that the UK's National Blood Service could be using the device by the end of this year. Peter Edwardson, ProMetic's vice-president of medical technologies, says that Ireland's clinical trial, aiming to confirm that the filtered red blood cells are just as effective as untreated blood when transfused into humans, should be complete in a few months.

More at the *Royal Society of Chemistry*...

ProMetic Life Sciences...

Flashbacks: Leukotrap® Affinity Prion Reduction Filter

Posted on April 9, 2008 09:14 AM

CLOSE

別紙様式第2-1

No. 4

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Terpstra FG, van 't Wout AB, Schuitemaker H, van Engelenburg FA, Dekkers DW, Verhaar R, de Korte D, Verhoeven AJ. Transfusion. 2008 Feb;48(2):304-13. Epub 2007 Nov 19.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			オランダ

研究報告の概要

○血小板濃厚液の病原体を不活化するUVC照射の効果と限界

背景:輸血による疾患伝播を引き起こす血液製剤の病原体汚染は現在も懸念されている。本試験において、血小板濃厚液のUVC照射の病原体不活化能を検討した。複数のウイルス・細菌不活化の用量依存性を血小板の質への影響と比較した。

試験デザインおよび方法:さまざまな脂質エンベロープ(LE)、非脂質エンベロープ(NLE)ウイルスおよび細菌を用いてUVC照射の効力を調べた。

LEウイルスは、ウシ・ウイルス性下痢ウイルス(BVDV)、ヒト免疫不全ウイルス(HIV)、仮性狂犬病ウイルス(PRV)、伝播性胃腸炎ウイルス(TGEV)と水疱性口内炎ウイルス(VSV)とし、NLEウイルスは、イヌ・パルボウイルス(CPV)とシミアンウイルス40(SV40)、細菌は、表皮ブドウ球菌、黄色ブドウ球菌、大腸菌とセレウス菌とした。スパイクおよび照射後の検体について、残存する感染性と減少率(RF)を調べた。さらに、in vitroでUVC照射が血小板の品質に及ぼす影響を調べた。

結果:UVC量500J/㎡では、血小板の品質の変化は許容できるものであった(pH、乳酸産生、CD62P発現、ホスファチジルセリン曝露にて測定)。CPV、TGEV、VSV、表皮ブドウ球菌、黄色ブドウ球菌、大腸菌のRFは高く(>4log)、BVDV、PRV、セレウス菌のRFは中等度(約3log)であり、HIVおよびSV40のRFは低かった(約1log)。cell-freeウイルス、cell-associatedウイルス間で、ウイルスの軽減に差は認められなかった。

結論:UVC照射は、血小板の品質に影響を及ぼさずに、細菌および広範なウイルス(HIVを除く)を不活化することのできる、血小板濃厚液の有望な病原体低減技術である。しかし、HIVのような血液感染性ウイルスに対応するには、UVC法をさらに最適化することが必要である。

報告企業の意見	今後の対応
UVC照射は、血小板の品質に影響を及ぼさずに、細菌および広範なウイルス(HIVを除く)を不活化することができるが、HIVのような血液感染性ウイルスに対応するには、UVC法をさらに最適化することが必要であるとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(18)

Potential and limitation of UVC irradiation for the inactivation of pathogens in platelet concentrates

Fokke G. Terpstra, Angélique B. van 't Wout, Hanneke Schuitemaker, Frank A.C. van Engelenburg, David W.C. Dekkers, Robin Verhaar, Dirk de Korte, and Arthur J. Verhoeven

BACKGROUND: Pathogen contamination, causing transfusion-transmitted diseases, is an ongoing concern in transfusion of cellular blood products. In this explorative study, the pathogen-inactivating capacity of UVC irradiation in platelet (PLT) concentrates was investigated. The dose dependencies of inactivation of several viruses and bacteria were compared with the effect on PLT quality.

STUDY DESIGN AND METHODS: The potential of UVC irradiation was studied with a range of lipid-enveloped (LE) and non–lipid-enveloped viruses (NLE) and bacteria. LE viruses were bovine viral diarrhea virus (BVDV), human immunodeficiency virus (HIV), pseudorabies virus (PRV), transmissible gastroenteritis virus (TGEV), and vesicular stomatitis virus (VSV). NLE viruses were canine parvovirus (CPV) and simian virus 40 (SV40). Bacteria were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*. After spiking and irradiation, samples were tested for residual infectivity and reduction factors (RFs) were calculated. Furthermore, the effect of UVC irradiation on PLT quality was determined by measuring in vitro quality variables.

RESULTS: A UVC dose of 500 J per m^2 resulted in acceptable PLT quality (as measured by pH, lactate production, CD62P expression, and exposure of phosphatidylserine) and high RFs (>4 log) for CPV, TGEV, VSV, *S. epidermidis*, *S. aureus*, and *E. coli*. Intermediate RFs (approx. 3 log) were observed for BVDV, PRV, and *B. cereus*. Low RFs (approx. 1 log) were found for HIV and SV40. No differences in virus reduction were observed between cell-free and cell-associated virus.

CONCLUSION: UVC irradiation is a promising pathogen-reducing technique in PLT concentrates, inactivating bacteria, and a broad range of viruses (with the exception of HIV) under conditions that have limited effects on PLT quality. Further optimization of the UVC procedure, however, is necessary to deal with blood-borne viruses like HIV.

Next to careful selection of donors, safety of cellular blood products is primarily based on screening systems to detect markers for viral contamination (e.g., specific antibody testing and nucleic acid testing [NAT]) and bacterial contamination (e.g., BacT/ALERT culturing). In donors experiencing primary virus infections, however, antibodies are not yet detectable in the early phase of infection and NAT might also score negative (the so-called window phase). Moreover, in case of emerging infections, both safety measures will fail. Also, despite screening for bacteria, cases of bacterial transmission have been reported, due to the limited sensitivity of the system.[1] Hence, there is a strong need for in-process steps with broad pathogen-inactivating capacity. An additional requirement for such a step is that the quality of the appropriate blood products is not compromised.

For pathogen inactivation in platelet concentrates (PCs), a number of different techniques involving ultraviolet (UV) light have recently been described. One method utilizes the psoralen compound S-59 (amotosalen hydrochloride) in combination with UVA light. In this photochemical process S-59 intercalates into and binds to

ABBREVIATIONS: BVDV = bovine viral diarrhea virus; CA virus = cell-associated virus; CPV = canine parvovirus; LE virus = lipid-enveloped virus; NLE virus = non–lipid-enveloped virus; PC(s) = platelet concentrate(s); PRV = pseudorabies virus; PS = phosphatidylserine; RF(s) = reduction factor(s); SV40 = simian virus 40; TGEV = transmissible gastroenteritis virus; VSV = vesicular stomatitis virus.

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nucleic strands. Upon irradiation with UVA light (320-400 nm), this binding becomes irreversible and the strands are cross-linked, resulting in inactivation of the pathogen.[2]

Another method takes advantage of the properties of a naturally occurring vitamin supplement, riboflavin. Riboflavin interacts between the bases of DNA or RNA and upon irradiation with broadband UV light (265-370 nm), riboflavin oxidizes guanine in nucleic acids, resulting in irreversible damage to the pathogen.[3]

A third method applies a two-step procedure of a photodynamic treatment with thionine and/or light to inactivate free viruses, followed by low-dose UVB treatment to inactivate white blood cells (WBCs) and bacteria.[4] Thionine has a high binding affinity to nucleic acids and specifically binds strongly in G-C–rich regions, but also formation of singlet oxygen is important.[5]

A disadvantage of most of the photochemical and photodynamic treatments developed to date is the need to add and to remove the sensitizer and/or its breakdown products. An alternative in this field is the use of UV light alone. The theoretically optimal wavelength for DNA damage without the need for a photosensitizer is 254 nm, that is, in the UVC range. A UVC technique applying light of 254 nm has, among others, been described by Caillet-Fauquet and colleagues[6] and shown to be effective in treating purified plasma-derived products. UVC mainly causes dimerization of adjacent pyrimidines[7] and the resultant intranucleotide cross-link abrogates subsequent pathogen replication. In addition, UVC also generates free radicals such as singlet oxygen.[8]

In this study, we explored the potential of UVC irradiation for pathogen inactivation in PCs. For a dose of 500 J per m^2, we found good pathogen reduction for bacteria and a broad range of viruses (with the exception of human immunodeficiency virus [HIV]) in a mixture of 10 percent plasma and additive solution (Composol-PS, Fresenius HemoCare, Emmer-Compascuum, the Netherlands), with a limited effect on several in vitro variables of platelet (PLT) quality.

MATERIALS AND METHODS

Preparation of PCs

PCs were prepared from whole blood–derived buffy coats by a modification of the standard protocol.[9,10] In short, 500 ± 50 mL of blood from nonremunerated, informed donors was collected in 70 mL of citrate-dextrose-phosphate in bottom-and-top blood collection systems (Fresenius HemoCare, Emmer-Compascuum, the Netherlands). After collection, the blood was stored for 12 to 16 hours at 20 ± 2°C.[11] After centrifugation (2780 × *g*, 8 min), the blood was separated in a plasma, buffy coat, and red cell (RBC) fraction with the aid of a semiautomated component preparation device (Compomat G4, Fresenius HemoCare).[12] Three buffy coats were mixed together and 240 mL of Composol-PS (consisting of 90 mmol/L NaCl, 5 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 27 mmol/L acetate, 23 mmol/L gluconate, 3.2 mmol/L citrate, pH 7.0; Fresenius HemoCare)[13] was added by means of sterile docking. Instead of a low-speed centrifugation step to prepare the PC, the pooled buffy coats were subjected to a high-speed centrifugation (2780 × *g*, 8 min), the supernatant was removed, and an additional 180 mL of Composol-PS was added to lower the plasma content of the final product. The buffy coats were then subjected to a low-speed centrifugation (5 min, 260 × *g*), and the PLT-rich supernatant was transferred across a leukoreduction filter (Compostop CS, Fresenius HemoCare) to an empty 1.3-L polyvinylchloride (PVC)-citrate storage bag (Compoflex, Fresenius HemoCare) with the use of a Compomat G4. This procedure had a yield of 60 to 65 percent and resulted in PCs with a residual plasma content of less than 15 percent, as determined by the protein content of the cell-free supernatant and a PLT count of 0.9×10^9 to 1.2×10^9 per mL. Residual WBCs and RBCs were less than 0.1×10^6 and 0.01×10^9 per mL, respectively, the detection limits of the hematologic counter used (Ac·T 10, Beckman Coulter, Mijdrecht, the Netherlands). After overnight storage at 22 ± 2°C (horizontally shaking with 1 cycle/second; PLT incubator, Model PF96, Helmer, Noblesville, IN), the PC was split into two equal volumes and the plasma concentration was adjusted to 10 percent (by addition of Composol-PS) or 30 percent (by addition of autologous plasma) with equal PLT counts. The final PLT counts were 0.6×10^9 to 0.8×10^9 per mL. The PLT was then subjected to UVC irradiation in portions of 5 mL, with or without pathogens added, as described below.

Bench-scale UVC irradiation

The UVC irradiation was performed as described previously.[6] Briefly, unless indicated otherwise, 5 mL of spiked material was irradiated from above in an open petri dish (84 mm in diameter) placed on an orbital shaker (50 r.p.m.). This resulted in a fluid layer with a thickness of approximately 1 mm. The irradiation device consisted of a UVC lamp with a low-pressure mercury arc (emission line at 254 nm, Germicidal 15T/8, General Electric, Fairfield, CT), a ventilator, filter, photoradiometer with UV sensor, and toothed rack.

In vitro measurements of PLT quality

After UVC irradiation, 5 mL of PC was transferred to 50-mL culture flasks with the addition of 10 mmol per L glucose, 12 mmol per L HCO_3^-, and 5 percent CO_2 in the gas phase and stored for 5 days at 22 ± 2°C (horizontal

shaking with 1 cycle/second). Control experiments had indicated that this down-scaled version of PLT storage results in similar loss of PLT quality as observed in whole PCs stored in PVC bags (data not shown). After the storage period, the PLTs were analyzed for pH (at 37°C), CD62P expression, exposure of phosphatidylserine (PS), and lactate production.

The expression of the activation antigen CD62P was measured essentially as described previously[14] with minor revisions. Briefly, PLTs were diluted to a concentration of 3×10^8 per mL with an electrolyte solution (Isoton II, Beckman Coulter, Mijdrecht, the Netherlands), and 5 µL of PLT suspension was then incubated with 45 µL of Isoton containing CD62P antibody (clone CLB-Thromb/6 conjugated with fluorescein isothiocyanate [FITC], Immunotech, Marseille, France) for 30 minutes at 22°C in the dark. Afterward, the PLTs were fixed by adding 0.5 mL of 0.5 percent (vol/vol) methanol-free formaldehyde (Polyscience, Inc., Warrington, PA; diluted in PBS) and analyzed via flow cytometry (FACScan, Becton Dickinson, Franklin Lakes, NJ). A parallel incubation with FITC-labeled murine immunoglobulin G (Sanquin Reagents, Amsterdam, the Netherlands) was used as negative control.

PS exposure after treatment and storage was determined by means of annexin V binding as follows. PLTs were diluted to a concentration of 1×10^8 per mL in HEPES medium (136 mmol/L NaCl, 3.2 mmol/L KCl, 2 mmol/L $MgSO_4$, 1.2 mmol/L K_2HPO_4, 10 mmol/L HEPES, pH 7.4), and 20 µL of PLT suspension was incubated with 180 µL of HEPES medium in the presence of annexin V-FITC (0.6 µg/mL, added from a stock solution of 250 µg/mL, VPS Diagnostics, Mijdrecht, the Netherlands) and 2.5 mmol per L $CaCl_2$. As negative control, all samples were also stained in the presence of 2.5 mmol per L ethylene glycol-bis(beta-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

Lactate was measured in cell-free supernatants (obtained by centrifugation of PLT samples for 5 min at 14,000 × *g*) by enzymatic conversion with lactoperoxidase (Trinity Biotech plc, Bray, Ireland). Rates of lactate production were calculated by comparison with cell-free supernatants obtained just before UVC irradiation.

Selection of viruses and cells

Viruses were selected for being blood-borne and pathogenic and/or to represent various genome types (RNA or DNA, single- or double-stranded) and sizes and with or without lipid envelopes[15,16] (Table 1). Five lipid-enveloped (LE) viruses were used: bovine viral diarrhea virus (BVDV; model for hepatitis C virus [HCV]), HIV (relevant blood-borne virus), pseudorabies virus (PRV; model virus for LE DNA viruses like hepatitis B virus [HBV]), transmissible gastroenteritis virus (TGEV; model for the corona virus causing severe acute respiratory syndrome), and vesicular stomatitis virus (VSV; model for LE RNA viruses). Two non–lipid-enveloped (NLE) viruses were used: canine parvovirus (CPV) and simian virus 40 (SV40), a specific model for parvovirus B19 and a general model for NLE DNA viruses, respectively.

BVDV, strain NADL (VR-534; ATCC, Rockville, MD), was cultured on MDBK cells (CCL-22; ATCC) and titrated on EBTr cells (ID-Lelystad, the Netherlands). HIV, strain HTLV-IIIB (National Cancer Institute, Bethesda, MD), was cultured on H9 cells (National Cancer Institute) and titrated on MT2 cells (Wellcome, Beckenham, UK). PRV, strain Aujeszki Bartha K61 (Duphar, Weesp, the Netherlands), was cultured and titrated on VERO cells (CCL-81; ATCC). TGEV, strain Purdue (VR-763; ATCC), was cultured and titrated on ST cells (CRL-1746; ATCC). VSV, strain Indiana (Sanquin Pharmaceutical Services, Amsterdam, the Netherlands), was cultured and titrated on BHK21 cells (CCL-10; ATCC). CPV, strain 780916 (Erasmus University Rotterdam, Rotterdam, the Netherlands), was cultured and titrated on A72 cells (Erasmus University Rotterdam). SV40, strain PML-2 (VR-821; ATCC), was cultured and titrated on BSC-1 cells (Organon, Oss, the Netherlands).

Test for cytotoxicity and interference

Before the actual spiking experiments, assays were performed to determine cytotoxic effects of the plasma-Composol mixture on the cell lines used for virus titrations and interference of the plasma-Composol mixture with the titration assays, as described previously.[17] Briefly, threefold serial dilutions of the plasma-Composol mixture

TABLE 1. Properties of the viruses used in UVC irradiation studies

Virus group	Virus	Size (nm)	Virus family	Genome	Size (kb)	Model virus for
LE	BVDV	37-50	Flavi	ss RNA	10-12	HCV
	HIV	100	Retro	2 ss RNA	2 × 10	Relevant virus
	PRV	100-200	Herpes	ds DNA	140	Large LE ds DNA viruses
	TGEV	100-120	Corona	ss RNA	27-32	SARS
	VSV	75 × 180	Rhabdo	ss RNA	11-15	Large LE ss RNA viruses
NLE	CPV	18-26	Parvo	ss DNA	5	Human parvovirus B19
	SV40	45	Polyoma	ds DNA	30	Human parvovirus B19

ss = single-stranded; ds = double-stranded; SARS = severe acute respiratory syndrome.

were prepared and tested on cells to determine cytotoxicity. Then, with the first dilution of the plasma-Composol mixture without cytotoxic effects, a known amount of virus was spiked and incubated. Subsequently, the infectivity of the virus inoculum and the spiked plasma-Composol mixture was measured to determine possible interference with the detection system. In none of the virus assays, interfering effects were observed for the plasma-Composol mixtures used.

Virus assays

Infectivity was measured in $TCID_{50}$ assays and bulk culture tests. For $TCID_{50}$ assays, 1-in-3 serial dilutions of samples were prepared in culture media and 50-µL (or 0.5 mL for HIV) volumes were tested in eight replicates. To detect small amounts of virus, up to 60 mL of prediluted sample was tested in duplicate bulk culture tests, with 25- and 175-cm^2 flasks. BVDV, CPV, PRV, SV40, TGEV, and VSV cultures were inspected microscopically for cytopathic effects at 6, 7, 5, 21, 3, and 4 days postinfection, respectively. HIV cultures were inspected microscopically twice a week for the formation of syncytia until 21 days postinfection. In all experiments, virus titers were calculated by the Spearman-Kärber method[18] with the exception of the factorial design experiment, in which the most probable number[19] method was used, and titers were expressed as $TCID_{50}$ per mL. If all cultures were negative, the titer ($TCID_{50}$/mL) was considered to be less than 1 ÷ total test volume (mL).

Reduction factors (RFs) were calculated by the formula

RF = log (total amount of virus spiked as derived from the reference sample ÷ total amount of virus recovered from the treated sample).

Bacteria assays

For these studies four different bacteria, representing potential contaminants in PCs as derived from the screening results in the Netherlands,[20] were selected: *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus* (for each species, two different clinical isolates were used). Bacterial stocks were produced by overnight culture on regular blood agar plates, and colonies were picked and diluted in PBS to a concentration of approximately 3×10^7 colony-forming units (CFUs) per mL. After treatment, samples were collected and titrated in CFU assays: samples were serially diluted 1 in 10 with ice-cold saline. For *B. cereus*, after UVC irradiation an extra sample was taken and given an additional 10 minutes' incubation at 80°C to check for spores. Subsequently, 10-µL samples were tested on blood agar plates in duplicate and incubated at 35°C during 18 to 24 hours. All samples were tested in serial dilutions and for selected samples also a 200-µL sample was plated, in duplicate. Subsequently the number of bacteria was counted and CFU values were calculated.

RFs were calculated by the formula

RF = log (total amount of bacteria spiked as derived from the reference sample ÷ total amount of bacteria recovered from the treated sample).

RESULTS

In a first explorative study, efficacy of UVC irradiation for virus inactivation was tested by spiking BVDV in Composol containing varying amounts of residual plasma (5, 10, and 30%) in the absence of PLTs. Given the poor penetration of UVC light in plasma, an experimental setting with a thin plasma layer of approximately 1 mm was chosen, an orbital mixing speed of 50 r.p.m., UVC irradiation at a light intensity of 0.25 mW per cm^2, and doses ranging from 250 up to 1000 J per m^2 (1000 J/m^2 equals exposure for 400 sec). For both the 5 and the 10 percent plasma suspensions, reductions of approximately 6 log were observed at 1000 J per m^2, although the kinetics for the 10 percent plasma suspension was clearly slower than the 5 percent plasma suspension. In case of 30 percent plasma, the reduction was approximately 2.6 log at 1000 J per m^2 (Fig. 1). Although these data clearly indicated that the plasma concentration should be kept as low as possible, we used 10 and 30 percent plasma in subsequent experiments to avoid compromising PLT quality beyond acceptable limits.

The effect of UVC irradiation on PLT quality was evaluated in a similar experimental setup as above. Before analysis, the PLTs were stored for 5 days to allow detection of long-term effects of the treatment. In Fig. 2, pH, lactate production, CD62P expression, and PS exposure (as

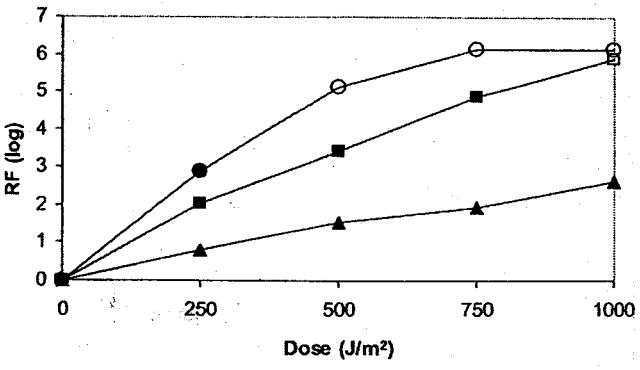

Fig. 1. Effect of UVC irradiation on BVDV inactivation in 5 (●), 10 (■), and 30 percent (▲) plasma. BVDV inactivation was tested with UVC irradiation in Composol containing 5, 10, or 30 percent plasma; RF values (log) are shown. Light intensity was 0.25 mW per cm^2, suspension depth 1 mm, and orbital mixing 50 r.p.m. Open symbols indicate maximal reduction.

measured by annexin V binding) are shown, as indicators of PLT quality. Increasing doses of UVC resulted in a clear deterioration in all of these variables, especially in the PCs suspended in 10 percent plasma. The pH had decreased from 7.23 to 6.52 with the highest UVC dose tested (Fig. 2A), concomitant with an increase in lactate production (Fig. 2B). CD62P expression increased up to 40 percent after a dose of 500 J per m^2 and leveled off at higher doses (Fig. 2C), probably due to shedding of the antigen. PS exposure also increased with increasing doses of UVC, but in 10 percent plasma and with 500 J per m^2 of UVC, the PS exposure remained below 30 percent (Fig. 2D). In 30 percent plasma, all PLT quality indicators remained within an acceptable range at all UVC doses tested, but, as noted above, under these circumstances virus inactivation may be limited.

To further optimize the UVC irradiation, three variables, light intensity, plasma percentage, and PLT concentration, were tested in a full factorial design (in duplicate) with inactivation of BVDV as readout, as described previously.[17] In this factorial design, light intensity was tested at 0.25 and 1.0 mW per cm^2, percentage plasma at 10 and 30 percent, and PLT concentration at 0 and 1.0×10^9 cells per mL. The irradiation dose was fixed at 500 J per m^2 based on the results obtained in the PLT quality experiments, and the depth was fixed at approximately 1 mm. Results for this series of experiments are shown in Table 2. Linear regression analysis of the complete data set showed that the percentage plasma was the most important determinant of UVC efficacy, with the highest virus reduction in the presence of 10 percent plasma. In addition, light intensity was also a significant parameter with highest virus reduction at 0.25 mW per cm^2. Finally, the presence or absence of PLTs did have a significant effect on the virus reduction obtained, but the contributing effect of the PLT concentration was slightly smaller than the other two variables. Given the relative low contribution of the PLT concentration, all subsequent virus studies were performed with the optimal setting as determined by the factorial design (i.e., 10% plasma, 0.25 mW/cm^2), but in the absence of PLTs.

To determine the generality of the results obtained with BVDV, we tested BVDV and six additional viruses with different genome type, size, and envelope status in a kinetic setting with irradiation values up to 1000 J per m^2. For CPV, TGEV, and VSV, high virus kill was observed, resulting in RF values of greater than 4 log already at 500 J per m^2 (sensitive viruses; Fig. 3A). For BVDV and PRV, the

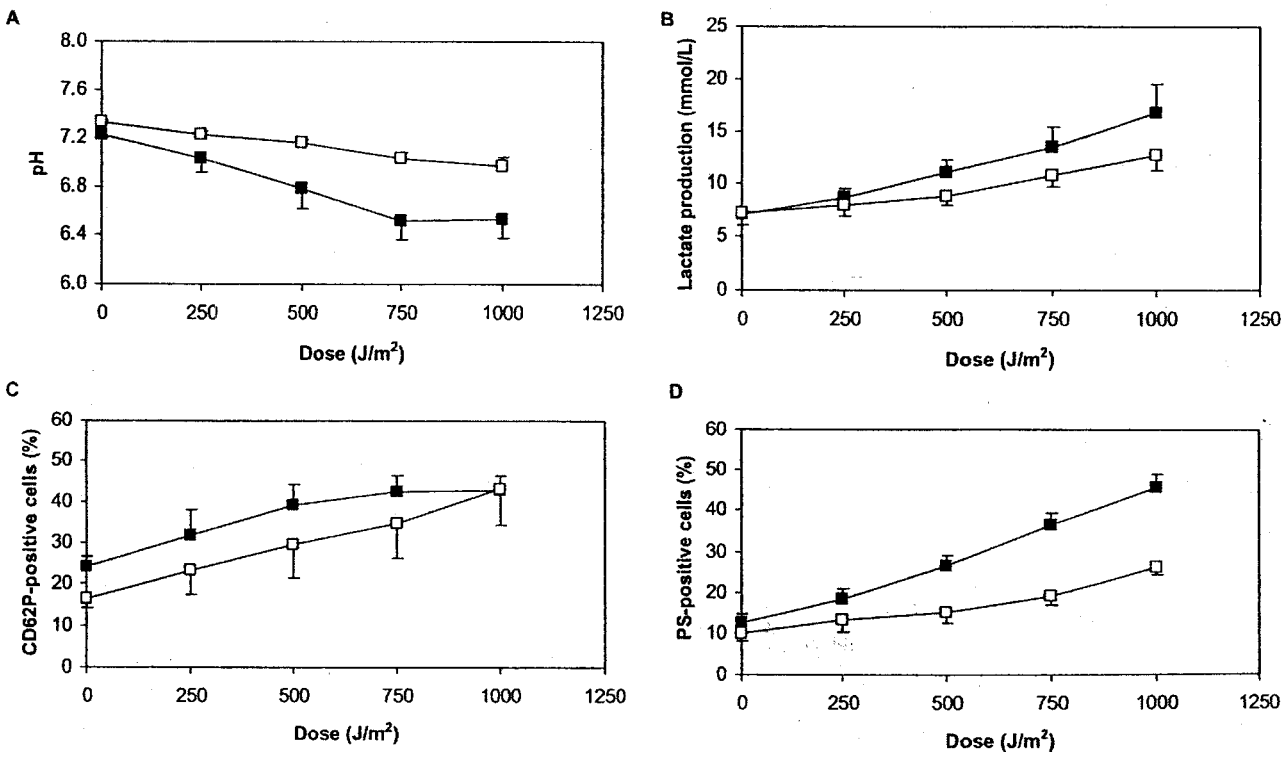

Fig. 2. Effect of UVC irradiation on PLT quality. PCs were subjected to UVC irradiation as described in the legend to Fig. 1. PLT quality variables after subsequent storage for 5 days were determined as described under Materials and Methods. (A) Medium pH; (B) lactate production; (C) CD62P expression; (D) PS-positive cells. (□) PCs in 30 percent plasma; (■) PCs in 10 percent plasma. Results shown are the mean ± SEM of 6 PCs.

TABLE 2. Factorial design for three variables (plasma, light intensity, and PLTs)*

Run	Plasma [%]	Light intensity (mW/cm²)	PLTs (cells/mL) × 10^9	RF (log)
1	10	0.25	0	5.81
2	30	0.25	0	3.03
3	10	1.0	0	4.63
4	30	1.0	0	1.90
5	10	0.25	1.0×10^9/mL	4.53
6	30	0.25	1.0×10^9/mL	2.47
7	10	1.0	1.0×10^9/mL	2.85
8	30	1.0	1.0×10^9/mL	1.66
9	10	0.25	0	4.63
10	30	0.25	0	2.88
11	10	1.0	0	4.32
12	30	1.0	0	1.89
13	10	0.25	1.0×10^9/mL	3.80
14	30	0.25	1.0×10^9/mL	2.51
15	10	1.0	1.0×10^9/mL	2.95
16	30	1.0	1.0×10^9/mL	1.50

* Factorial design to determine the influence of three variables (percentage of plasma, light intensity, and absence or presence of PLTs) on the efficacy of UVC irradiation for the inactivation of BVDV in PCs. The percentage of plasma was tested at 10 and 30 percent, the light intensity at 0.25 and 1.0 mW per cm², and the PLT concentration at 0 and 1.0 (cells/mL) × 10^9. The irradiation dose was fixed at 500 J per m², the depth was fixed at 1 mm, and orbital mixing was 50 r.p.m. The results for BVDV are shown as RF (log) calculated with the most probable number method.[19]

virus kill was somewhat lower, resulting in approximately 3 to 4 log at 500 J per m², but eventually more than 5 log at 1000 J per m² (medium-resistant viruses; Fig. 3B). For HIV and SV40, the virus kill was limited to approximately 1 log at 500 J per m² and approximately 2 log with doses up to 1000 J per m² (resistant viruses; Fig. 3C). Because cell-associated (CA) virus (intracellular and/or bound to the cellular membrane) may be more resistant to UVC damage, we also tested CA virus with HIV as a resistant virus and with VSV as a sensitive virus. In both cases, however, the CA-virus results mimicked those obtained with the corresponding cell-free viruses, that is, CA HIV was UVC-resistant and CA VSV was UVC-sensitive (Fig. 3D).

To further explore the resistance of HIV to UVC irradiation, we tested the effect of irradiation doses up to 4000 J per m². Although the virus kill slowly improved with increasing doses, infectious virus was still present even after irradiation at 4000 J per m² (RF = 3.5 log; data not shown).

Finally, we investigated the effect of UVC irradiation on the survival of bacteria. Experiments were performed with the same settings as for the virus studies. Irradiation at 250 J per m² resulted in greater than 4 log reduction for *S. epidermidis*, *S. aureus*, and *E. coli* and greater than 5 log at 500 J per m². In the case of *B. cereus*, the kill at 500 J per m² was limited to approximately 3 log (Fig. 4). An increase to 1000 J per m² resulted in a reduction of 3.7 log (data not shown). When after UVC irradiation, however, the *B. cereus* samples were incubated for 10 minutes at 80°C, all samples were below the detection limit (<0.5 log).

DISCUSSION

The risk of transmission of pathogens via cellular products, especially for PCs, is still a concern. Here we describe the potential of a UVC irradiation technique for pathogen inactivation in PCs.

Because of the high absorbance of UVC light by human plasma, we chose to study only PCs suspended in synthetic medium with 10 or 30 percent residual plasma. From the extinction coefficient per percentage of plasma (experimentally determined by us in a 1-cm cuvet as being close to 0.3), it can be calculated that, with a 1-mm light path as used in most of our experiments, 50 and 10 percent of the UVC light will reach the bottom of the suspension with, respectively, 10 or 30 percent plasma present. To avoid "dead" volumes not exposed to UVC, we chose the relative short light path of 1 mm, realizing that special containers of UV-permeable plastic of similar thickness would be required when the technique should be further developed into a blood bank procedure.

In our experimental setup, we then investigated whether varying a number of variables like percentage of plasma, irradiation dose, and light intensity would result in conditions with good pathogen inactivation in combination with good PLT quality. An acceptable compromise was found for 10 percent plasma in Composol, in combination with a depth of 1 mm, a light intensity of 0.25 mW per cm², and a total dose of 500 J per m². These conditions resulted in good (3-4 log) inactivation for the majority of pathogens tested with only limited effects on in vitro PLT quality. Evidently, this set of conditions can only be taken as a rough indication, because in this explorative study neither the irradiation nor the storage resembled blood bank conditions and the set of PLT quality variables was limited. The only quality variable for PCs mentioned in guidelines is the pH, which should be between 6.4 and 7.4 (at 22°C) according to European blood bank regulations.[21] Although this condition was met under all conditions tested, the results of the other in vitro quality variables indicated that in 10 percent plasma with doses higher than 500 J per m², PLT quality was seriously affected. An increase of 10 to 30 percent in CD62P-positive PLTs has been reported for standard PC at the end of their shelf life (5-7 days),[14,22,23] whereas with CD62P values higher than 50 percent the in vivo survival seems affected.[24] PS exposure during storage of standard PC usually remains below 20 percent at the end of the

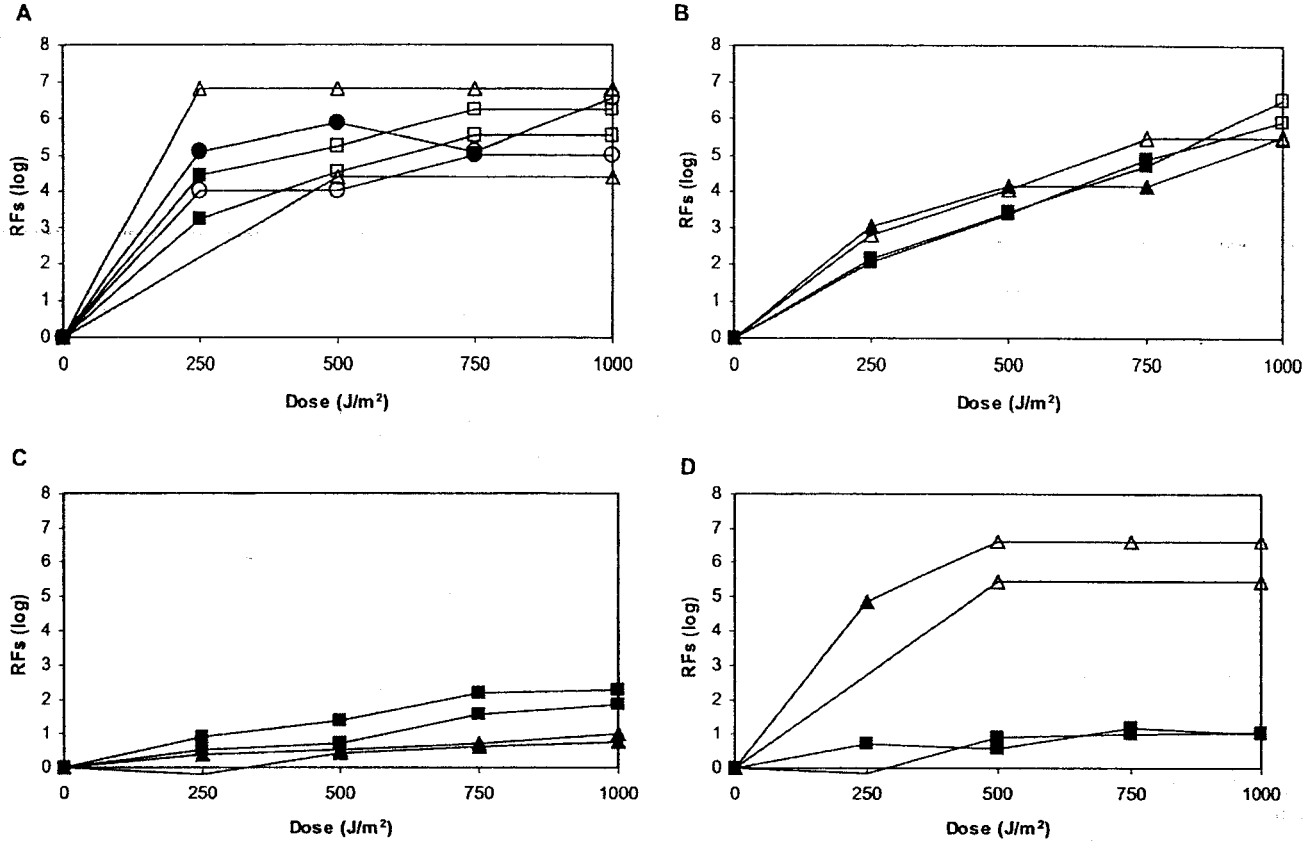

Fig. 3. Inactivation of different viruses by UVC irradiation. Virus inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. (A) Sensitive viruses (■ = CPV; ▲ = TGEV; ● = VSV); (B) medium-resistant viruses (■ = BVDV; Δ = PRV); (C) resistant viruses (■ = HIV; ▲ = SV40); (D) CA viruses (■ = CA HIV; ▲ = CA VSV). Open symbols indicate maximal reduction. Results shown are representative for at least two experiments.

shelf life,[22,23] but so far there are no data about correlation of PS exposure and in vivo survival.

In a factorial design, it was shown that the percentage of residual plasma was the major variable affecting the outcome of the UVC irradiations. As expected, the percentage of plasma resulted in opposite effects on pathogen inactivation and PLT quality. To a lesser extent, this opposite effect was also found for the light intensity, whereas the presence or absence of PLTs had a relatively minor, although significant, effect on pathogen inactivation. The presence of PLTs, resulting in a decrease of approximately 1 log of pathogen inactivation, should be taken into account in interpreting much of our inactivation data. Furthermore, the necessity to lower the residual plasma concentration to guarantee sufficient pathogen inactivation will require adjustment of current procedures to produce suitable PCs, but it has been shown in earlier studies[25,26] that this may be achievable. Because UVC irradiation results in extra glucose consumption, additional measures should be taken to ensure provision of glucose.

It was anticipated that in case of bacteria, high inactivation values would be observed[6] and this was indeed the case for all bacteria tested, with the exception of *B. cereus*. The reason for the decreased sensitivity of *B. cereus* is not quite clear, but one might speculate that formation of spores plays a role. It has been shown that *Bacillus* spores are 10 to 20 times more resistant to UVC irradiation;[27] thus formation of spores can cause a suboptimal kill of *Bacillus*. Because freshly prepared bacteria cultures were used containing relatively low amounts of spores (as determined by specific staining of spores, data not shown), however, a higher resistance toward UVC irradiation of *B. cereus* itself compared to other bacteria is also a possible explanation.[28,29] Moreover, the UVC-surviving bacteria were killed upon incubation at 80°C for 10 minutes, a treatment that spores will survive, also indicating that *B. cereus* itself has a higher resistance toward UVC.

We found a broad spectrum of viral sensitivity to UVC irradiation. For CPV, TGEV, and VSV we found very high inactivation, whereas the inactivation for BVDV and PRV

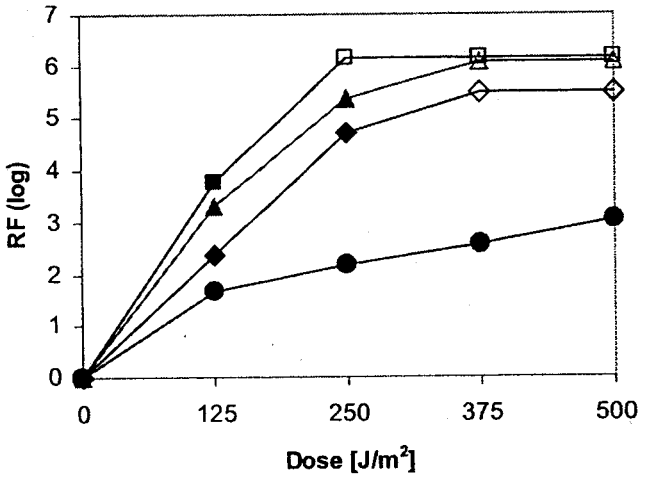

Fig. 4. Inactivation of different bacteria by UVC irradiation. (◆) *S. epidermidis*; (■) *S. aureus*; (▲) *E. coli*; and (●) *B. cereus*. Bacteria inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. Open symbols indicate maximal reduction. Results shown are representative of two experiments.

was less and slower. In the case of HIV and SV40, the inactivation was very limited and only 1 to 2 log was observed. Increasing doses of UVC irradiation also induced increased damage to HIV. Even a dose of 4000 J per m^2, however, did not result in complete inactivation. Interestingly, the efficacy of UVC was not different for cell-free and CA virus because CA HIV and CA VSV showed susceptibilities very similar to the corresponding cell-free virus. This indicates that the presence of infected cells is not an impediment for virus inactivation with UVC irradiation, provided that the virus in question is sensitive to UVC irradiation.

Based on our results we would rank the viruses in the following order with respect to UVC sensitivity: TGEV > VSV > CPV > PRV > BVDV > HIV > SV40. This ranking is exactly in line with previous observations and theoretical considerations postulating that UVC is especially effective on viruses with large genomes (i.e., PRV)[30] and on viruses with single-stranded nucleic acid genomes (i.e., TGEV, VSV, and CPV).[31] Furthermore, it has been described that RNA is less severely damaged than DNA, because pyrimidine dimers and more specifically thymine are the most frequent lesions caused by UVC irradiation.[7] Our results are also in line with Caillet-Fauquet and coworkers[6] who determined a sensitivity of MVM > EMC > BHV and Li and colleagues[32] who showed a sensitivity of CPV > BVDV > HAV > PRV. Wang and coworkers,[33] however, reported SV40 to be highly sensitive, more or less comparable to parvovirus. In contrast, we found that SV40 is very resistant, similar to the resistance found for HIV, as was also predicted by Lytle and Sagripanti.[34] The reason for this discrepancy remains unclear, especially because the possible explanation of cell line–dependent repair can be ruled out, because both studies propagated the virus in the cell line BSC.

Considering the ranking of virus inactivation as observed in this study and as predicted by Lytle and Sagripanti,[34] it can be concluded that single-stranded DNA or RNA viruses are effectively inactivated by UVC irradiation. This confirms that UVC is distinct from several other techniques with respect to its capacity to inactivate the NLE viruses like parvovirus B19 and HAV. This effective elimination of NLE viruses, combined with B19 contamination in several blood and plasma products, renders this technique interesting for further consideration.

SV40 has been regarded to be a very resistant virus and was often used in the past as a general model for NLE viruses with a DNA genome (like parvovirus B19). At present, however, specific model viruses for parvovirus B19 are applied and/or parvovirus B19 itself. Given the fact that CPV is very effectively inactivated by UVC irradiation, the relevance of SV40 as model virus for parvovirus B19 can be questioned. We do recommend, however, continuing studies with SV40 as a general model virus as this virus may be representative for new currently unknown threats to the blood supply. Indeed, the inability of UVC irradiation to inactivate viruses with small double-stranded genomes like SV40 illustrates possible limitations of this treatment.

BVDV and PRV are effectively inactivated, although the kinetics are slower compared to the sensitive group of viruses. Therefore, it is expected that UVC is capable of inactivating problematic blood-borne viruses like HBV and HCV. The inability of UVC irradiation to sufficiently inactivate HIV, a very relevant virus, however, is a major disadvantage. There seem to be several reasons for the resistance of HIV to UVC irradiation. HIV is a retrovirus with a small RNA genome. It has a single-stranded genome, but each virion encapsulates two copies of the viral RNA that are tightly linked and might serve as each other's back-up in case of UVC-induced damage. Indeed, strand transfers during reverse transcription are an integral part of the HIV life cycle.[35]

The observation that UVC does not effectively inactivate HIV may be partially compensated by careful and efficient donor screening for HIV. Both specific antibody and NAT are routinely performed and the risk of HIV transmission via cellular products is estimated to be less than 1 in 1 million.[36,37] One should again keep in mind, however, that new viruses may emerge with similar characteristics as HIV that would not be affected by this treatment in its current state. Given the broad inactivation of bacteria and viruses, we believe, however, that UVC irradiation for PCs is a promising technology that warrants further investigation.

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研究報告の概要

○アモトサレンおよび紫外線A波による光化学的プロセス:欧州の血液センター3施設での検証

背景:治療用血漿中の病原体および白血球(WBC)を不活化する光化学処理(PCT)プロセスが開発された。欧州の血液センター3施設において通常の稼動状況下でプロセスバリデーション試験を実施した。

試験デザインおよび方法:各センターで、アフェレーシス血漿30、PCT用全血由来血漿製剤30～36を用意した。全血由来血漿はいずれも、適合する供血血液2～3を混合したものであった。未処置対照検体(対照新鮮凍結血漿C-FFP)を除いてから、6 mmol/Lのアモトサレン15mLおよびUVA 3J/cm²で血漿546～635 mLを処理し、吸着装置を用いて残存アモトサレンを除去した。プロセス後、血漿検体(PCT-FFP)を採取し、採血後8時間まで-60℃で冷凍保存し、凝固因子および残存アモトサレンの測定を行った。

結果:合計186本の血漿にプロセスを実施した。C-FFPと比較してPCT-FFPの平均プロトロンビン時間(12.2±0.6秒)および活性化部分トロンボプラスチン時間(32.1±3.2秒)は若干延長した。フィブリノゲンおよび第VIII因子は、PCTへの感受性がもっとも高かった(平均減少率26%)。しかし、PCT処理-FFPを実施しても、治療用血漿に必要なフィブリノゲン(217±43mg/dL)と第VIII因子(97±29 IU/dL)は十分保持された。PCT-FFP中の第II、V、VII、IX、X、XI、XIII因子の平均値はC-FFP(活性保持81～97%)と同等であった。抗血栓性のタンパク質は、PCTによる有意な影響を受けず、83%～97%の範囲で保持された。アモトサレン平均残存量は、0.6±0.1 μmol/Lであった。

結論:欧州の3つのセンターにおけるプロセスバリデーション試験は、治療用血漿に関する欧州規制およびそれぞれの国内基準の範囲内で、PCT-FFP中の凝固因子の活性は保持されていた。

報告企業の意見	今後の対応
欧州の3つの血液センターにおけるアモトサレンおよび紫外線A波による不活化工程のプロセスバリデーション試験は、処理済FFP中の凝固因子が治療用血漿に関する欧州規制および国内基準の範囲内に保持されることを示したとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(3)

Photochemical treatment of plasma with amotosalen and UVA light: process validation in three European blood centers

Peter Schlenke, Tor Hervig,* Hervé Isola, Marie-Louise Wiesel, Daniel Kientz, Linda Pinkoski, Yasmin Singh, Lily Lin, Laurence Corash, and Jean-Pierre Cazenave**

BACKGROUND: A photochemical treatment (PCT) process has been developed to inactivate pathogens and white blood cells (WBCs) in therapeutic plasma. Process validation studies were performed in three European blood centers under routine operating conditions.

STUDY DESIGN AND METHODS: Each center prepared 30 apheresis and 30 to 36 whole blood–derived plasma units for PCT. Each whole blood–derived plasma unit contained a mixture of two to three matched donations. After removal of pretreatment control samples (control fresh-frozen plasma [C-FFP]), 546 to 635 mL of plasma was treated with 15 mL of 6 mmol per L amotosalen, 3 J per cm^2 UVA treatment, and removal of residual amotosalen with a compound adsorption device. After processing, plasma samples (PCT-FFP) were withdrawn, frozen at –60°C within 8 hours of collection, and assayed for coagulation factors and residual amotosalen.

RESULTS: A total of 186 units of plasma were processed. The mean prothrombin time (12.2 ± 0.6 sec) and activated partial thromboplastin time (32.1 ± 3.2 sec) of PCT-FFP were slightly prolonged compared to C-FFP. Fibrinogen and Factor (F)VIII were most sensitive to PCT (26% mean reduction). PCT-FFP, however, retained sufficient levels of fibrinogen (217 ± 43 mg/dL) and FVIII (97 ± 29 IU/dL) for therapeutic plasma. Mean levels of FII, FV, FVII, F IX, FX, FXI, and FXIII in PCT-FFP were comparable to C-FFP (81%-97% retention of activity). Antithrombotic proteins were not significantly affected by PCT with retention ranging between 83 and 97 percent. Mean residual amotosalen levels were 0.6 ± 0.1 µmol per L.

CONCLUSION: Process validation studies in three European centers demonstrated retention of coagulation factors in PCT-FFP within the required European and respective national standards for therapeutic plasma.

The INTERCEPT Blood System for plasma (Cerus Europe B.V., Leusden, the Netherlands) received CE Mark registration based on extensive studies demonstrating pathogen inactivation,[1] preclinical safety,[2] and clinical efficacy.[3-5] This system uses a photochemical treatment (PCT) process with amotosalen and long-wavelength ultraviolet UVA light (320-400 nm). Amotosalen is a synthetic psoralen molecule that reversibly intercalates into the helical regions of DNA and RNA. Upon illumination with UVA light, amotosalen forms irreversible covalent bonds with pyrimidine bases of the nucleic acid.[6] The genomes of pathogens and white blood cells (WBCs) modified by amotosalen can no longer replicate.

ABBREVIATIONS: aPTT(s) = activated partial thromboplastin time(s); AP = α2-antiplasmin; AT = antithrombin; CAD = compound adsorption device; C-FFP = control fresh-frozen plasma; PC = protein C; PCT = photochemical treatment; PCT-FFP = photochemically treated plasma samples; PS = protein S; PT(s) = prothrombin time(s).

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There are two components in the PCT system intended for commercial use: an integrated disposable set and a UVA illuminator. The integrated disposable set is a closed system composed of sequentially connected container with amotosalen, illumination container, a flowthrough compound adsorption device (CAD), and three plastic storage containers (Fig. 1). The UVA illuminator is a microprocessor-controlled device capable of delivering the target UVA dose and illuminating two plasma units simultaneously. The PCT system is capable of treating plasma units within the volume range of 385 to 650 mL.

Plasma units for PCT processing can be obtained by apheresis collection or by mixing 2 to 3 matched units of whole blood–derived plasma, analogous to whole blood–derived platelets (PLTs). After sterile connection to the PCT disposable set, plasma is passed through the series of containers. The PCT steps include addition of amotosalen to plasma, illumination of the plasma mixture with UVA light, and the removal of residual amotosalen. Although this system has been evaluated for preparation of plasma in specialized centers during clinical trials, process validation studies were conducted in blood centers to verify the performance of the PCT system under different routine operating conditions. Blood centers in three European countries (France, Norway, Germany) participated in these studies. Each center collected 30 units of apheresis plasma and prepared 30 to 36 units of whole blood–derived plasma with a target volume ranging from 600 to 655 mL. Residual amotosalen concentrations in photochemically treated plasma samples (PCT-FFP) were measured to assess CAD performance. The performance of the PCT system was assessed based on in vitro coagulation function assays in treated plasma (PCT-FFP) compared to the plasma before treatment (C-FFP) as well as to European and national regulatory requirements for therapeutic plasma.

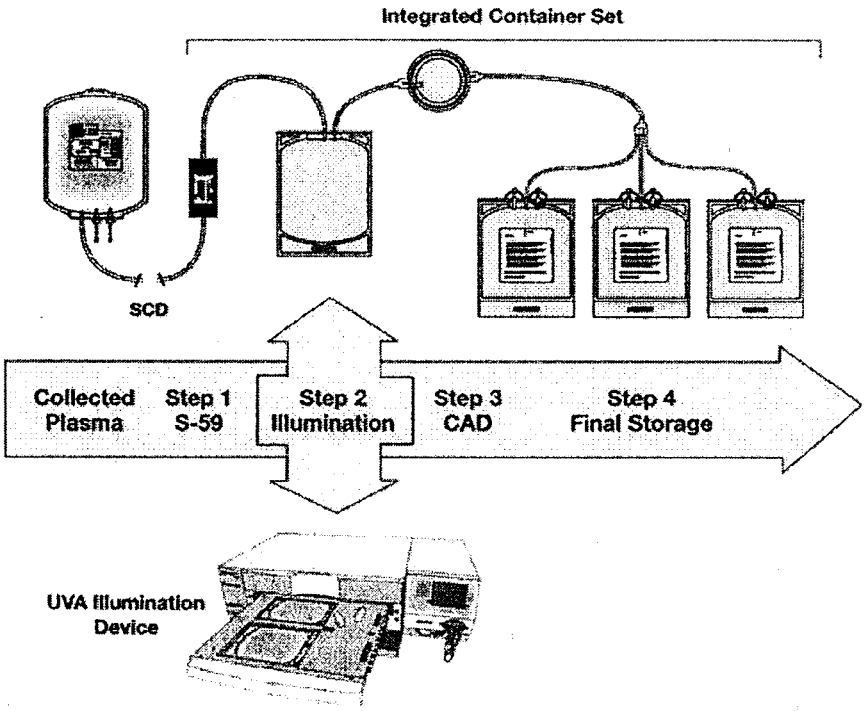

Fig. 1. The PCT system for plasma. The PCT system consists of a UVA illumination device and an integral disposable set. The UVA illuminator can illuminate 2 units of plasma per processing cycle. The disposable set provides a single-use, closed, integrated system for pathogen inactivation treatment of a plasma unit. The integrated disposable set is comprised of the following sterile components: a container with amotosalen (also known as S-59), a plastic illumination container, a CAD, and three plastic storage containers. The processing steps are as described under Materials and Methods section. SCD = sterile connection device.

MATERIALS AND METHODS

Plasma collection

Plasma collections were performed in three European blood centers and the collections methods varied slightly. In the Etablissement Français du Sang-Alsace in Strasbourg, France (Site S), apheresis plasma was collected on the Haemonetics PCS platform (Haemonetics Corp., Braintree, MA) in acid citrate dextrose anticoagulant. In blood centers of the University of Lübeck, Institute of Immunology and Transfusion Medicine, Lübeck, Germany (Site L), and Haukeland University Hospital, Bergen, Norway (Site B), apheresis plasma was collected on the Autopheresis-C platform (Baxter Healthcare Corp., Deerfield, IL) in citrate or in half-strength citrate-phosphate-dextrose (CPD) anticoagulant, respectively. Each site collected 30 units of apheresis plasma. Of the 90 apheresis units processed for this study, 27 were blood group A, 24 group AB, 22 group B, and 17 group O. The target volume of plasma was 600 to 655 mL. Fresh apheresis plasma units were kept at ambient temperature before and during the PCT process.

Whole blood was collected in CPD anticoagulant. Plasma was prepared by centrifugation with local standard operating procedures. A leukofiltration step was incorporated in the whole blood process only in Etablissement Français du Sang-Alsace in Strasbourg. Sites S and B each prepared 30 units of whole blood–derived plasma and Site L prepared 36 units. Of the 96 units, 44 were

blood group O, 42 group A, 7 group B, and 3 group AB. The target volume for the whole blood plasma unit before removing baseline samples was 600 to 655 mL and was obtained by mixing the appropriate volume of 2 to 3 ABO-matched whole blood–derived plasma units with a pooling set (FTC 0061, Baxter Healthcare Corp.). The plasma units were maintained at ambient temperature before and during the PCT process.

PCT disposable sets and UVA illumination device

The PCT disposable set for treatment of plasma (INT 3103 and INT 3104, Cerus Europe B.V.) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen HCl solution in saline packaged inside a plastic container (PL 2411, Baxter Healthcare Corp.) and protected from UVA light; a 1.3-L plastic container (PL 2410, Baxter Healthcare Corp.) for illumination of plasma; a flowthrough CAD consisting of an adsorbent disk composed of a copolymer of polystyrene and divinylbenzene particles fused with an ultrahigh-molecular-weight polyethylene plastic enclosed in an acrylic housing to reduce the concentration of amotosalen and its photoproducts; and three 400-mL plastic containers (PL 269, Baxter Healthcare Corp.) for storage of the treated plasma.

Illumination of plasma was performed in a UVA illumination device (Model INT100, Cerus Europe B.V.). The device was capable of illuminating 2 units of plasma per processing cycle. During illumination plasma units were reciprocally agitated at approximately 70 cycles per minute.

PCT process

For these studies, the entire process was completed to allow frozen storage of treated plasma within 8 hours of the start of plasma collection. A Luer adapter was sterile-connected to each plasma unit. Baseline coagulation factor samples (C-FFP) of approximately 20 mL were collected from each unit before PCT.

During the treatment process, plasma was passed through each component of the PCT set in a series of steps (Fig. 1). In Step 1, the plasma unit was sterile-connected to the amotosalen container, and the entire plasma volume was passed through the amotosalen container into the illumination container. In Step 2, the plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA treatment. In Step 3, the illuminated plasma mixture was passed by gravity flow through the CAD into the storage containers.

After processing, plasma samples (PCT-FFP) were withdrawn and frozen in 1.5-mL aliquots in 2-mL polypropylene tubes at or below –60°C within 8 hours of the start of plasma or whole blood collection. The C-FFP and PCT-FFP samples were assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), Factor (F)I (fibrinogen), FII, FV, FVII, FVIII, F IX, FX, FXI, FXIII, protein C (PC), protein S (PS), antithrombin (AT), and α2-antiplasmin (AP). Samples were also withdrawn after addition of amotosalen before UVA illumination and after the entire PCT process (including CAD) for measurement of amotosalen concentrations. To minimize assay variability, samples collected from the three processing centers were shipped on dry ice to a single location for analysis. All assays were performed at Cerus Corp. with the exception of quantification of AP, which was performed by a reference laboratory (Esoterix Laboratories, Aurora, CO).

Measurement of the levels of amotosalen

The initial and residual amotosalen levels in each plasma unit were quantified. A 200-µL volume of plasma was diluted to 1 mL with 35 percent methanol in buffer. After centrifugation, the supernatant was filtered and 100 µL were analyzed on a C-18 (Zorbax) reverse-phase column (Agilent Technologies, Palo Alto, CA) with a gradient of increasing methanol in KH_2PO_4 buffer. Amotosalen was detected by optical absorption (300 nm).[7]

Measurement of in vitro coagulation function

Clottable fibrinogen (FI) was measured with a modified Clauss assay. Coagulation factors were assayed with one-stage PT-based clotting assays (FII, FV, FVII, FX) or one-stage aPTT-based clotting assays (FVIII, F IX, FXI). The clotting time of a mixture of diluted test plasma and plasma deficient in the factor being quantified was compared with a reference curve constructed from the clotting times of 5 dilutions, ranging from 1:5 to 1:320, of plasma with known activity mixed with deficient plasma. These coagulation assays, as well as the PT and aPTT, were performed on an automated coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co., Lexington, MA). Reagents included brain thromboplastin (Hemoliance, Instrumentation Laboratory Co.), Platelin L (bioMérieux, Durham, NC), and congenitally depleted factor-deficient substrate (Helena Laboratories, Beaumont, TX; George King Bio-Medical, Overland Park, KS). The endpoint of all tests was the formation of a clot detected photooptically and measured in seconds. Factor Assay Control Plasma (FACT; George King Bio-Medical) was used as the reference standard for the procoagulation factor assays.

FXIII was measured with a commercially available FXIII kit (Berichrom, Dade Behring, Marburg, Germany). FXIII, activated by thrombin, releases an activation product that leads to a series of reactions resulting in a decrease in nicotinamide adenine dinucleotide, detected by monitoring absorbance at 340 nm. The assay was per-

formed on a Behring Clot Timer (BCT, Dade Behring), and standard human plasma (Dade Behring) was used as the reference standard.

PC and PS were measured with commercially available PC and PS kits (Staclot, both from Diagnostica Stago, Asnieres, France). PC and PS assays were based on prolongation of the aPTT resulting from inactivation of FV and FVIII by activated PC. The activator in the PC assay was an extract of *Agkistrodon contortrix* snake venom; the activator in the PS assay was activated PC. The tests were performed on the Behring Clot Timer. Unicalibrator (Diagnostica Stago) was used as the reference standard.

AT was measured with a commercially available ATIII kit (Stachrom, Diagnostica Stago). Plasma containing AT was incubated with a known excess of thrombin. A chromogenic substrate, imidolyzed by the remaining thrombin, was detected photooptically on a coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co.). Factor assay control plasma was used as the reference standard.

α2-AP was quantified with reagents from Diagnostica Stago. In this chromogenic method, plasmin was added in excess to the test plasma, resulting in the formation of antiplasmin–plasmin complexes. The concentration of residual plasmin is measured by its amidolytic activity with a chromogenic substrate measured at 405 nm. α2-AP concentration is inversely proportional to the residual plasmin concentration and is determined by color intensity. This analysis was performed by Esoterix Laboratories (Aurora, CO) with an STA analyzer (Diagnostica Stago).

The mean and standard deviation (SD) were determined for each coagulation variable. All factor activities were expressed in IU per dL with the exception of fibrinogen, which is expressed in mg per dL. The activity of each coagulation variable remaining after PCT was also expressed as proportional (%) retention compared to the pretreatment (baseline) activity. Comparison of the PT and aPTT was based on the prolongation of the clotting time in seconds after PCT relative to baseline. Significant differences were determined by the t test at a p value of 0.05. Reference ranges for each assay were defined as the mean ± 2 SD for untreated plasma samples.[1]

RESULTS

Processing of plasma

A total of 186 units of plasma of approximately 600 to 655 mL was prepared in this study. After removal of control samples, the volume per plasma unit for PCT processing ranged from 546 to 635 mL. The mean pretreatment amotosalen concentration was 143 ± 8 µmol per L (Table 1). The illumination of a 3 J per cm^2 UVA treatment took 7 to 9 minutes. The mean time required for the plasma mixture to completely pass through the CAD was 21 ± 3 minutes (range, 15-30 min). After CAD, the mean residual amotosalen concentration was 0.6 ± 0.1 µmol per L (Table 1). All values were below the target performance value of less than 2.0 µmol per L. The PLT and RBC concentrations for both apheresis and whole blood–derived plasma before treatment and after treatment were less than 50×10^9 and less than 6×10^9 per L, respectively, and were within the European guidelines for therapeutic FFP.[8] The WBC concentrations for apheresis plasma were less than 0.1×10^9 per L before and after treatment. The WBC concentrations for nonleukofiltered whole blood plasma in Site L and Site B were higher before treatment ($6 \times 10^9 \pm 7 \times 10^9$ and $38 \times 10^9 \pm 22 \times 10^9$/L, respectively); however, after treatment, the WBC concentrations in PCT-FFP were within the European guidance limits.

TABLE 1. Amotosalen concentrations before illumination and after CAD treatment*

Measure	Amotosalen (µmol/L) Before illumination	After CAD
Target range	110-225	<2
Apheresis plasma		
Site S† (n = 30)	139 ± 4	0.5 ± 0.1
Site B† (n = 30)	151 ± 6	0.5 ± 0.1
Site L† (n = 30)	142 ± 2	0.7 ± 0.1
Whole blood–derived plasma		
Site S (n = 30)	136 ± 8	0.6 ± 0.1
Site B (n = 30)	136 ± 4	0.5 ± 0.1
Site L (n = 36)	150 ± 3	0.6 ± 0.1
Overall (n = 186)	143 ± 8	0.6 ± 0.1

* Data are reported as mean ± SD.
† Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.

The effect of PCT on global coagulation assays (PT and aPTT)

The mean C-FFP and PCT-FFP PT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP PTs were prolonged by a mean of 0.3 ± 0.2 seconds for apheresis plasma (n = 90) and 0.5 ± 0.1 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4).

Similarly, the mean C-FFP and PCT-FFP aPTT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP aPTTs were prolonged by a mean of 3.5 ± 1.3 seconds for apheresis plasma (n = 90) and 4.6 ± 0.9 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4). The overall (apheresis and whole blood combined for an n = 186) mean PCT-FFP PT and aPTT compared to C-FFP was prolonged by 0.4 ± 0.2 and 4.1 ± 1.2 seconds, respectively (Table 4).

TABLE 2. Apheresis plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	12.2 ± 0.6	12.7 ± 0.7	0.5 ± 0.2	12.3 ± 0.7	12.5 ± 0.7	0.2 ± 0.2	12.0 ± 0.4	12.4 ± 0.5	0.3 ± 0.2
aPTT (sec)	23-35	27.9 ± 2.5	32.4 ± 3.2	4.5 ± 1.1	26.1 ± 2.5	28.5 ± 2.7	2.3 ± 1.0	27.8 ± 2.5	31.5 ± 3.1	3.8 ± 0.8
FI (mg/dL)	200-390	295 ± 71	208 ± 55	70 ± 5	313 ± 48	261 ± 43	83 ± 3	287 ± 57	216 ± 43	75 ± 3
FII (IU/dL)	80-120	102 ± 17	89 ± 15	88 ± 7	117 ± 15	107 ± 16	91 ± 3	103 ± 14	91 ± 12	88 ± 3
FV (IU/dL)	95-170	120 ± 29	116 ± 26	97 ± 3	144 ± 23	142 ± 20	99 ± 5	125 ± 19	119 ± 17	95 ± 3
FVII (IU/dL)	70-175	116 ± 25	93 ± 21	80 ± 4	112 ± 27	92 ± 22	82 ± 3	111 ± 23	91 ± 19	81 ± 3
FVIII (IU/dL)	85-235	140 ± 34	99 ± 25	70 ± 3	161 ± 42	130 ± 35	81 ± 4	123 ± 39	91 ± 30	74 ± 3
F IX (IU/dL)	75-145	90 ± 13	75 ± 10	84 ± 4	101 ± 18	88 ± 14	87 ± 3	93 ± 12	78 ± 11	84 ± 4
FX (IU/dL)	75-130	108 ± 21	95 ± 19	88 ± 4	117 ± 20	106 ± 19	91 ± 3	112 ± 14	99 ± 12	88 ± 2
FXI (IU/dL)	60-150	93 ± 16	79 ± 13	85 ± 8	111 ± 19	101 ± 7	91 ± 6	103 ± 20	90 ± 18	87 ± 2
FXIII (IU/dL)	85-135	114 ± 22	110 ± 22	97 ± 9	126 ± 18	121 ± 18	96 ± 5	115 ± 21	108 ± 20	94 ± 3
PC (IU/dL)	80-140	122 ± 25	102 ± 24	84 ± 9	122 ± 20	107 ± 19	88 ± 4	119 ± 22	101 ± 20	85 ± 2
PS (IU/dL)	85-135	108 ± 25	107 ± 25	100 ± 9	108 ± 18	103 ± 17	95 ± 6	92 ± 20	88 ± 19	96 ± 3
AT (IU/dL)	85-105	102 ± 9	97 ± 9	95 ± 4	105 ± 9	101 ± 8	96 ± 4	95 ± 10	91 ± 10	95 ± 3
AP (IU/dL)	80-150	97 ± 11	81 ± 8	83 ± 10	108 ± 13	89 ± 8	83 ± 6	101 ± 12	82 ± 6	82 ± 8

* Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 3. Whole blood–derived plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable†	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.5 ± 0.3	12.0 ± 0.3	0.4 ± 0.1	11.5 ± 0.4	12.0 ± 0.4	0.6 ± 0.1
aPTT (sec)	23-35	29.5 ± 1.8	34.2 ± 2.4	4.7 ± 0.7	28.6 ± 1.8	32.9 ± 2.4	4.3 ± 0.9	28.2 ± 1.4	33.1 ± 2.1	4.9 ± 0.8
FI (mg/dL)	200-390	290 ± 25	207 ± 23	71 ± 3	291 ± 30	222 ± 24	76 ± 3	272 ± 40	191 ± 30	70 ± 3
FII (IU/dL)	80-120	106 ± 8	95 ± 9	89 ± 3	100 ± 9	89 ± 8	89 ± 3	101 ± 7	89 ± 7	88 ± 3
FV (IU/dL)	95-170	123 ± 14	119 ± 13	97 ± 2	125 ± 15	121 ± 14	97 ± 3	119 ± 18	114 ± 17	96 ± 3
FVII (IU/dL)	70-175	113 ± 15	91 ± 12	80 ± 3	108 ± 14	86 ± 11	80 ± 3	114 ± 17	90 ± 14	79 ± 3
FVIII (IU/dL)	85-235	127 ± 24	91 ± 19	71 ± 3	118 ± 20	91 ± 17	77 ± 4	119 ± 23	84 ± 19	71 ± 4
F IX (IU/dL)	75-145	93 ± 10	78 ± 8	84 ± 3	92 ± 11	78 ± 10	85 ± 2	95 ± 9	78 ± 6	82 ± 4
FX (IU/dL)	75-130	108 ± 9	94 ± 8	87 ± 1	106 ± 12	92 ± 11	87 ± 3	105 ± 10	90 ± 9	85 ± 2
FXI (IU/dL)	60-150	83 ± 14	69 ± 12	83 ± 4	101 ± 11	87 ± 12	86 ± 6	94 ± 9	81 ± 9	85 ± 3
FXIII (IU/dL)	85-135	104 ± 12	101 ± 12	97 ± 2	121 ± 10	111 ± 10	92 ± 3	113 ± 14	106 ± 12	94 ± 3
PC (IU/dL)	80-140	115 ± 14	97 ± 12	85 ± 5	114 ± 19	102 ± 16	90 ± 5	116 ± 16	99 ± 14	85 ± 7
PS (IU/dL)	85-135	111 ± 15	107 ± 14	96 ± 3	117 ± 14	114 ± 14	97 ± 4	114 ± 16	109 ± 15	96 ± 5
AT (IU/dL)	85-105	101 ± 7	97 ± 7	96 ± 2	96 ± 4	93 ± 4	96 ± 2	91 ± 6	86 ± 6	95 ± 2
AP (IU/dL)	80-150	100 ± 8	85 ± 5	85 ± 5	95 ± 5	78 ± 4	82 ± 4	93 ± 6	76 ± 4	82 ± 3

* Site S: = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 4. Combined clotting times and coagulation factor activity before and after PCT (mean ± SD): comparison apheresis plasma and whole blood–derived plasma

		Apheresis plasma (n = 90)			Whole blood plasma (n = 96)			Overall (n = 186)		
Variable	Reference range*	C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†
PT (sec)	11.1-13.5	12.2 ± 0.6	12.5 ± 0.6	0.3 ± 0.2	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.8 ± 0.6	12.2 ± 0.6	0.4 ± 0.2
aPTT (sec)	23-35	27.3 ± 2.6	30.8 ± 3.4	3.5 ± 1.3	28.7 ± 1.7	33.4 ± 2.3	4.6 ± 0.9	28.0 ± 2.3	32.1 ± 3.2	4.1 ± 1.2
FI (mg/dL)	200-390	299 ± 60	228 ± 52‡	76 ± 7‡	284 ± 34	206 ± 29‡	72 ± 4‡	291 ± 49	217 ± 43	74 ± 6
FII (IU/dL)	80-120	107 ± 17‡	95 ± 16	89 ± 5	102 ± 8‡	91 ± 8	89 ± 3	105 ± 13	93 ± 13	89 ± 4
FV (IU/dL)	95-170	130 ± 26‡	126 ± 24‡	97 ± 4	122 ± 16‡	118 ± 15‡	97 ± 3	126 ± 22	122 ± 20	97 ± 3
FVII (IU/dL)	70-175	113 ± 25	92 ± 21‡	81 ± 4‡	112 ± 15	89 ± 12‡	80 ± 3‡	112 ± 21	90 ± 17	81 ± 3
FVIII (IU/dL)	85-235	141 ± 41‡	107 ± 35‡	75 ± 6‡	121 ± 23‡	88 ± 18‡	73 ± 5‡	131 ± 34	97 ± 29	74 ± 5
F IX (IU/dL)	75-145	95 ± 15‡	80 ± 13‡	85 ± 4‡	94 ± 10‡	78 ± 8‡	84 ± 3‡	94 ± 13	79 ± 11	84 ± 4
FX (IU/dL)	75-130	112 ± 18‡	100 ± 17‡	89 ± 4‡	106 ± 10‡	92 ± 9‡	86 ± 2‡	109 ± 15	96 ± 14	88 ± 3
FXI (IU/dL)	60-150	102 ± 19‡	90 ± 18‡	88 ± 7‡	93 ± 13‡	79 ± 13‡	85 ± 5‡	97 ± 17	84 ± 17	86 ± 6
FXIII (IU/dL)	85-135	119 ± 21‡	113 ± 21‡	96 ± 6	113 ± 14‡	106 ± 12‡	94 ± 3	116 ± 18	110 ± 17	95 ± 5
PC (IU/dL)	80-140	121 ± 22	103 ± 21	85 ± 6	115 ± 16	99 ± 14	87 ± 6	118 ± 20	101 ± 18	86 ± 6
PS (IU/dL)	85-135	103 ± 22‡	99 ± 22‡	97 ± 7‡	114 ± 15‡	110 ± 15‡	96 ± 4‡	109 ± 20	105 ± 19	97 ± 5
AT (IU/dL)	85-105	100 ± 12‡	95 ± 12	95 ± 3	95 ± 7‡	91 ± 7	96 ± 2	98 ± 9	94 ± 9	96 ± 3
AP (IU/dL)	80-150	102 ± 12‡	83 ± 8‡	82 ± 7	96 ± 7‡	79 ± 6‡	83 ± 4	99 ± 11	82 ± 7	83 ± 6

* The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
† For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.
‡ Significant differences between apheresis and whole blood plasma were detected at a p value of less than 0.05.

The effect of PCT on procoagulant factors

The mean procoagulant factor activities in apheresis C-FFP obtained in each processing site were within reference ranges (Table 2). Apheresis plasma processed with the PCT system retained mean factor activity ranging from 70 to 83 percent and 70 to 81 percent of C-FFP for fibrinogen (FI) and FVIII, respectively. Retentions of FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 80 to 99 percent (Table 2). After PCT, the mean procoagulant factor activities were also within the reference ranges. Mean FVIII activities were 99 ± 25, 130 ± 35, and 91 ± 30 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

Similarly, the mean activity levels of the procoagulant factors in whole blood–derived plasma were within reference ranges (Table 3). Whole blood plasma processed with the PCT system retained mean coagulation factor activities ranging from 70 to 76 and 71 to 77 percent of C-FFP for FI and FVIII, respectively. The mean retentions for FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 79 to 97 percent (Table 3). After PCT, the mean factor activities were also within the reference ranges with the exception of FI and FVIII in Site L. Mean FVIII activities were 91 ± 19, 91 ± 17, and 84 ± 19 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). Whole blood–derived plasma generally exhibited statistically significantly lower factor activities compared to apheresis plasma (see Table 4). All mean values, however, fell within the reference ranges. The mean FVIII activity in apheresis PCT-FFP (107 ± 35 IU/dL, n = 90) and whole blood–derived plasma (88 ± 18 IU/dL, n = 96) met the national and European Pharmacopoeia requirements for therapeutic plasma.

The overall (n = 186) mean level of fibrinogen in PCT-FFP was 217 ± 43 mg per dL, which is 74 ± 6 percent of the C-FFP values. The overall (n = 186) mean activity of FVIII in PCT-FFP was 97 ± 29 IU per dL, which is 74 ± 5 percent of the C-FFP values. The retention of other factors in PCT-FFP was consistently higher (81%-97%; Table 4).

The effect of PCT on antithrombotic and fibrinolytic protein activity

All pretreatment activities of PC, PS, AT III, and α2-AP of apheresis plasma (Table 2) and whole blood–derived plasma (Table 3) were within reference ranges. After PCT, the mean activities of these antithrombotic proteins were still within the reference ranges with two exceptions: the

mean levels of AP activity in PCT-FFP from Site B (78 ± 4 IU/dL) and Site L (76 ± 4 IU/dL) were slightly outside the lower limit (80 IU/dL) of the reference range.

After PCT, the mean retention of antithrombotic proteins ranged from 84 to 90 percent for PC and 82 to 85 percent for AP (Tables 2 and 3). No differences were observed between apheresis and whole blood–derived plasma. Similarly, the mean retention in PCT-FFP ranged from 95 to 100 percent for PC and 95 to 96 percent for AT (Tables 2 and 3). The results were comparable between apheresis and whole blood–derived plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). There were no significant differences in the activity of PC of either C-FFP or PCT-FFP between apheresis and whole blood–derived plasma. Whole blood–derived plasma contained significantly higher levels of PS before and after PCT than apheresis plasma (see Table 4). In contrast, the levels of AT and AP were generally lower in whole blood–derived plasma compared to apheresis plasma and reached significance (see Table 4).

The overall (n = 186) mean activities of the antithrombotic proteins in C-FFP and PCT-FFP were within the reference ranges. After PCT, mean retention of PC and AP was 86 and 83 percent, respectively, whereas mean retention of PS and AT was 97 and 96 percent (Table 4).

DISCUSSION

The PCT process for preparation of pathogen-inactivated FFP involves the addition of amotosalen to a nominal concentration of 150 µmol per L (range, 110-225 µmol/L), illumination of the plasma mixture with a 3 J per cm² UVA treatment, and removal of residual amotosalen to less than 2.0 µmol per L by a flow CAD. Three European centers participated in this study to validate the process under routine blood bank operation conditions. Each center processed 30 units of apheresis plasma and 30 to 36 units of whole blood–derived plasma with integral disposable sets that have received CE Mark approval and commercial UVA illuminator. The PCT process was completed within the time frame for FFP, allowing units to be frozen within 8 hours of collection.

The mean pretreatment amotosalen concentration from all three sites was 143 ± 8 µmol per L (n = 186), which is well within the target system performance range of 110 to 225 µmol per L. The use of the microprocessor-controlled UVA illuminator ensured delivery of the UVA treatment dose of 3 J per cm². All three centers demonstrated the addition of the correct amotosalen concentration, combined with a 3 J per cm² UVA treatment dose, thus ensuring robust pathogen inactivation. The mean amotosalen level after the CAD treatment for all centers was 0.6 ± 0.1 µmol per L (n = 186), which is significantly below the target performance value of 2.0 µmol per L. The mean residual amotosalen levels among the three sites ranged from 0.5 ± 0.1 to 0.7 ± 0.1 µmol per L with no units having residual amotosalen higher than 1.2 µmol per L, demonstrating the consistency and the efficacy of the CAD. These results demonstrate that the PCT process can be performed consistently under blood bank conditions.

The quality of PCT-FFP was assessed for activity of FVIII with respect to meeting national and European regulatory guidelines.[8] The consistency of the PCT process was assessed by the retention of all factor activities in PCT-FFP compared to levels in pretreatment plasma samples.

The factor most affected by PCT was FVIII with a mean of 26 percent reduction in activity. However, residual activity is within the current requirement for FFP as the level of FVIII is greater than 50 IU per dL in the European Pharmacopoeia standard for therapeutic FFP.[9] The mean FVIII activity after PCT was 107 ± 35 IU per dL (n = 90) for apheresis plasma and 88 ± 18 IU per dL (n = 96) for whole blood–derived plasma or an overall mean of 97 ± 29 IU per dL (n = 186). All units had FVIII activity greater than 50 IU per dL. In France, greater than 90 percent of quality control samples must have greater than 70 IU per dL in FVIII. Preliminary studies measuring the thrombin generation time for PCT-FFP have shown no difference from untreated plasma for peak thrombin levels, lag time to start of thrombin generation, or total thrombin produced.[10] These observations suggest that the reduction in FVIII levels are not critical to generation of thrombin and the ultimate conversion of fibrinogen to fibrin. These observations of normal thrombin generation are in contrast to those recently reported for plasma prepared with methylene blue and visible light.[11]

Fibrinogen was also affected by PCT with a mean of 26 percent reduction in the clottable fibrinogen levels. Although there is no required standard for the level of fibrinogen in FFP, the mean levels retained in PCT-FFP (217 ± 43 mg/dL, n = 186) were within the reference range. Prior clinical studies with PCT-FFP for support of liver transplant with massive transfusion have shown no increased requirement for plasma or cryoprecipitate, indicating that the levels of fibrinogen in PCT-FFP are sufficient.[4] These patients have a significant period of fibrinolytic activity after unclamping of the transplanted liver. The study examined the use of conventional FFP, cryoprecipitate, and PCT-FFP for support of these patients and observed no differences to indicate that the reduced levels of fibrinogen in PCT-FFP were clinically relevant. In addition, the levels of AP activity are reasonably conserved in PCT-FFP.[1] Although the levels of fibrinogen are reduced by the treatment, the levels appear adequate to support hemostasis in patients with active fibrinolysis.

Other coagulation factors (FII, FV, FVII, F IX, FX, FXI, FXIII) were less affected by PCT. The mean factor activities in PCT-FFP were within the reference ranges. Retention of activity after PCT ranged from 81 to 97 percent. Of specific importance were the levels of FVII, which is the factor with the shortest half-life and thus the most critical for transfusion support of acquired complex coagulopathy. In addition, levels of the anticoagulant PC and PS and AT were relatively unaffected by PCT and α2-AP was well conserved.

There was slight prolongation in PT and aPTT. PCT-FFP, however, retained PT and aPTT within the reference range. The slight changes in PT and aPTT after PCT were not associated with any adverse clinical observations in controlled clinical trial settings, and treatment of congenital coagulation defects has demonstrated consistent correction of both the PT and aPTT after transfusion with PCT-FFP.[3-5,12]

The results of this study demonstrate that there is good retention of relevant coagulation factor activities and antithrombotic protein function in PCT-FFP from either apheresis or whole blood and that these products meet the requirements for therapeutic plasma. In a separate study, the effect of storage on FI, FII, FV, FVII, FVIII, F IX, FX, and FXI has been evaluated.[13] The results show that therapeutic levels of these factors were conserved in PCT-FFP after 12 months of storage at −18°C and after 18 months of storage at −25°C. Similar results were obtained in storage studies conducted at one of the three centers (Site S, data not shown) with PCT-FFP prepared from apheresis plasma frozen up to 1 year. In addition, clinical trials with PCT-FFP have shown that this product is sufficient for therapeutic support of patients with each of the major clinical indications for plasma transfusion.

The effect of PCT on plasminogen and von Willebrand factor (VWF) has also been evaluated.[14,15] After treatment, plasminogen was within normal ranges and retained 94 percent. The von Willebrand antigen, VWF:ristocetin cofactor, components of the von Willebrand complex, including multimers and VWF:CP activity, remained within normal ranges and demonstrated greater than 98 percent retention. Because of the stability of these factors after treatment, they were not included in the current validation study.

When the results were compared between sites or between types of plasma, significant differences were observed, although the differences were small, not likely of clinical relevance, and did not appear to follow a specific pattern. The observed differences could simply be due to the geographic variation in the plasma characteristics and the slight variation in the processing techniques. Of particular interest is that the FVIII activities as well as the retention for apheresis PCT-FFP in Site B were significantly higher than the values obtained in the other two sites. This difference could not be completely explained by the different apheresis collection platforms. Site S used the Haemonetics platform, but the same Baxter platform was used in both Site B and Site L. The observed difference between Site B and Site L was most likely due to the variation in donor population and processing techniques. Different anticoagulants may introduce variability but were poorly defined and not well evaluated. Overall, PCT-FFP manufactured in the three different geographic locations were of comparable quality. All met the respective national and European standards for transfusable FFP.

Previous studies with cryoprecipitate prepared from photochemically treated plasma yielded approximately 95 and 88 percent retention of fibrinogen and FVIII, respectively, compared to cryoprecipitate prepared from untreated plasma.[16] Cryosupernatant prepared from photochemically treated plasma retained adequate levels of critical plasma proteins for plasma exchange therapy in acute thrombocytopenic purpura. These data indicate good preservation of hemostasis control proteins such as PS, α2-AP, and VWF-cleaving protease activity.[17]

In summary, the results of process validation studies from three European centers demonstrated the consistency of the PCT process for FFP. From a blood center perspective, scaleup manufacturing of PCT-FFP in routine is feasible by the ability to treat individual large-volume units of fresh apheresis plasma and small pools of whole blood–derived plasma. The mixture of whole blood plasma from two or three matched donations is similar to the procedure for whole blood–derived PLT components. Since adult patients will require 4 to 6 FFP units (200 mL each) for a therapeutic episode, donor exposures are consistent with current practice in which whole blood plasma units are processed individually. A similar PCT system utilizing amotosalen and UVA light for PLT components has been in routine use in some blood centers in European countries.[18] Both PLT and plasma components are treated with the same UVA illumination device thus simplifying the logistics of implementation of two pathogen inactivation systems in one blood center.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 5. 26	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Blue DE, Cruz J, Limiac A, Spinola S, Davis TE, Waxman D, McCarthy L, Smith D. American Society for Microbiology 108th General Meeting; 2008 Jun 1-5; Boston.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○輸血による*Babesia microti*死亡例

輸血によって*Babesia microti*に感染し死亡する例は赤血球を含む輸血100万単位あたり1件未満と見積もられている。疾患は通常無症候性だが、無脾症、高齢、免疫抑制状態の患者では感染によって死に至ることがある。

症例:腎臓疾患で透析を必要としていた61歳の女性患者。入院45日前に赤血球2単位を輸血され、その後更に2単位追加輸血された。入院前日、吐き気と発熱を訴えたため、血液培養をオーダーし、抗生物質が投与された。リハビリ施設に戻る際に、体温は39.4℃を示し、低血圧で、昇圧剤を必要とし、敗血症の症状を呈した。血液塗抹標本では、赤血球の5~15%にトロフォゾイト(栄養体)があり、*Plasmodium falciparum*か*B. microti*と考えられた。静注キニジン及びクリンダマイシン投与が開始された。赤血球交換により寄生虫血症は1%まで低下した。投薬は適切だったが、播種性血管内凝固症候群(DIC)を発症し6日後に死亡した。外出や旅行はしていなかったため、唯一のリスクファクターは輸血と考えられた。

結果:*Babesia*はCDCで形態学的に確認された。患者の入院時の検体では6%の寄生虫血症と*B. microti* PCR陽性が認められた。輸血された製剤の供血者4名のうち1名がIFAで*B. microti*陽性となった。供血者はダニに噛まれた記憶はなく、流行地域に旅行したこともなかった。

結論:上の臨床症状と転帰は*Babesia*の輸血伝播による死亡例の中では珍しいものではないが、中西部で発生したという点が他と異なっている。ベクターIxodes scapularisが寄生する中西部のオジロジカの頭数増加に伴い、供血者における*Babesia microti*抗体陽性率を解明する為の研究を行うべきである。

報告企業の意見	今後の対応
輸血によると考えられる*Babesia microti*に感染し死亡した症例の報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Abstract Title: Fatal Transfusion-Transmitted *Babesia microti*

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Presentation Number: C-198

Poster Board Number: 0335

Keywords: Babesia,transfusion-transmitted

Background: Fatal transfusion-transmitted *B. microti* has an estimated incidence of <1:1,000,000 per unit of transfused red cell containing blood products. The disease is usually asymptomatic; however, fatal infections occur in asplenic, elderly or immunosuppressed individuals. **Case Report:** The 61-year-old female patient had renal disease requiring dialysis. Forty-five days prior to admission she received two units of packed red cells and then two more. One day prior to admission, the patient complained of nausea and fever. Blood cultures were ordered and antibiotics administered. Upon returning to the rehabilitation facility, she spiked temperatures to 103°F and was admitted to the hospital. She was hypotensive, requiring vasopressor support, and appeared to be septic. The blood smear revealed trophozoites in 5 to 15% of red cells, probable species: *Plasmodium falciparum* vs. *B. microti*. Treatment with intravenous quinidine and clindamycin was begun. A red cell exchange reduced parasitemia to 1%. Despite appropriate medication, the patient developed disseminated intravascular coagulation and expired 6 days later. Since she was confined indoors and did not travel, the only risk factor was transfusion. **Results:** *Babesia* was confirmed morphologically by CDC with 6% parasitemia and PCR positivity for *B. microti* from the patient's specimen at admission. The three donors available for testing were negative for *B. microti* and all samples were negative for *P. falciparum* by PCR. One blood donor and the patient were positive for *B. microti* by immunofluorescent antibody (IFA). The seropositive donor had no recollection of a tick bite and did not travel to endemic areas. **Conclusion:** The above clinical presentation and course is not atypical for rare fatal cases of transfusion-transmitted *Babesia*. This is an unusual case as it arose in the Midwest. With the expanding Midwest white-tailed deer populations harboring the vector, *Ixodes scapularis*, studies to determine the regional incidence of *Babesia microti* seropositive blood donors may be warranted.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
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一般的名称	人赤血球濃厚液	研究報告の公表状況	飯岡大, 前追善智, 中村文彦, 林孝昌, 津田勝代. 第56回日本輸血・細胞治療学会総会; 2008 Apr 25-27; 福岡.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○血小板濃厚液の輸血後に、急性呼吸不全と*Bacillus cereus* (*B. cereus*)による髄膜炎を併発した症例

【緒言】輸血後細菌感染症は、診断・治療に難渋し、時に致命的な状態になることもある。我々は、*B. cereus*の輸血後感染症で急性呼吸不全および重症髄膜炎を併発した症例を経験し、その治療および診断経過が今後の対策につき有用と考えられここに報告する。

【症例】76歳女性。64歳に再生不良性貧血と診断、免疫抑制療法に不応で、71歳よりは赤血球および血小板輸血が定期的に必要となった。平成19年4月、血小板輸血を施行中、発熱・悪寒、その後急速な呼吸不全を認め、胸部X線・心エコー検査より、輸血関連肺障害(TRALI)と判断した。メチルプレドニゾロン500mg投与で呼吸状態は改善し発熱も消退した。しかし発症12時間後、嘔気・頭痛の出現と共に再び発熱を認めた。感染症を考え直ちに抗生剤を開始したが悪化し、発症16時間後には右方への眼球偏位と意識障害(昏睡)が出現した。髄液検査にて細胞数・蛋白の増加を認め、脳波でも異常波を認めたことから、細菌性髄膜炎および症候性てんかんと診断した。その後、抗生剤および抗てんかん薬が奏効し、発症第13日には意識清明となり、発症第25日には後遺障害なく退院できた。輸血関連感染の診断目的に当院で各種培養検査を施行したところ、血小板残液の鏡検・培養検査で*B. cereus*が検出された。髄液では、初回抗生剤投与後に採取した影響もあり鏡検・培養検査は陰性であったが、遺伝子検査PCR法にて、血小板製剤と同一菌株の*B. cereus*が検出され、今症例が輸血後感染症から髄膜炎に進展したと考えられた。一方で、凍結処理された供血者保存血漿では、培養検査・遺伝子検査共に陰性であった。

【考察】TRALI様の急性呼吸不全を呈した際は、輸血後感染症も視野に入れた対応が必要である。髄膜炎併発例の報告はこれまでに無いが、輸血後感染症治療では髄液移行性も考慮した抗生剤選択が求められる。培養検査だけでなく、遺伝子検査まで施行することが、診断及び同一菌株の証明に重要である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

自発報告:2007年5月28日付1-07000033

報告企業の意見	今後の対応
血小板濃厚液の輸血後に、TRALI様の急性呼吸不全と髄膜炎を併発し、血小板残液から*Bacillus cereus*が検出された症例の報告である。 本症例について、日本赤十字社では抗白血球抗体、抗顆粒球抗体検査を実施し、臨床経過及び診断基準よりTRALIであると評価した。患者の血液培養が陰性で、当該血小板製剤と同一採血時の凍結血漿では無菌試験陰性であったことから、輸血による細菌感染があったかどうかは不明である。	日本赤十字社では、輸血による細菌感染予防対策として平成18年10月より血小板製剤について、また、平成19年3月より全血採血由来製剤について、初流血除去を導入した。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。さらに、輸血情報リーフレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

(19)

WS-3-3 血小板濃厚液の輸血後に，急性呼吸不全と Bacillus cereus (B.cereus) による髄膜炎を併発した症例

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【緒言】輸血後細菌感染症は，診断・治療に難渋し，時に致命的な状態になることもある．我々は，*B.cereus* の輸血後感染症で急性呼吸不全および重症髄膜炎を併発した症例を経験し，その治療および診断経過が今後の対策につき有用と考えられここに報告する．【症例】76 歳女性．64 歳に再生不良性貧血と診断，免疫抑制療法に不応で，71 歳よりは赤血球および血小板輸血が定期的に必要となった．平成 19 年 4 月，血小板輸血を施行中，発熱・悪寒，その後急速な呼吸不全を認め，胸部 X 線・心エコー検査より，輸血関連肺障害（TRALI）と判断した．メチルプレドニゾロン 500mg 投与で呼吸状態は改善し発熱も消退した．しかし発症 12 時間後，嘔気・頭痛の出現と共に再び発熱を認めた．感染症を考え直ちに抗生剤（ビアペネム）を開始したが悪化し，発症 16 時間後には右方への眼球偏位と意識障害（昏睡）が出現した．髄液検査にて細胞数・蛋白の増加を認め，脳波でも異常波を認めたことから，細菌性髄膜炎および症候性てんかんと診断した．その後，抗生剤（バンコマイシンも併用）および抗てんかん薬が奏効し，発症第 13 日には意識清明となり，発症第 25 日には後遺障害なく退院できた．輸血関連感染の診断目的に当院で各種培養検査を施行したところ，血小板残液の鏡検・培養検査で *B.cereus* が検出された．髄液では，初回抗生剤投与後に採取した影響もあり鏡検・培養検査は陰性であったが，遺伝子検査 PCR 法にて，血小板製剤と同一菌株の *B.cereus* が検出され，今症例が輸血後感染症から髄膜炎に進展したと考えられた．一方で，凍結処理された供血者保存血漿では，培養検査・遺伝子検査共に陰性であった．【考察】TRALI 様の急性呼吸不全を呈した際は，輸血後感染症も視野に入れた対応が必要である．髄膜炎併発例の報告はこれまでに無いが，輸血後感染症治療では髄液移行性も考慮した抗生剤選択が求められる．培養検査だけでなく，遺伝子検査まで施行することが，診断及び同一菌株の証明に重要である．

別紙様式第2-1

No. 9

医薬品 研究報告 調査報告書

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				2008. 4. 9	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		SignOnSanDiego.com. 2008 Mar 26.	米国

研究報告の概要

○カリフォルニア州サンディエゴ郡の梅毒症例急激に増加

カリフォルニア州サンディエゴ郡の年間梅毒症例数は、最低となった2000年の28例から昨年(2007年)は340例まで急増した。州の他の大都市の郡と比べて非常に急激な増加である。増加率は州全体の2倍以上、全国の3倍以上になる。州から派遣された5名の専門家チームは、梅毒と診断された人々と連絡をとって、性的パートナーを探し、検査を受けるよう勧めている。

全国的に、梅毒感染者の大部分は他のHIV陽性の男性と無防備な性交渉を行うHIV陽性の男性であり、サンディエゴ郡でも同様の傾向が見られる。昨年、当郡で梅毒と診断された人の84パーセントは男性と性交渉がある男性(MSM)であり、大半はHIV陽性だった。米国疾病対策予防センター(CDC)は、医師に対し、MSMの患者が年に1度梅毒のルーチンの検査を受けるよう奨励することを求めている。

連邦当局は、梅毒と他の性感染症(STD)の蔓延に対しても懸念を抱いている。先週、CDCは全国の梅毒症例数が7年連続で増加したと発表した。CDCは連邦の予算配分を変更してSTD教育と予防の取り組みに力を入れるよう要求している。AIDSや癌、心臓病と比較すると、米国のSTDの症例数は多くはない。しかし、連邦、州および地域の当局は、STD症例の増加が重要な公衆衛生問題であると認識している。

使用上の注意記載状況・その他参考事項等

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照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
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vCJD等の伝播のリスク

報告企業の意見	今後の対応
カリフォルニア州サンディエゴ郡の梅毒症例数が、2000年以降急増しているとの報告である。	日本赤十字社では、輸血感染症対策として問診時に梅毒を含む性感染症の既往歴を確認し、梅毒の既往歴、治療歴のある人は献血不適としている。また、献血血液に対して梅毒血清学的検査を実施し、陽性血液を排除している。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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More Health news

Syphilis cases up sharply in county

STD prevention, education efforts to be heightened

By Cheryl Clark
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Alarmed by San Diego County's more than 1,100 percent rise in syphilis cases between 2000 and last year, state health officials are using five investigators to help the region stem the spread.

"San Diego's increase is a cause for concern because we're just not understanding why it's being transmitted in the frequency that we're seeing," said Dr. Douglas Hatch, chief of California's division of communicable disease control.

The search for causes and solutions includes debate about whether safe-sex education needs to be explicit and targeted at men who have sex with men. That group accounts for most of the syphilis infections in the county and nationwide.

San Diego County's annual syphilis caseload skyrocketed from 28 in 2000 – when the infection total hit a low – to 340 last year. It is a much sharper rise than in the state's other large urban counties, including San Francisco, Los Angeles, Orange and Alameda. It's also more than double the statewide percentage of increase and triple that of the nation.

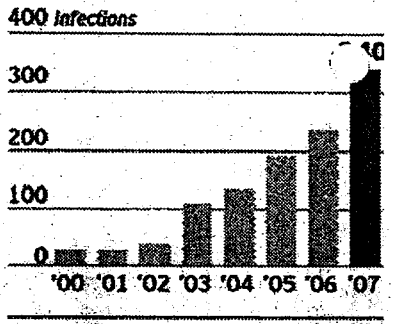
The team that the state dispatched to San Diego County consists of three investigators who recently started aiding county officials and two who were already working with them but have increased their level of assistance. They are contacting people who were diagnosed with syphilis, finding their sexual partners and urging those people to get tested so they can receive treatment and not transmit the bacteria.

Federal officials are also concerned by the spread of syphilis and other sexually transmitted diseases. Last week, the U.S. Centers for Disease Control and Prevention said the national syphilis case count had increased for the seventh consecutive year.

The agency wants to change federal funding formulas so states and counties can obtain more money for STD education and prevention efforts.

The United States' STD numbers aren't huge when compared to those for AIDS, cancer or heart disease.

REPORTED SYPHILIS INFECTIONS COUNTYWIDE



SOURCE: California Dept. of Public Health
MATT PERRY / Union-Tribune

REPORTED SYPHILIS INFECTIONS BY COUNTY

Sampling of counties with large populations

County	2000	2007	Change
San Diego	28	340	1,1 %
Los Angeles	152	827	444%
Orange	28	141	404%
San Francisco	54	202	274%
Alameda	14	39	179%
Statewide	330	2,002	507%

SOURCE: California Dept. of Public Health
MATT PERRY / Union-Tribune

But officials at the federal, state and local levels all have identified the growth of STDs as an important public health issue Public agencies and nonprofit groups spend hundreds of millions of dollars each year on STD prevention and testing.

Nationwide, an increasingly large portion of people infected with syphilis are HIV-positive men who have unprotected se with other HIV-positive men.

The trend is reflected in San Diego County. Last year, 84 percent of the people diagnosed with syphilis in San Diego County were men who had sex with men, and a big segment of them were HIV-positive, state health officials said.

Medical experts said the region's proximity to the border and its substantial tourist trade also could be contributing to

unsafe sex, which leads to higher numbers of syphilis cases and other STDs.

Syphilis likely has spread in San Diego County because at-risk men aren't telling health care providers about their sexual behavior and don't get tested, said Terry Cunningham, chief of the county's HIV, STD and Hepatitis branch.

He also blames health care providers who don't consistently address the issue with their patients.

The CDC is asking physicians to be more diligent in encouraging male patients who have sex with men to undergo routine screenings for syphilis, perhaps once a year.

Public health officials and various nonprofits diverge on a major point – how best to emphasize STD prevention. They ask:

▪How explicit must the anti-STD message be to draw enough attention?

▪Will public-service ads that are candid about sexual practices turn off or offend some people?

▪Should awareness campaigns aggressively focus on men who have sex with men, perhaps by using images of male couples?

San Diego County's approach is to address all individuals who face exposure to sexually transmitted diseases, said Holly Crawford, a spokeswoman for the county's Health and Human Services Agency.

"Targeting only specific populations . . . could lead to a false sense of security among the general population," she said. "STDs do not recognize gender, age, sexual orientation or location."

Greg Cox, chairman of the county Board of Supervisors, said the county is "addressing the issue of sexually transmitted diseases among all at-risk populations through bilingual education campaigns, alerts to doctors and STD education and testing at county clinics.

"I'm concerned about any disease or illness that affects the people of our region . . . but I am optimistic that our efforts wil succeed in bringing down the disturbing case numbers."

The county has allocated more than $1.5 million in state funds for this fiscal year to five nonprofit groups that work on STD prevention. They are the Family Health Centers of San Diego; San Diego Lesbian, Gay, Bisexual Transgender Community Center; San Diego Youth and Community Center; San Ysidro Health Center; and Vista Community Clinic.

The Vista clinic does outreach in neighborhoods where people who engage in high-risk sexual activities congregate, said director Barbara Mannino. The four other groups declined to discuss how they're using their grants.

The county also tries to reach high-risk populations in central San Diego by placing safe-sex ads in bus shelters and gay-themed publications.

Despite those efforts, some who work on STD prevention said more must be done to connect with specific risk groups.

"San Diego County's message is, 'We're all at risk.' That's true, but some people are much more at risk than others. What are they doing to target gay men and their sexual practices in bathhouses, for example?" said Oscar de la O, a founding member and president of Bienestar.

The nonprofit group tries to educate high-risk individuals in Southern California about syphilis and other STDs.

Bienestar outreach worker Abigail Madariaga is atransgendered woman, which means she was born a man but identifies and lives as a woman. On a recent evening, she and fellow STD educator Antonio Munoz talked to people watching a drag queen performance at Urban Mo's bar and nightclub in Hillcrest.

Madariaga said her orientation allows her to spread the safe-sex message more effectively to many of the estimated 1,500 other transgendered people in the region, as well as to gays, lesbians and bisexuals watching the drag show.

In San Francisco, public health workers spend time in Internet chat rooms, where gay men often make their connections. They began doing so after discovering how syphilis was spreading in many cases, said Dr. Jeffrey Klausner, director of STD prevention for the San Francisco Department of Public Health.

San Francisco has established medical clinics friendly to the gay community. Its health officials also conduct public awareness campaigns that show sex "as a positive, normal and healthy activity, rather than use fear and avoidance as a motivator," Klausner said.

One of those initiatives was the "Healthy Penis" program, which featured outreach workers dressed in costumes resembling penises and syphilis sores. That got people's attention, Klausner recalled.

The city has seen a drop in syphilis cases – from 348 cases in 2004 to 202 last year.

Klausner blamed the spread of syphilis elsewhere on reduced funding for anti-STD efforts and said health leaders in each community need to target their messages to those most at risk. "Increasing rates of STDs are what you can expect when there are no resources," he said.

San Diego County's share of state money for STD education and prevention this fiscal year is down $280,000 from last year.

Beyond money, many safe-sex campaigns are thwarted by politicians' fear of candor, said Michael Weinstein, president of the AIDS Healthcare Foundation in Los Angeles.

"They must make it obvious they are talking about sex," he said. "The sooner we stop treating this as a dirty little secret, the better off we'll be."

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感染症定期報告の報告状況(2008/6/1～2008/8/31)

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
80101	2008/06/06	ベネシス	ポリエチレングリコール処理抗破傷風人免疫グロブリン 乾燥抗破傷風人免疫グロブリン	破傷風抗毒素	人血液	米国	有効成分	有	無	無
80102	2008/06/10	バイエル薬品	pH4 処理酸性人免疫グロブリン	人免疫グロブリンG	ヒト血液	米国	有効成分	有	無	無
80103	2008/06/17	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液	人血液	日本	有効成分	有	有	無
80104	2008/06/17	日本赤十字社	人全血液	人全血液	人血液	日本	有効成分	有	無	無
80105	2008/06/17	日本赤十字社	抗HBs人免疫グロブリン	抗HBs人免疫グロブリン	人血液	日本	有効成分	有	無	無
80106	2008/06/17	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液	人血液	日本	有効成分	有	有	無
80107	2008/06/24	化学及血清療法研究所	乾燥スルホ化人免疫グロブリン	スルホ化人免疫グロブリンG	ヒト血液	①米国、②日本	有効成分	有	無	無
80108	2008/06/27	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	無	無
80109	2008/06/27	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	無	無
80110	2008/07/03	ベネシス	人ハプトグロビン	人ハプトグロビン	人血液	日本	有効成分	有	無	無
80111	2008/07/15	化学及血清療法研究所	乾燥濃縮人血液凝固第Ⅷ因子	血液凝固第Ⅷ因子	ヒト血液	日本	有効成分	有	無	無
80112	2008/07/16	富士フィルムRIファーマ	テクネチウム大凝集人血清アルブミン(99mTc)	テクネチウム大凝集人血清アルブミン(99mTc)	ヒト血液	米国	有効成分	有	無	無
80113	2008/07/24	CSLベーリング	乾燥濃縮人アンチトロンビンⅢ	乾燥濃縮人アンチトロンビンⅢ	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
80114	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ新生仔血清	ウシ血液	ニュージーランド	製造工程	無	無	無
80115	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ胎仔血清	ウシ血液	ニュージーランド、オーストラリア、米国及びカナダ	製造工程	無	無	無
80116	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ブタ膵臓由来トリプシン	ブタ膵臓(抽出物)	不明	製造工程	無	無	無
80117	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	該当しない	有効成分	無	無	無
80118	2008/07/28	日本製薬	乾燥人血液凝固第Ⅸ因子複合体	血液凝固第Ⅸ因子複合体	人血液	日本	有効成分	有	無	無
80119	2008/07/29	日本メジフィジックス	放射性医薬品基準ガラクトシル人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc)注射液	ガラクトシル人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc)	生物学的製剤基準人血清アルブミン	日本	有効成分	無	無	無
80120	2008/07/29	日本赤十字社		合成血	人血液	日本	有効成分	有	無	無
80121	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-1)	ウシ血液	米国	製造工程	無	有	無
80122	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ウシ血清アルブミン	ウシ血液	米国	製造工程	無	有	無

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
80123	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	有	無
80124	2008/07/31	バクスター	加熱人血漿たん白	人血清アルブミン	人血漿	米国	有効成分	無	有	無
80125	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ膵臓	米国	製造工程	無	有	無
80126	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	人血清アルブミン	人血漿	米国	添加物	無	有	無
80127	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	アプロチニン	ウシ肺	ニュージーランド	製造工程	無	有	無
80128	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ血液	オーストラリア	製造工程	無	有	無
80129	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	補養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-2)	ウシ肝臓	米国又はカナダ	製造工程	無	有	無
80130	2008/08/14	富士フィルムRIファーマ	ヨウ化人血清アルブミン(131I)	ヨウ化人血清アルブミン(131I)	ヒト血液	日本	有効成分	有	無	無
80131	2008/08/25	日本製薬	①加熱人血漿たん白 ②人血清アルブミン(5%) ③人血清アルブミン(20%) ④人血清アルブミン(25%) ⑤乾燥ポリエチレングリコール処理人免疫グロブリン ⑥トロンビン ⑦乾燥濃縮人アンチトロンビンⅢ ⑧人免疫グロブリン ⑨乾燥人血液凝固第Ⅸ因子複合体	ヘパリン	ブタ腸粘膜	ブラジル	①～⑧製造工程、⑨添加物・製造工程	無	無	無
80132	2008/08/27	CSLベーリング	人血清アルブミン 破傷風抗毒素 フィブリノゲン加第XIII因子 乾燥濃縮人アンチトロンビンⅢ	ヘパリンナトリウム	ブタ腸粘膜	中国	製造工程	無	無	無
80133	2008/08/27	CSLベーリング	人C1-インアクチベーター	人C1-インアクチベーター	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
80134	2008/08/28	化学及血清療法研究所	乾燥抗破傷風人免疫グロブリン	抗破傷風人免疫グロブリン	ヒト血液	米国	有効成分	有	無	有
80135	2008/08/28	化学及血清療法研究所	乾燥人血液凝固第Ⅸ因子複合体 乾燥濃縮人血液凝固第Ⅸ因子 乾燥濃縮人アンチトロンビンⅢ 人免疫グロブリン フィブリノゲン加第ＸⅢ因子 乾燥濃縮人活性化プロテインC ヒスタミン加人免疫グロブリン製剤 トロンビン 乾燥スルホ化人免疫グロブリン 人血清アルブミン 乾燥ペプシン処理人免疫グロブリン	ヘパリンナトリウム	ブタ腸粘膜	中国、フランス、米国、カナダ	製造工程	無	無	無
80136	2008/08/28	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	無	無

別紙様式第4

感染症発生症例一覧

番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	感染症および寄生虫症	菌血症	日本	男	51	2008/3/29	①回復	症例報告	当該製品	2008/4/15提出、識別番号1-08000029　未完了報告
2	感染症および寄生虫症	細菌感染	日本	女	64	2008/3/10	②軽快	症例報告	当該製品	2008/3/28提出、識別番号1-07000289　未完了報告
3	感染症および寄生虫症	細菌感染	日本	女	90	2008/3/5	⑤死亡	症例報告	当該製品	2008/3/21提出、識別番号1-07000278　未完了報告
4	感染症および寄生虫症	B型肝炎	日本	男	65	2008/2/19	③未回復	症例報告	当該製品	2008/3/11提出、識別番号1-07000249　未完了報告
5	感染症および寄生虫症	C型肝炎	日本	女	39	2008/2/19	⑥不明	症例報告	当該製品	2008/3/25提出、識別番号1-07000279　未完了報告
6	感染症および寄生虫症	C型肝炎	日本	女	39	2008/2/19	③未回復	症例報告	当該製品	2008/4/8提出、識別番号1-07000279　未完了報告（5番と同一症例）
7	感染症および寄生虫症	細菌感染	日本	男	67	2008/2/18	①回復	症例報告	当該製品	2008/3/4提出、識別番号1-07000244　未完了報告
8	感染症および寄生虫症	C型肝炎	日本	女	28	2008/2/16	④回復したが後遺症あり	症例報告	当該製品	2008/3/28提出、識別番号1-07000290　未完了報告
9	感染症および寄生虫症	敗血症性ショック	日本	女	82	2008/2/5	②軽快	症例報告	当該製品	2008/3/4提出、識別番号1-07000242　未完了報告
10	感染症および寄生虫症	敗血症	日本	女	82	2008/2/5	②軽快	症例報告	当該製品	2008/3/4提出、識別番号1-07000242　未完了報告（9番と同一症例）
11	感染症および寄生虫症	B型肝炎	日本	男	81	2008/2/4	③未回復	症例報告	当該製品	2008/2/21提出、識別番号1-07000231　未完了報告
12	感染症および寄生虫症	B型肝炎	日本	男	81	2008/2/4	⑤死亡	症例報告	当該製品	2008/3/24提出、識別番号1-07000231　未完了報告（12番と同一症例）
13	感染症および寄生虫症	C型肝炎	日本	女	75	2008/1/30	③未回復	症例報告	当該製品	2008/3/18提出、識別番号1-07000253　未完了報告
14	感染症および寄生虫症	C型肝炎	日本	女	85	2008/1/29	③未回復	症例報告	当該製品	2008/3/11提出、識別番号1-07000250　未完了報告
15	感染症および寄生虫症	C型肝炎	日本	女	72	2008/1/23	②軽快	症例報告	当該製品	2008/3/21提出、識別番号1-07000277　未完了報告
16	感染症および寄生虫症	C型肝炎	日本	男	35	2008/1/21	③未回復	症例報告	当該製品	2008/2/15提出、識別番号1-07000227　未完了報告
17	感染症および寄生虫症	C型肝炎	日本	男	57	2008/1/16	⑥不明	症例報告	当該製品	2008/2/14提出、識別番号1-07000222　未完了報告
18	感染症および寄生虫症	C型肝炎	日本	男	57	2008/1/16	⑥不明	症例報告	当該製品	2008/3/31提出、識別番号1-07000222　取り下げ（17番と同一症例）
19	感染症および寄生虫症	細菌感染	日本	男	74	2008/1/14	①回復	症例報告	当該製品	2008/2/5提出、識別番号1-07000200　未完了報告
20	感染症および寄生虫症	B型肝炎	日本	男	71	2008/1/9	⑥不明	症例報告	当該製品	2008/1/25提出、識別番号1-07000181　未完了報告
21	感染症および寄生虫症	B型肝炎	日本	男	66	2008/1/9	③未回復	症例報告	当該製品	2008/1/30提出、識別番号1-07000186　未完了報告
22	感染症および寄生虫症	B型肝炎	日本	男	65	2008/1/8	⑥不明	症例報告	当該製品	2008/2/5提出、識別番号1-07000202　未完了報告
23	感染症および寄生虫症	C型肝炎	日本	女	76	2007/12/26	③未回復	症例報告	当該製品	2008/4/16提出、識別番号1-08000030　未完了報告
24	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	2	2007/12/25	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000229　未完了報告
25	感染症および寄生虫症	C型肝炎	日本	女	42	2007/12/19	③未回復	症例報告	当該製品	2008/1/9提出、識別番号1-07000171　未完了報告
26	感染症および寄生虫症	敗血症	日本	男	74	2007/12/16	③未回復	症例報告	当該製品	2008/1/7提出、識別番号1-07000165　未完了報告
27	感染症および寄生虫症	C型肝炎	日本	女	97	2007/12/14	④回復したが後遺症あり	症例報告	当該製品	2008/1/7提出、識別番号1-07000166　未完了報告
28	感染症および寄生虫症	B型肝炎	日本	男	84	2007/12/14	⑥不明	症例報告	当該製品	2008/1/25提出、識別番号1-07000182　未完了報告
29	感染症および寄生虫症	C型肝炎	日本	女	80	2007/12/12	③未回復	症例報告	当該製品	2008/1/9提出、識別番号1-07000170　未完了報告
30	感染症および寄生虫症	C型肝炎	日本	男	62	2007/12/11	③未回復	症例報告	当該製品	2008/1/8提出、識別番号1-07000169　未完了報告
31	感染症および寄生虫症	C型肝炎	日本	男	53	2007/12/7	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000162　未完了報告
32	感染症および寄生虫症	C型肝炎	日本	女	84	2007/12/7	⑥不明	症例報告	当該製品	2008/3/6提出、識別番号1-07000247　未完了報告
33	感染症および寄生虫症	細菌感染	日本	女	83	2007/11/19	①回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000135　未完了報告
34	感染症および寄生虫症	B型肝炎	日本	男	83	2007/11/16	③未回復	症例報告	当該製品	2007/12/5提出、識別番号1-07000139　未完了報告

別紙様式第4

感染症発生症例一覧

第10回

35	感染症および寄生虫症	B型肝炎	日本	男	83	2007/11/16	⑥不明	症例報告	当該製品	2008/2/1提出、識別番号1-07000139　取り下げ（34番と同一症例）
36	感染症および寄生虫症	B型肝炎	日本	男	62	2007/11/15	③未回復	症例報告	当該製品	2007/12/14提出、識別番号1-07000145　未完了報告
37	感染症および寄生虫症	B型肝炎	日本	男	69	2007/11/14	③未回復	症例報告	当該製品	2007/12/19提出、識別番号1-07000149　未完了報告
38	感染症および寄生虫症	B型肝炎	日本	男	69	2007/11/14	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000149　未完了報告（37番と同一症例）
39	感染症および寄生虫症	伝染性紅斑	日本	女	55	2007/11/12	②軽快	症例報告	当該製品	2007/12/28提出、識別番号1-07000161　未完了報告
40	感染症および寄生虫症	細菌感染	日本	男	90	2007/11/9	②軽快	症例報告	当該製品	2007/11/27提出、識別番号1-07000129　未完了報告
41	感染症および寄生虫症	B型肝炎	日本	女	61	2007/11/9	③未回復	症例報告	当該製品	2007/11/27提出、識別番号1-07000130　未完了報告
42	感染症および寄生虫症	C型肝炎	日本	女	80	2007/11/7	②軽快	症例報告	当該製品	2007/11/28提出、識別番号1-07000132　未完了報告
43	感染症および寄生虫症	B型肝炎	日本	女	63	2007/11/6	⑥不明	症例報告	当該製品	2007/11/26提出、識別番号1-07000127　未完了報告
44	感染症および寄生虫症	B型肝炎	日本	女	26	2007/11/5	③未回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000137　未完了報告
45	感染症および寄生虫症	C型肝炎	日本	男	58	2007/10/26	②軽快	症例報告	当該製品	2007/11/19提出、識別番号1-07000123　未完了報告
46	感染症および寄生虫症	細菌感染	日本	男	50	2007/10/24	②軽快	症例報告	当該製品	2007/11/8提出、識別番号1-07000114　未完了報告
47	感染症および寄生虫症	B型肝炎	日本	女	34	2007/10/19	③未回復	症例報告	当該製品	2007/11/8提出、識別番号1-07000113　未完了報告
48	感染症および寄生虫症	B型肝炎	日本	男	56	2007/10/19	①回復	症例報告	当該製品	2007/11/13提出、識別番号1-07000117　未完了報告
49	感染症および寄生虫症	B型肝炎	日本	女	30	2007/10/15	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000136　未完了報告
50	感染症および寄生虫症	B型肝炎	日本	女	30	2007/10/15	⑥不明	症例報告	当該製品	2007/12/11提出、識別番号1-07000136　未完了報告（49番と同一症例）
51	感染症および寄生虫症	B型肝炎	日本	女	30	2007/10/15	③未回復	症例報告	当該製品	2008/3/31提出、識別番号1-07000136　完了報告（49番、50番と同一症例）
52	感染症および寄生虫症	B型肝炎	日本	男	84	2007/10/12	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000107　未完了報告
53	感染症および寄生虫症	B型肝炎	日本	女	62	2007/10/12	③未回復	症例報告	当該製品	2007/11/19提出、識別番号1-07000124　未完了報告
54	感染症および寄生虫症	B型肝炎	日本	男	82	2007/10/1	⑥不明	症例報告	当該製品	2007/11/19提出、識別番号1-07000122　未完了報告
55	感染症および寄生虫症	B型肝炎	日本	男	82	2007/10/1	③未回復	症例報告	当該製品	2008/1/7提出、識別番号1-07000122　未完了報告（54番と同一症例）
56	感染症および寄生虫症	C型肝炎	日本	女	78	2007/9/22	②軽快	症例報告	当該製品	2007/11/5提出、識別番号1-07000108　未完了報告
57	感染症および寄生虫症	B型肝炎	日本	男	59	2007/9/10	③未回復	症例報告	当該製品	2007/11/26提出、識別番号1-07000125　未完了報告
58	感染症および寄生虫症	C型肝炎	日本	女	70	2007/9/3	⑤死亡	症例報告	当該製品	2007/12/4提出、識別番号1-07000095　取り下げ 第10回症例番号58は前回報告における第9回症例番号4において報告したものの追加報告
59	感染症および寄生虫症	C型肝炎	日本	男	67	2007/8/30	③未回復	症例報告	当該製品	2007/12/18提出、識別番号1-07000097　取り下げ 第10回症例番号59は前回報告における第9回症例番号6において報告したものの追加報告
60	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/11/20提出、識別番号1-07000104　未完了報告 第10回症例番号60は前回報告における第9回症例番号8において報告したものの追加報告
61	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	②軽快	症例報告	当該製品	2007/11/13提出、識別番号1-07000116　未完了報告
62	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	①回復	症例報告	当該製品	2008/2/1提出、識別番号1-07000116　取り下げ（61番と同一症例）
63	感染症および寄生虫症	B型肝炎	日本	女	34	2007/8/6	②軽快	症例報告	当該製品	2007/12/28提出、識別番号1-07000160　未完了報告
64	感染症および寄生虫症	B型肝炎	日本	女	34	2007/8/6	②軽快	症例報告	当該製品	2008/3/31提出、識別番号1-07000160　取り下げ（63番と同一症例）

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別紙様式第4

感染症発生症例一覧

155	65	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000106　未完了報告
	66	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/11/20提出、識別番号1-07000106　未完了報告（65番と同一症例）
	67	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000106　完了報告（65番、66番と同一症例）
	68	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	②軽快	症例報告	当該製品	2007/11/6提出、識別番号1-07000078　取り下げ 第10回症例番号68は前回報告における第9回症例番号18において報告したものの追加報告
	69	感染症および寄生虫症	B型肝炎	日本	男	62	2007/7/6	⑥不明	症例報告	当該製品	2007/11/6提出、識別番号1-07000064　取り下げ 第10回症例番号69は前回報告における第9回症例番号19において報告したものの追加報告
	70	感染症および寄生虫症	B型肝炎	日本	男	58	2007/7/4	②軽快	症例報告	当該製品	2007/11/26提出、識別番号1-07000126　未完了報告
	71	感染症および寄生虫症	B型肝炎	日本	男	58	2007/7/4	③未回復	症例報告	当該製品	2008/2/6提出、識別番号1-07000126　未完了報告（70番と同一症例）
	72	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/11/6提出、識別番号1-07000055　取り下げ 第10回症例番号72は前回報告における第9回症例番号24において報告したものの追加報告
	73	感染症および寄生虫症	C型肝炎	日本	男	84	2007/6/1	⑥不明	症例報告	当該製品	2007/12/18提出、識別番号1-07000053　取り下げ 第10回症例番号73は前回報告における第9回症例番号30において報告したものの追加報告
	74	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000029　取り下げ 第10回症例番号74は前回報告における第9回症例番号39において報告したものの追加報告
	75	感染症および寄生虫症	B型肝炎	日本	女	82	2007/5/1	⑤死亡	症例報告	当該製品	2007/12/20提出、識別番号1-07000038　完了報告 第10回症例番号75は前回報告における第9回症例番号42において報告したものの追加報告
	76	感染症および寄生虫症	B型肝炎	日本	女	71	2007/4/26	③未回復	症例報告	当該製品	2008/2/14提出、識別番号1-07000223　未完了報告
第9回	4	感染症および寄生虫症	C型肝炎	日本	女	70	2007/9/3	⑤死亡	症例報告	当該製品	2007/9/28 提出、識別番号 1-07000095 未完了報告
	6	感染症および寄生虫症	C型肝炎	日本	男	67	2007/8/30	③未回復	症例報告	当該製品	2007/10/9 提出、識別番号 1-07000097 未完了報告
	8	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/10/19 提出、識別番号 1-07000104 未完了報告
	18	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	③未回復	症例報告	当該製品	2007/8/16 提出、識別番号 1-07000078 未完了報告
	19	感染症および寄生虫症	B型肝炎	日本	男	62	2007/7/6	⑥不明	症例報告	当該製品	2007/7/25 提出、識別番号 1-07000064 未完了報告
	24	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/7/5 提出、識別番号 1-07000055 未完了報告
	30	感染症および寄生虫症	C型肝炎	日本	男	84	2007/6/1	③未回復	症例報告	当該製品	2007/7/5 提出、識別番号 1-07000053 未完了報告
	39	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30 提出、識別番号 1-07000029 未完了報告
	42	感染症および寄生虫症	B型肝炎	日本	女	82	2007/5/1	⑤死亡	症例報告	当該製品	2007/6/14 提出、識別番号 1-07000038 未完了報告

80103	2008/06/17	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	感染症および寄生虫症	敗血症	日本	男	48	2008/3/25	②軽快	症例報告	当該製品	2008/4/15提出、識別番号1-08000028　未完了報告

80106	2008/06/17	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液

別紙様式第3

感染症の種類別発生状況

感染症の種類		前回調査期間終了時までの状況	当該調査期間	合計	外国製品による症例の内数	備考
器官別大分類	基本語					
感染症および寄生虫症	サイトメガロウイルス感染	1(0)	0(0)	1(0)	1	
感染症および寄生虫症	B型肝炎	2(0)	1(0)	3(0)	3	
感染症および寄生虫症	C型肝炎	18(0)	1(0)	19(0)	19	
感染症および寄生虫症	HIV感染	2(0)	0(0)	2(0)	2	
感染症および寄生虫症	ウイルス性肝炎	1(0)	0(0)	1(0)	1	
感染症および寄生虫症	伝染性紅斑	1(0)	0(0)	1(0)	1	
臨床検査	C型肝炎抗体陽性	3(0)	0(0)	3(0)	3	
臨床検査	C型肝炎RNA陽性	5(0)	0(0)	5(0)	5	
臨床検査	サイトメガロウイルス検査陽性	1(0)	0(0)	1(0)	1	
臨床検査	サイトメガロウイルス抗体陽性	2(0)	0(0)	2(0)	2	
臨床検査	B型肝炎表面抗原陽性	2(0)	0(0)	2(0)	2	
臨床検査	抗HBc抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBs抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBcIgG抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBe抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	C型肝炎陽性	3(0)	0(0)	3(0)	3	
臨床検査	ウイルス負荷	1(0)	0(0)	1(0)	1	

別紙様式第４　　感染症発生症例一覧　　別紙(5)

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 完了報告提出日2008年2月19日
	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 追加報告提出日2008年3月19日
	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 追加報告提出日2008年4月1日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 未完了報告提出日2008年4月24日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 完了報告提出日2008年5月29日
第9回	報告なし										

MedDRA /J Ver.10.1

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8. 0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8. 1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8. 0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7. 1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7. 1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6. 1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

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感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	9-2	感染症および寄生虫症	Ｂ型肝炎	日本	女	26歳	2007/11/5	未回復	症例報告	当該製品	識別番号：07000143 報告日：2008年1月28日 第9回症例番号9-2において報告したもの（完了報告）の取り下げ報告 MedDRA: Version(10.1)
	10-1	感染症および寄生虫症	Ｃ型肝炎	日本	男	不明	1993	不明	症例報告	当該製品	識別番号：07000248 報告日：2008年3月10日 MedDRA: Version(10.1)
	10-1	感染症および寄生虫症	Ｃ型肝炎	日本	男	不明	1993	不明	症例報告	当該製品	識別番号：07000248 報告日：2008年3月21日 MedDRA: Version(10.1) 第10回　症例番号10-1（完了報告）の追加報告
第9回	9-1	感染症および寄生虫症	Ｃ型肝炎	日本	女	40歳代	不明	不明	症例報告	当該製品	識別番号：07000164 報告日：2007年12月28日 MedDRA: Version(10.1)
	9-2	感染症および寄生虫症	Ｂ型肝炎	日本	女	26歳	2007/11/5	未回復	症例報告	当該製品	識別番号：07000143 報告日：2007年12月13日 MedDRA: Version(10.1)
第8回	該当なし										
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第6回	該当なし										
第5回	該当なし										
第4回	1-2	臨床検査	Ｃ型肝炎抗体陽性	日本	男	76歳	2003/9/19	不明	症例報告	当該製品	登録番号：A03-120 報告日：2005年4月28日 第1回症例番号1-2において報告したもの（未完了報告）の取り下げ報告 MedDRA: Version(7.1)
第3回	該当なし										
第2回	該当なし										
第1回	1-1	臨床検査	Ｂ型肝炎表面抗原陽性	日本	男性	72歳	2003/7/18	不明	症例報告	当該製品	識別番号：A03-40 報告日：2003年9月5日 MedDRA: Version(7.1)
	1-2	臨床検査	Ｃ型肝炎抗体陽性	日本	男性	76歳	2003/9/19	不明	症例報告	当該製品	登録番号：A03-120 （未完了報告 報告日：2003年10月3日） MedDRA: Version(7.1)

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第 1 回は該当なし。