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
				2008. 12. 17	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Shimasaki N, Kiyohara T, Totsuka A, Nojima K, Okada Y, Yamaguchi K, Kajioka J, Wakita T, Yoneyama T. Vox Sang. 2009 Jan ; 96(1):14-19	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社)					

研究報告の概要

○加熱および高静水圧によるA型肝炎ウイルスの不活化:実験株間の差異

背景および目的:感染した血液製剤によるA型肝炎ウイルス(HAV)の伝播が報告されている。HAV細胞馴化株(Cell-adapted HAV strains)は、通常、血液製剤製造時のウイルス不活化の確認に用いられるが、これらは不活化処理に対する感度が異なると考えられる。ウイルス・バリデーションに適切なHAV細胞馴化株を選ぶため、2種類の物理的不活化処理法下(加熱および高静水圧)で、4株間の不活化効率を比較した。

材料および方法:本試験で使用したHAV細胞馴化株は、KRM238、KRM003(subgenotype IIIB)、KRM031(IA)、TKM005(IB)であった。60℃(～10時間)の加熱、または高静水圧下(～420 MPa)にて、これらの株に処理を行った。immunofocus-staining法でHAV感染力の低下を測定した。

結果:加熱(60℃10時間)処理はHAV感染性を3～5 $\log_{10}$の範囲で低下させたが、KRM238およびTKM005は他2株と比べ不活性化が困難であった。高静水圧処理(420 MPa)も感染性を3～5 $\log_{10}$の範囲で低下させ、KRM031は他の株と比べて不活性化が容易であった。

結論:加熱処理および高静水圧処理によりHAV細胞馴化株間の不活化効果の差が明らかとなり、処理によって各株の反応は異なった。KRM238は不活化が困難で、他の細胞株よりも細胞培養での複製が良好であるため、血液製剤のウイルス汚染に対する安全性を評価するのにもっとも適した候補と考えられる。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
加熱および高静水圧の物理的不活化処理法で4株のA型肝炎ウイルスの不活化を行ったところ、それぞれの処理はHAV感染性を3～5 $\log_{10}$の範囲で低下させた。また、血液製剤のウイルス汚染に対する安全性を評価するのにもっとも適した株は耐熱性のKRM238であったとの報告である。 これまで、本剤によるHAV感染の報告はない。さらに最終製品についてHAV-NAT陰性であることを確認している事から本剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、本剤の重要なウイルス除去・不活化工程である液状加熱に抵抗性のある遺伝子型の存在が示唆されたので、今後も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。なお、日本赤十字社は、輸血感染症対策として、問診で肝炎の既往があった場合、A型肝炎については治癒後6ヶ月間、家族に発症した人がいる場合は1ヶ月間献血不適としている。

MedDRA/J Ver.11.0J

Vox Sanguinis (2009) 96, 14–19
Journal compilation © 2008 International Society of Blood Transfusion
DOI: 10.1111/j.1423-0410.2008.01013.x

ORIGINAL PAPER

Inactivation of hepatitis A virus by heat and high hydrostatic pressure: variation among laboratory strains

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Vox Sanguinis

Background and Objectives Hepatitis A virus (HAV) transmission via contaminated blood products has been reported. Cell-adapted HAV strains are generally used to confirm virus inactivation in manufacturing blood products, but the strains may differ in their sensitivity to inactivation treatment. To select an appropriate cell-adapted HAV strain for virus validation, we compared the inactivation efficiency among four strains under two different physical inactivation treatments: heat and high hydrostatic pressure.

Materials and Methods The cell-adapted HAV strains used here were KRM238, KRM003 (subgenotype IIIB), KRM031 (IA), and TKM005 (IB). The strains were treated at 60°C for up to 10 h or under high hydrostatic pressure (up to 420 MPa). The reduction in HAV infectivity was measured by an immunofocus-staining method.

Results The heat treatment at 60°C for 10 h reduced HAV infectivity in the range of 3 to 5 $\log_{10}$ among the strains; KRM238 and TKM005 were harder to inactivate than the other two. The high hydrostatic pressure treatment at 420 MPa also reduced infectivity in the range of 3 to 5 $\log_{10}$ among the strains, and KRM031 was easier to inactivate than the other strains.

Conclusion Heat treatment and high hydrostatic pressure treatment revealed differences in inactivation efficiencies among cell-adapted HAV strains, and each strain reacted differently depending on the treatment. KRM238 may be the best candidate for virus validation to ensure the safety of blood products against viral contamination, as it is harder to inactivate and it replicates better in cell culture than the other strains.

Key words: heat inactivation, hepatitis A virus, high hydrostatic pressure inactivation, variation among strains, virus validation.

Received: 2 May 2008,
revised 19 September 2008,
accepted 20 September 2008,
published online 2 November 2008

Introduction

Hepatitis A virus (HAV), which is responsible for acute viral hepatitis, is transmitted primarily by the fecal-oral route, either through the ingestion of contaminated food or water or through person-to-person contact [1,2]. On the other hand, parenteral HAV transmission has also been reported via contaminated blood [3] or blood products [4,5]. Moreover, *in vivo* HAV infection via blood reportedly has a much higher HAV infection efficiency than does oral HAV infection [6]. In developed countries such as Japan, HAV infections have become less common, owing to improved hygiene resulting from the maintenance of water and sewage facilities. Infections in early childhood are relatively rare, and thus the majority

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Table 1 Characteristics of HAV strains used

Strain	Subgenotype	Source	Year of recovery	Number of passages on African green monkey kidney cells	Titre of stock virus (FFU/ml)	Reference	Accession no.
KRM238	IIIB	Outbreak	1977	59	$1{\cdot}5 \times 10^8$	[21]	AB300205
KRM003	IIIB	Sporadic	1979	72	$1{\cdot}5 \times 10^8$	[15,18]	AB425339
KRM031	IA	Outbreak	1977	47	$1{\cdot}5 \times 10^8$	[15]	AB300206
TKM005	IB	Travel-associated	1981	48	$0{\cdot}5 \times 10^8$	[15]	AB300207

of adults remain susceptible to infection, because they lack the immunity to HAV [7]. As this could potentially facilitate massive outbreaks of hepatitis A in the general population, treatment to inactivate HAV in blood and blood products should be improved.

Previous results have demonstrated that, because HAV is a non-enveloped virus, it is quite resistant against chemical inactivation approaches, such as solvent/detergent treatments used in the preparation of blood products [8]. HAV can be inactivated however by pasteurization [9], γ-irradiation [10], and short wavelength ultraviolet light irradiation [11].

Because environmental HAV strains that have just isolated from human generally grow poorly in cell culture, cell-adapted HAV strains are generally used to test virus inactivation. As extensive genetic variation is found among cell-adapted strains [12], the strains may differ in their sensitivity to inactivation treatments. But no studies have considered the variation among cell-adapted HAV strains in testing the efficiency of inactivation treatments.

HAV strains recovered from different parts of the world have been classified into six genotypes (I–VI). Genotypes I, II and III are found in humans, and each of them is further divided into subgenotypes A and B. Most human HAV strains belong to genotypes I and III [13–15]. Subgenotype IA appears to be the predominant virus of hepatitis A cases worldwide, whereas subgenotypes IB and IIIA have been found in Scandinavia and in the Mediterranean region [16,17]. Subgenotype IIIB is unique to Japan [15,18].

To select an appropriate HAV laboratory strain for use in virus validation, we compared the rates of inactivation efficiency among cell-adapted HAV strains by using two different physical inactivation treatments – heat treatment at 60°C and high hydrostatic pressure treatment – among four cell-adapted HAV strains belonging to three subgenotypes. Heat treatment was used as a conventional inactivation treatment for blood products. High hydrostatic pressure treatment is a promising new virus-inactivating technique that is applicable to human immunodeficiency virus in blood products [19] and has been applied to HAV in food [20]. It is expected to be useful for inactivating a broad range of micro-organisms in blood products under conditions without applying high temperatures.

Materials and methods

Virus strains and propagation

Four laboratory HAV strains (KRM238, KRM003, KRM031, and TKM005) were isolated from patients with hepatitis A in Japan [15,21], and these strains were adapted by numerous passages on African green monkey kidney cells. Table 1 shows each strain's subgenotype, passage history, and stock virus titre. All four strains were propagated on an established African green monkey kidney cell line, GL37 [18].

GL37 cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml gentamycine. To prepare the virus stocks, GL37 cells were infected at a multiplicity of infection of 0·1 focus forming units (FFU) per cell in Eagle's minimum essential medium containing 2% FBS, and were incubated for 2 weeks at 36·5°C in the presence of 5% CO_2. The infected cells were harvested by replacing the medium with phosphate-buffered saline containing 2% FBS. Virus stocks were obtained as supernatants of centrifugation at 2380 *g* for 5 min after release of the viruses by three freeze-thaw cycles and sonication of infected cells. The virus stocks were then stored at −80°C until use.

Infectivity assay

The infectious titre of each HAV strain was measured by the immunofocus-staining method described previously [21]. Briefly, a 100 μl portion of the virus dilution was inoculated into duplicate GL37 cells cultures in six-well plates at 36·5°C in the presence of 5% CO_2. After 60 min adsorption, 5 ml of the medium containing 0·6% agarose and 2% FBS was overlaid on each well. The plates were incubated at 36·5°C in the presence of 5% CO_2 for 9 days. The cells were fixed with 80% methanol containing 0·03% H_2O_2 after removal of the agarose medium. HAV foci were revealed by anti-HAV rabbit serum and horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG) (MBL, Nagoya, Japan) followed by colour development with DAB substrate solution (0·5 mg/ml diaminobenzidine, 0·03% $(NH_4)_2Ni(SO_4)_2$, 0·03% $CoCl_2$, and 0·03% H_2O_2 in phosphate-buffered saline).

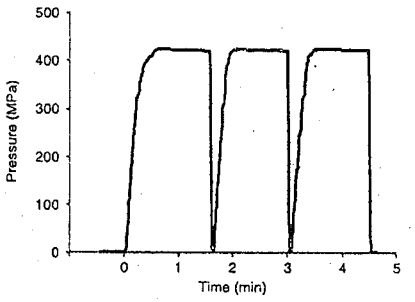

Fig. 1 The pattern of pressure change with high hydrostatic pressure at 420 MPa. Samples were treated at 25–30°C by three cycles of pressurization at the indicated pressure for 1 min followed by immediate release of the pressure. Essentially similar patterns were obtained at other hydrostatic pressures.

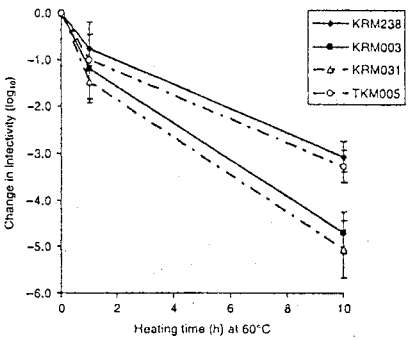

Fig. 2 Inactivation of HAV strains by heat at 60°C. The cell-adapted strains in 25% human serum albumin were treated by heat at 60°C for the indicated times. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity (log_{10}) = log_{10} (titre of treated samples) − log_{10} (titre of untreated samples).

Heat treatment

The samples used for the heat treatment were prepared by adding one volume of each virus stock to 9 volumes of 25% human serum albumin (Benesis Corporation, Osaka, Japan). The samples were divided into microcentrifuge tubes in amounts of approximately 0·8 ml, and the tubes were sealed. The samples were heated at 60°C for 1 or 10 h and were then cooled on ice rapidly to arrest the heating process.

Two or three independent trials were conducted for all samples. The 95% confidence limits of these data were statistically determined and assessed; the difference was significant if it was over the 95% confidence limits.

High hydrostatic pressure treatment

The samples used for the high hydrostatic pressure treatment were prepared by adding one volume of each virus stock to 9 volumes of 5% human serum albumin. The samples were divided into ultra-centrifuge tubes (Beckman Coulter, Fullerton, CA, USA) in amounts of approximately 1·5 ml, and the tubes were sealed. The sealed tubes were placed in the chamber of a laboratory-sized high hydrostatic pressure instrument designed for food processing (Echigo Seika, Co., Ltd, Niigata, Japan). High hydrostatic pressure was controlled by water filled in the chamber. The samples were treated at 25–30°C by repeating three cycles of pressurization at the indicated pressure for 1 min and then immediately releasing the pressure. Three different pressures (300, 350, or 420 MPa) were used. At 420 MPa, the pattern of pressure change with treatment is shown in Fig. 1.

Two or three independent trials were conducted for all samples. The 95% confidence limits of these data were statistically determined and assessed; the difference was significant if it was over the 95% confidence limits.

Results

Inactivation by heat treatment at 60°C

The four cell-adapted HAV strains were treated in 25% human serum albumin with heat at 60°C for 1 or 10 h. The infectious titres of HAV in the samples were measured after heat treatment, and the reduction in HAV infectivity was then calculated. For all four strains, infectivity was reduced by approximately 1 log_{10} after heat treatment at 60°C for 1 h, indicating that HAV was resistant to heat inactivation as compared, for example, to poliovirus, which Barrett *et al.* reported was much more thermolabile than HAV [22].

With heat treatment at 60°C for 10 h, the reduction of HAV infectivity ranged from approximately 3 to 5 log_{10} among the four strains, as shown in Fig. 2. The reduction in the infectivity of KRM238 was 3·1 log_{10}, that of KRM003 was 4·7 log_{10}, that of KRM031 was 5·1 log_{10}, and that of TKM005 was 3·3 log_{10}. In other words, two strains (KRM238 and TKM005) were more resistant to inactivation by heat treatment than the other two (KRM003 and KRM031). There was 2·0 log_{10} difference between the most resistant strain KRM238 and the most sensitive strain KRM031. There was 1·6 log_{10} of variation in the inactivation rate between KRM238 and KRM003, even though they belong to the same IIIB strain subgenotype. These differences mentioned here were significant.

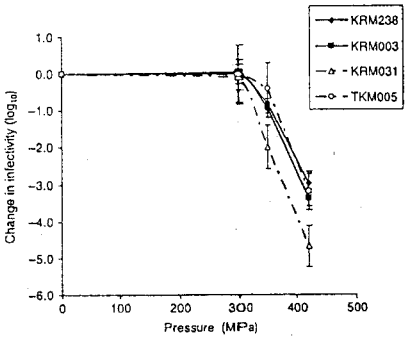

Fig. 3 Inactivation of HAV strains by high hydrostatic pressure. The cell-adapted strains in 5% human serum albumin were treated at the indicated pressures by repeating three cycles. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity (log_{10}) = log_{10} (titre of treated samples) − log_{10} (titre of untreated samples).

Inactivation by high hydrostatic pressure treatment

The four cell-adapted HAV strains were treated in 5% human serum albumin with high hydrostatic pressure at 300, 350, or 420 MPa. The infectious titres of HAV in the samples were measured after the treatment, and the reduction in HAV infectivity was then calculated.

None of the HAV strains were inactivated by high hydrostatic pressure of less than 300 MPa, but all of the strains began to show inactivation at pressures exceeding 300 MPa. At 420 MPa, the reduction of HAV infectivity ranged from approximately 3 to 5 log_{10} among the strains, as shown in Fig. 3. The reduction in the infectivity of KRM238 was 3·0 log_{10}, that of KRM003 was 3·4 log_{10}, that of KRM031 was 4·7 log_{10}, and that of TKM005 was 3·2 log_{10}. There was at least 1·3 log_{10} difference, which was significant, between the resistant strains and the sensitive strain KRM031. In other words, high hydrostatic pressure inactivation was more effective against KRM031 than against the other three strains. As with heat inactivation, high hydrostatic pressure inactivation showed variation among the strains.

Accumulative effects of inactivation by heat and pressurization

To evaluate efficiency of two such inactivation treatments in the manufacture of blood products, the combined effects of inactivation by heat at 60°C for 10 h and by high hydrostatic pressure at 420 MPa are calculated by addition as shown in Table 2.

With either treatment, the degree of variation in infectivity reduction between resistant and sensitive strains was approximately 2 log_{10}. KRM238 and TKM005 well resisted inactivation by either heat or high hydrostatic pressure.

The combined reduction in the infectivity of KRM238 was 6·1 log_{10}, that of KRM003 was 8·1 log_{10}, that of KRM031 was 9·8 log_{10}, and that of TKM005 was 6·5 log_{10}.

Discussion

Cell-adapted strains are useful in studies aimed at validating the virus-inactivation procedures used in manufacturing. We report here on variation in inactivation rates – whether by heat treatment or high hydrostatic pressure treatment – among laboratory HAV strains. As shown in Table 2, if both inactivation treatments could be combined, the variation between resistant and sensitive strains would increase. For example, the most sensitive strain, KRM031, showed an estimated total reduction of 9·8 log_{10} via the combined treatments; on the other hand, the most resistant strain, KRM238, showed only a 6·1 log_{10} reduction. The maximum variation among the HAV strains after combined treatment inactivation was predicted to be about 3·7 log_{10}. To ensure the safety of

Table 2 Inactivation among HAV strains by heat and pressurization

	Reduction in infectivity (log_{10})		
HAV strain	By heat at 60°C for 10 h	By high hydrostatic pressure at 420 MPa	By combination[b] of heat and high hydrostatic pressure
KRM238	3·1 (± 0·3 2)[a]	3·0 (± 0·25)	6·1
KRM003	4·7 (± 0·4 5)	3·4 (± 0·22)	8·1
KRM031	5·1 (± 0·6 1)	4·7 (± 0·56)	9·8
TKM005	3·3 (± 0·3 5)	3·2 (± 0·52)	6·5

[a]Parentheses indicate 95% confidential limits.
[b]Expected values calculated by addition.

manufactured blood products, it is important to avoid overestimating HAV-inactivation rates. Thus, the HAV strain that is most resistant to inactivation treatment should be used in virus validation.

Considering that KRM238 grows better in cell culture than TKM005 (Table 1), it can be concluded that, among the four strains used here, KRM238 is the best candidate for virus-validation to ensure the safety of blood products against viral contamination. In general, the evaluation of inactivation processes will depend on the strains used for testing.

Our results also indicated that we should evaluate carefully the efficiency of inactivation by selecting an appropriate strain that is resistant to inactivation treatment, and that a strain that is resistant to one particular inactivation treatment may not always be resistant to another. Here, KRM003 was easily inactivated by heat treatment, showing a 4·7 log_{10} reduction, but was more stubborn against high hydrostatic pressure, which resulted in only a 3·4 log_{10} reduction. Indeed, when a novel inactivation treatment is applied to the manufacture of blood products to prevent viral contamination, inactivation treatment must be validated carefully. In other words, the efficiency of inactivation should be evaluated not only by using a strain that has shown resistance to the standard inactivation treatment, but also by selecting an appropriate strain that is resistant to a newer inactivation treatment. A test strain of virus validation for a newer inactivation should be selected carefully for avoiding a risk of overestimating the resistance of the test strain to a newer inactivation.

Pressurization has emerged as a new technique for inactivating pathogenic viruses in blood plasma and plasma-derived products, as pressurization at 400 MPa exerted no effect on the recovery of biologically active plasma proteins, with the exception of factor XIII [19]. Most enveloped viruses are markedly inactivated at pressures below 400 MPa, as summarized by Grove *et al.* [23]. However, small RNA viruses can vary widely in their sensitivity to high pressure. For example, HAV and poliovirus are both members of the picornavirus family, but they exhibit quite different susceptibilities. HAV is inactivated by 3–5 log_{10} of infectivity at 420 MPa, whereas poliovirus remains essentially unaffected even at 600 MPa [24]. At this point in time, the mechanism underlying virus inactivation by pressurization is still poorly understood.

Heat inactivation is currently used to inactivate enveloped viruses in particular, such as human immunodeficiency virus, hepatitis B virus and hepatitis C virus, in blood products. Moreover, non-enveloped viruses such as HAV and poliovirus differ greatly in terms of their sensitivity to heat inactivation [22]. As with pressurization, in heat treatment the mechanism underlying inactivation of non-enveloped viruses remains unclear.

The cell-adapted HAV strains exhibited disparate sensitivities to the two different treatments used in this study. These findings are important in terms of ensuring safety in the manufacture of blood products. Further studies will be needed in order to validate the inactivation procedures for naturally occurring viral strains.

Acknowledgements

We thank Dr Takashi Shimoike, National Institute of Infectious Diseases, Japan, for his greatly enlightening discussions. We also thank Echigo Seika Co for kindly providing a laboratory-sized high hydrostatic pressure instrument. This study was supported in part by a grant (#H16-IYAKU-017) from the Ministry of Health, Labor, and Welfare of Japan.

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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	赤血球、血小板					公表国	
販売名（企業名）	－	研究報告の公表状況		Transfusion medicine (Oxford, England) (England) Dec 2008, 18 (6) p379-81		英国	

研究報告の概要	2006年11月、大阪赤十字社血液センターにおいて、繰り返し供血していた69歳の女性が20-NATでHBV DNA陽性であることが判明し、ルーチンの検査ではHBsAg、抗HBs抗体と抗HBc抗体は陰性で、EIA法による抗HBc抗体だけが陽性であり、供血者が抗HBc抗体低値の不顕性HBV感染者であることを示していた。 この供血者の凍結標本を調査したところ、1999年10月1日以降に供血された血清が個別（ID）-NATでHBV DNA陽性であり、13の供血のうち、11が輸血に使用されていた。 受血者のHBV検査記録を収集したが、4例は既に原疾患で死亡しており、2例は記録がなかった。残りの5例のうち3例では輸血前後で、2例では輸血後のみHBV検査が行われており、HBV感染のサインを示唆する情報はなかったが、HBV感染が起きたかどうか決定するには不十分である。 神奈川赤十字社血液センターは、繰り返し血小板を提供していたID-NATのHBV検出臨界付近でウイルス量が揺れ動いていた不顕性HBV感染症の症例からの200mlの血漿を含む濃厚血小板液でのHBV感染症を報告している。日赤血液センターによる最近のルックバック研究では、不顕性HBV陽性の供血者から得られた33の血液成分中の1つ（450mlの新鮮凍結血漿の輸注）でHBV感染症を起こしており、ミニプールNATのウインドピリオドの間に供血された22の血液成分中11の輸注でのHBV感染を明らかにした。 不顕性HBV感染者から血液成分の潜在的な危険を明確にするためには、さらに多くの症例が詳細に分析される必要があり、血液成分中のHBVの総輸注量、HBV免疫抗体の保有状態、受血者の免疫状況、HBV遺伝子型そして／あるいは突然変異の存在は算定されるべきである。	使用上の注意記載状況・その他参考事項等 重要な基本的注意 (1) 本剤の原材料となる（献血者の）血液については、HBs抗原、抗HCV抗体、・・・・・陰性で、かつALT（GPT）値でスクリーニングを実施している。さらに、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。
報告企業の意見	今後の対応	
不顕性HBV感染者（HBsAg陰性）からの輸血によるB型肝炎感染に関する報告である。 当社血漿分画製剤の製造工程におけるHBVのモデルウイルスに対するウイルスクリアランス指数は9以上である。なお、原料血漿はミニプール血漿におけるNAT検査でHBV DNA陰性を確認しており、最終製品においてもHBV DNA陰性を確認している。	今後ともにB型肝炎ウイルス感染に関する安全性情報に留意していく。	

Transfusion Medicine, 2008, 18, 379–381 doi: 10.1111/j.1365-3148.2008.00898.x

LETTER TO THE EDITOR

Transfusions of red blood cells from an occult hepatitis B virus carrier without apparent signs of transfusion-transmitted hepatitis B infection

Dear Sir

To minimize the risk of transfusion-transmitted hepatitis B virus (HBV) infections, the Japanese Red Cross (JRC) Blood Centers have adopted a multistep screening system to identify donors at risk of HBV infection. First, donors are examined for the hepatitis B surface antigen (HBsAg) by performing reverse passive haemagglutination tests with a sensitivity of 3 ng mL^{-1}. HBsAg-negative donations are screened for antibodies against HBsAg and the hepatitis B core antigen (anti-HBs and anti-HBc, respectively) by particle haemagglutination and haemagglutination inhibition (HI) tests, respectively. Donations with a high anti-HBs titre ($\geq 2^4$ dilution equivalent to 200 mIU mL^{-1}) or a low or zero anti-HBc titre ($\leq 2^4$ dilution) are defined as 'seronegative'. The cut off value for anti-HBc tests is relatively high compared to that of enzyme-linked immunoassays (EIAs) because HBV DNA was not detected by an in-house polymerase chain reaction (PCR) in donors who tested negative for HBsAg and positive for anti-HBc at an HI titre less than 2^5 (Iizuka *et al.*, 1992). Since the introduction of nucleic acid amplification test (NAT) technology, all seronegative donations are pooled (initially, at a pool size of 500 and a current pool size of 20, i.e. 20-NAT) and subjected to NAT (Ampli-NAT, Roche, IN, USA). If the 20-NAT tests positive, the pooled donations are further subjected to individual NAT (ID-NAT) to identify the blood donation that contains the viral genome. The 95% confidence interval of the detection range for HBV in ID-NAT is 22–60 copies of HBV per millilitre (Meng *et al.*, 2001). Donors who did not fall within the algorithm would be either categorized in the window period of 20 NAT or assigned an occult HBV status with a low viral load (reviewed by Raimondo *et al.*, 2007).

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In November 2006, the Osaka Red Cross Blood Center, Japan, identified a repeat donor, namely, a 69-year-old female, whose donation was found to be positive for HBV DNA when tested by the latest 20-NAT. According to the guidelines for the safety of transfusion in the JRC Blood Centers, the serological status of the donation was re-evaluated. The donated blood was found to be negative for HBsAg, anti-HBs and anti-HBc by routine testing methods and positive for only anti-HBc when tested using EIA (AxSYM; Abbott Laboratories, Abbott Park, IL, USA), indicating that the donor was an occult HBV carrier with a low anti-HBc titre. We retrieved frozen aliquots of previous donations by this donor and found that sera donated on and after 1 October 1999 tested positive for HBV DNA when tested by ID-NAT. The amount of HBV DNA in these donations was less than 100 copies per millilitre, except for two donations (Table 1). From the 13 donations made by this donor in the abovementioned period, 11 components were transfused into recipients (recipient number 1–11 in Table 1). We collected the HBV test records of some of the recipients from the medical institutions where each recipient had been hospitalized. Recipients 3, 6, 7 and 9 had succumbed to their primary disease, and no records were available for recipients 10 and 11. Of the remaining five cases, the HBV test was performed at both the pre- and post-transfusion stages in recipients 1, 4 and 5, but recipients 2 and 8 were tested only at the post-transfusion stage. Recipient 1 was a 70-year-old female who had tested negative for HBsAg and anti-HBc by EIA 2 days prior to transfusion. She was transfused with packed red blood cells (RBCs) and tested negative for HBsAg, anti-HBs and anti-HBc by EIA and negative for HBV DNA by PCR 7 months after the transfusion. These data suggest that the latest RBC component from this occult HBV donor did not cause transfusion-transmitted HBV infection. In recipients 2 and 8, the post-transfusion EIA test results for HBsAg were reported negative. Recipient 4 tested negative for HBsAg by EIA at 11 days before

Table 1. HBV status of the donor and recipients

Donor					Recipients				Pretransfusion		Post-transfusion	
Date of donation	Pooled NAT	ID-NAT	Copy number per mL	Component	Recipient number	Age (years)	Primary diseases*	HBsAg	Anti-HBc	HBsAg	Other markers
1 November 2006	+†	+	ND	—¶							
22 May 2006	–†	+	<100	RBCs	1	70	(1)	–	–	–	–, anti-HBs; –, anti-HBc; –, HBV DNA
15 April 2006	–†	+	140	RBCs	2	NA	(2)	NA	NA	–**	
26 September 2005	–†	+	210	RBCs	3	NA	NA	–	NA	–**	
27 June 2005	–†	+	<100	—¶							
10 April 2005	–†	+	<100	RBCs	4	86	NA	–	NA	–	–, HBV DNA
15 February 2004	–‡	+	<100	RBCs	5	60	(3)	–	NA	–	
15 September 2003	–‡	+	<100	—¶							
21 March 2003	–‡	+	<100	RBCs	6	69	(4)	–	NA	–**	
1 March 2002	–‡	+	<100	RBCs	7	51	(5)	NA	NA	–**	
1 July 2002	–‡	+	<100	RBCs	8	41	(6)	NA	NA	–	
15 January 2001	–‡	+	<100	RBCs	9	57	(7)	NA	NA	–**	
1 October 1999	–§	+	<100	RBCs	10	NA	NA	NA	NA	NA	
15 April 1999	–§	–	ND	RBCs	11	NA	NA	NA	NA	NA	

NA, not applicable; ND, not determined.
*Primary Diseases: (1), perforation of sigmoid diverticulum; (2), transverse colon cancer; (3), bleeding gastric ulcer; (4), operative diseases; (5) operative diseases; (6), gastric ulcer; and (7), ovarian cancer.
†20-pooled.
‡50-pooled.
§500-pooled.
¶Not used.
**Decease by the primary disease.

transfusion with RBCs. Furthermore, she tested negative for HBsAg at both 17 and 19 months after the transfusion. In addition, PCR results for this patient were negative for HBV DNA 21 months after transfusion. In recipient 5, it was reported that both pre- and post-transfusion sera tested negative for HBsAg by EIA. Although no further reports suggesting any signs of HBV transmission in recipients 2, 4, 5, and 8 have been filed with our blood centre, the HBV test records of these four recipients are insufficient to determine whether transfusion-transmitted HBV infection occurred.

Kanagawa Red Cross Blood Center, Japan, recently reported a case of transfusion-transmitted HBV infection caused by an individual with an occult HBV infection who had repeatedly donated platelets and whose viral load fluctuated around the limit of HBV detection level by the ID-NAT (Inaba *et al.*, 2006). It is noteworthy that the component transfused in this case was a platelet concentrate containing approximately 200 mL of plasma; on the other hand, in our subjects, the transfused component was packed RBCs including 10–15 mL of plasma. A more recent look-back study on transfusion-transmitted HBV infection conducted by the JRC Blood Center identified that only one of the 33 components obtained from occult HBV donors caused the HBV infection (Satake *et al.*, 2007). This particular patient was transfused with 450 mL of fresh frozen plasma. The same study also demonstrated that 11 of the 22 components donated during the mini-pool NAT window period resulted in transfusion-transmitted HBV infection. Although the results of recipient 1 in our case appear to be consistent with those in the look-back study, data available in the literature suggest that occult HBV infection is transmissible, especially in endemic areas (reviewed by Liu *et al.*, 2006). To clarify the potential risks of blood components from occult HBV donors, many more cases need to be analysed in detail, where the total amount of HBV in the component transfused, the presence or absence of HBV antibodies in the component, the immunological status of the recipient, the HBV genotype and/or the presence of mutation(s) should be assessed.

The peculiar criterion of seronegative used in the JRC Blood Centers was a practical solution to exclude donors with a risk of HBV infection, without excessively reducing the size of the donor pool. This criterion was introduced because the prevalence of HBV infection, when serological testing was introduced, was relatively higher in Japan than in other industrialized countries. Our serological screening, however, has failed to identify a few occult HBV carriers with a low anti-HBc titre and a low viral DNA. JRC has been re-evaluating the efficacy of our screening strategy by follow-up surveys, including the present study, and exploring options to be adopted to minimize the risk not only by the occult HBV carrier but also by donors in the 20-NAT window period.

Although we consider that the current possibility of HBV transmission by occult HBV carriers with a low anti-HBc titre is limited in Japan, this consideration cannot be generalized to countries with different HBV prevalence as mentioned above. Once the cut off value of the anti-HBc titre confirming the HBV-DNA-negative status of the donor blood is more rigorously determined, our serological screening algorithm may be an acceptable option in areas of intermediate or high HBV endemicity where NAT is unavailable.

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別紙様式第2−1　No. 19

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
				2009. 2. 18	該当なし		
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	小松 陽樹、乾 あやの、十河 剛、藤澤 知雄. 第40回日本小児感染症学会総会・学術集会; 2008 Nov 15-16; 名古屋市.	公表国	日本	使用上の注意記載状況・その他参考事項等
販売名（企業名）	クロスエイトM250（日本赤十字社） クロスエイトM500（日本赤十字社） クロスエイトM1000（日本赤十字社）						クロスエイトM250 クロスエイトM500 クロスエイトM1000
研究報告の概要	○母子感染以外のHBV感染によるHBV DNAの解析 【目的】小児における母子感染以外のHBV感染の実態を分子疫学的に把握する。 【方法】成人および小児HBVキャリア82名中で、母親がHBsAg陰性かつ患児以外にHBVキャリアが存在する7家族を対象とし、HBV全遺伝子解析を行い、分子系統樹を用い感染源を検索した。 【成績】HBsAg陽性例は、父親が4家族、兄弟のみが2家族（両親はHBVマーカー陰性）、祖母が1家族であった。血清が得られた7家族中3家族（2家族；父親HBsAg陽性、1家族；祖母HBsAg陽性）を対象にHBV遺伝子解析を行った。Family1は長女3歳がHBVキャリアと判明し、父親および長男5歳がHBsAg陽性、母親はHBsAb陽性であった。Family2は次男4歳がHBVキャリアと判明し、長男9歳、長女2歳がHBsAg陽性、母親がHBsAb陽性であった。Family3は、12歳女児がB型劇症肝炎と診断され、祖母がHBVキャリア、同居の従弟が同時期にB型急性肝炎と診断された。分子系統樹解析ではいずれも家族でも高い相同性を示し、それぞれ1つのクラスターを形成したため同じ感染源であると考えられた。 【考察】アジア諸国の中でHBV浸淫度が比較的低いと考えられる本邦でも、母子感染以外のHBV感染経路は無視できない。7家族中3家族で父親以外の感染源の可能性があり、祖母からの感染は分子疫学的に感染経路を証明できた。 【結語】母子感染など感染リスクが高い集団に対してのみワクチン接種を行う"target strategy"ではこのような水平感染を完全に防止することは不可能であり、本邦でuniversal vaccinationが必要と考えられた。						血液を原料とすることに由来する感染症伝播等 vCJD等の伝播のリスク
	報告企業の意見		今後の対応				
	母親がHBsAg陰性かつ家族内に患者以外のHBVキャリアが存在する成人および小児HBVキャリア7家族を対象とし、HBV全遺伝子解析に基づく分子系統樹を用い感染源を検索したところ、3家族で父親以外の感染源の可能性があり、祖母からの感染は分子疫学的に感染経路を証明できたとの報告である。 これまで、本剤によるHBV感染の報告はない。また本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHBV-NAT陰性であることを確認していることから、特別の対応を必要としないと考える。		今後も引き続き情報の収集に努める。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法（CLEIA）および新NATシステムを導入し、陽性血液を排除している。				

(3)

【研究奨励賞】

E-20　母子感染以外の HBV 感染による HBV DNA の解析

小松 陽樹、乾 あやの、十河 剛、藤澤 知雄

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【目的】小児における母子感染以外の HBV 感染の実態を分子疫学的に把握する。【方法】当科でフォロー中の成人および小児 HBV キャリア一82 名のなかで母親が HBs 抗原陰性かつ患児以外に HBV キャリアが存在する 7 家族を対象とした。HBs 抗原陽性の家族から得られた血清を用いて HBV 全遺伝子解析を行い、分子系統樹を用いて感染源の検索を行った。【成績】父親が HBs 抗原陽性例は 4 家族、兄弟のみ HBs 抗原陽性例は 2 家族（両親は HBV マーカー陰性）、祖母 HBs 抗原陽性例は 1 家族であった。 7 家族中 3 家族（2 家族；父親 HBsAg 陽性、 1 家族；祖母 HBsAg 陽性）にて家族から血清が得られ、この 3 家族を対象に HBV 遺伝子解析を行った。Family1 は長女 3 歳が伝染性単核球症罹患時の血液検査にて HBV キャリアが判明。家族内検索にて父親および長男 5 歳が HBsAg 陽性、母親は HBsAb 陽性。Family2 は次男 4 歳が胃腸炎症罹患時の血液検査にて HBV キャリアが判明。家族内検索にて父親、長男 9 歳、長女 2 歳が HBsAg 陽性、母親は HBsAb 陽性。Family 3 は、12 歳女児が黄疸と全身倦怠感を主訴に来院し、B 型劇症肝炎と診断された。祖母が HBV キャリアであり、同居していた。同時期に従弟は B 型急性肝炎と診断された。分子系統樹解析では、 3 家族においていずれも高い相同性を示すとともに、各家族がそれぞれ 1 つのクラスターを形成し、同じ感染源であると考えられた。【考案】アジア諸国の中で HBV 浸淫度が比較的低いと考えられる本邦でも、父子感染など母子感染以外の HBV 感染経路は無視できない。 7 家族中 3 家族で父親以外の感染源の可能性があり、祖母からの感染は分子疫学的に感染経路を証明できた。【結語】母子感染など感染リスクが高い集団に対してのみワクチン接種を行う”target strategy”ではこのような水平感染を完全に防止することは不可能であり、本邦で universal vaccination が必要と考えられた。

E-21　治療後もβ-D-グルカン高値が持続するカンジダ血症の一例

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β-D-グルカンは真菌細胞壁の主要成分であり、特にカンジダ感染症では感度の高い検査法として頻用されている。今回我々は、カンジダ血症に対して抗真菌剤の治療を行い、臨床症状、培養を含む検査データから治癒と考えられる状態に至ったにもかかわらず、β-D-グルカン異常高値のみが持続する男児例を経験したので報告する。 症例は生来健康な 4 歳男児。腹痛、下痢、嘔吐のため近医数回受診の後、第 8 病日前医入院。第 12 病日皮下出血斑に気づかれ、アレルギー性紫斑病と診断されプレドニン 2mg/kg/d 開始されたところ翌日には腹痛消失。しかし食事開始すると腹痛・血便が再燃するため食止・再開を繰り返し、PSL 開始 15 日目に当院転院。PSL と第 13 因子製剤で治療継続していたところ、入院 3 日目より発熱。血液培養で *Candida parapsilosis* が検出されたため MCFG にて治療開始。入院 11 日目の血液培養で再度同菌が検出されたためボリコナゾールを併用し、その後解熱したが入院 13 日目の血液培養でも陰性化していなかったため、眼科受診、腹部超音波、腹部 CT、頭部 CT、心臓超音波などで全身検索を行ったが、膿瘍形成や感染性心内膜炎を示唆する所見は得られなかった。抗真菌剤は 2 週間点滴で使用した後 VCZ＋FCZ 内服に変更、再発熱や炎症反応の増悪がみられないことを確認して外来フォローとした。血中 β-D-グルカンは入院時すでに 1610pg/ml と高値であったがその後も増加し、退院前の最高値 3460pg/ml、退院後も発熱などの症状はないが、7400pg/ml まで上昇した。抗真菌剤開始後は腹部症状消失していたがむしろ便秘傾向であったため、緩下剤を開始し、抗真菌剤は合計約 2 ヶ月で中止とした。その後は緩やかに低下傾向であるが、発症から 8 ヶ月経った段階でまだ 884pg/ml と高値が続いている。

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
				2008. 11. 20	該当なし		
一般的名称	人血清アルブミン		研究報告の公表状況	古居 保美、五十嵐 正志、蕎麦田 理英子、蕎俣 尋史、星 友二、福田俊洋、松本 千恵子、鈴木 光、柚木 久雄、内田 茂治、三枝 英子、田所 憲治 日本赤十字社NAT研究グループ. 第32回 日本血液事業学会総会; 2008 Oct 2-4; 大阪市.	公表国		
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社）					日本	

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
○NATスクリーニング検査で検出されたHCV-RNA陽性検体の解析 【はじめに】1999年7月のNATスクリーニング検査（以下「NAT」という）導入以降、2008年3月までに111本のHCV-RNA陽性検体が検出され、Genotypeの分類結果について献血者情報等をもとに解析を行い、HCVの感染動向を調査した。 【対象と方法】NATで検出されたHCV-RNA陽性検体のGenotypeについて、Core領域196bpの塩基配列をRT-PCR direct sequence法で決定し、分子系統樹解析により分類した。 【結果】HCV-RNA陽性検体のGenotypeは、1b：30本（27.0%）、2a：52本（46.8%）、2b：29本（26.1%）で、その他のGenotypeは検出されなかった。献血者の性別は男性71人（64.0%）、女性40人（36.0%）で、平成18年度の全献血者男女比（男性64.5%、女性35.5%）と完全に一致した。Genotypeの男女比は1bが15：15、2aは33：19、2bは23：6で、Genotype 2bで男性の割合が高かった。献血者の年齢別では、10代～20代で平成18年度の献血者の年代別構成比よりも高かった。また献血者100万人あたりの陽性者数を求めたところ、1bは中部地方以西で多く、関東地方以北では少なかった。2aは中部地方及び東北地方で若干多いものの、北海道を除き、その他の地域ではあまり差は見られなかった。2bは関東地方で多く、中部地方及び東北地方では検出されていない。 【考察】NATで検出されたHCV-RNA陽性検体はGenotype 2aが最も多く、1bと2bがほぼ同数だった。RNA陽性献血者のGenotypeを分類して感染傾向を調査していくことは、日本の急性肝炎患者の動向を予測するのに有用であると思われるので、引き続き行っていきたい。	赤十字アルブミン20 赤十字アルブミン25 血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
1999年7月からの2008年3月までにNATで検出された111本のHCV-RNA陽性検体のGenotype解析の結果、Genotype 2aが最も多く、1bと2bがほぼ同数だったとの報告である。 これまで、本剤によるHCV感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。また最終製品についてHCV-NAT陰性であることを確認していることから、本剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、NATでの陽性となる献血者は新規感染者の可能性があるため、Genotypeを分類して感染傾向を調査していくことは、日本の急性肝炎患者の動向を予測するのに有用であり、今後もGenotypeの調査を継続するとともに、情報の収集に努める。なお、献血時のHCVスクリーニング法としてより感度の高い化学発光酵素免疫測定法（CLEIA）および新NATシステムを導入した。

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HBVの一過性感染におけるeAg/eAb セロコンバージョンとプレコア領域の変異

埼玉県赤十字血液センター

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〈目的〉HBVの慢性持続感染者においては，一般的にプレコア変異，プロモーター変異が生じることによりe抗原の産生が低下し，e抗原期からe抗体期へセロコンバージョンすることが報告されている。HBVの一過性感染でも同様な現象が生じているかどうかを献血者のNAT陽性者を追跡調査した結果から調べたので報告する。〈対象と方法〉1999年から2003年までの間に日赤の血清学的検査陰性でNAT陽性になった349症例の内，e抗原陽性期からe抗体にセロコンバージョンしている追跡可能な症例を対象とした。塩基配列はプレコア領域のPCRを行い，PCR-ScriptAmpCloningKit（STRATAGENE）を用いてクローニングした。得られたクローンはグラスミッドをQIAprepMiniprepKit（QIAGEN）にて抽出しDNAシークエンスを解析した。〈結果と考察〉野生株に一過性感染した献血者のe抗原陽性期の検体から7クローン，e抗体にセロコンバージョンした検体から17クローンを調べたところプレコア変異部位の塩基配列に変異は生じていなかった。一方プレコア変異株の一過性感染では，感染当初はe抗原もe抗体も認められないものの，コア抗体出現に伴いe抗体が認められるようになったが塩基配列の変異は認められなかった。一過性感染では，慢性持続感染の場合と異なり，核酸の変異をほとんど伴わず，野生株のままe抗原からe抗体にセロコンバージョンし，血中HBV-DNA量も定量限界以下に減少することが確かめられた。

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NATスクリーニング検査で検出された HCV-RNA陽性検体の解析

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松本千恵子[1]，鈴木　光[1]，柚木久雄[2]，
内田茂治[3]，三根英子[3]，田所憲治[1]，
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【はじめに】1999年7月のNATスクリーニング検査（以下「NAT」という）導入以降，2008年3月までにHCV-RNA陽性検体111本が検出された。その111本についてGenotype分類を行ってきたので，その結果について献血者情報等を基に解析を行いHCVの感染動向を探ることとした。【対象と方法】NATで検出されたHCV-RNA陽性検体111本を対象とした。GenotypeはCore領域196bpの塩基配列をRT-PCR direct sequence法で決定し，分子系統樹解析により分類した。【結果】HCV-RNA陽性検体111本のGenotypeは，1b：30本（27.0%），2a：52本（46.8%），2b：29本（26.1%）で，その他のGenotypeは検出されなかった。献血者の性別は男性71人（64.0%），女性40人（36.0%）と男性が多かったが，平成18年度の全献血者男女比（男性64.5%，女性35.5%）と完全に一致した。Genotypeの男女比は1bが15：15，2aは33：19，2bは23：6で，Genotype 2bで男性の割合が高かった。献血者の年齢別では，10代～20代で平成18年度の献血者の年代別構成比よりも高かった。また地域別に献血者100万人あたりの陽性者数を求めたところ，1bについては中部地方より西の地方で多く，関東以北では少なかった。2aについては，中部地方で若干多いものの，北海道を除くその他の地域ではあまり差は見られなかった。2bについては関東地方で多く，中部地方及び東北地方では検出されていない。【考察】NATで検出されたHCV-RNA陽性検体はGenotyp2aが最も多く，1bと2bがほぼ同数であった。NATで検出されたHCV検体のGenotypeを分類して感染傾向を調査していくことは，日本の急性肝炎患者の動向を予測するのに有用であると思われるので，引き続き行なっていきたい。

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
				2008. 11. 20	該当なし	公表国	
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Ikeda H, Matsubayashi K, Sakata H, Takeda H, Kon E, Sato S, Kato T, Abe I, Satoru H, Tadokoro K. AABB Annual Meeting and TXPO 2008; 2008 Oct 4-7; Montreal.		日本	使用上の注意記載状況・その他参考事項等
販売名（企業名）	クロスエイトM250（日本赤十字社） クロスエイトM500（日本赤十字社） クロスエイトM1000（日本赤十字社）						クロスエイトM250 クロスエイトM500 クロスエイトM1000

研究報告の概要

○北海道の献血者におけるHEV感染症

背景：日本を含む先進工業国でHEVの輸血伝播が複数認められているが，献血者のHEV感染は未解明である。一方，日本のHEV感染は，主に人畜獣共通伝染病の食物介在経路であると考えられており，E型肝炎の散発性症例を引き起こしている。

方法：2005～2007年まで，北海道においてプールNATによりHEV RNAの有無についてスクリーニングを実施した。HEV -NAT RNA陽性（RT-PCR）献血者および抗HEV抗体陽性（ELISA）献血者について遡及及び追跡調査を実施した。HEV遺伝子型はダイレクトシーケンス法により決定した。また，献血者の食事歴についてアンケート調査を実施した。

結果：献血者834,843名のうち，HEV-NAT陽性献血者が100名特定され，男女比72/28，平均年齢41.0±2.5，遺伝子3/4型比92/6であった。74名からは抗HEV抗体は検出されず，20名からIgM抗HEV抗体が検出された。6ヵ月以内の前回献血歴がある39名の前回検体中からは，HEVマーカーは検出されなかった。献血時に肝炎の臨床症状を示す者はなかった。追跡調査を実施できた23名中13名は，ALT値の一過性の上昇（60IU/L超）が見られ，内2名がE型肝炎を発症した（最大ALT値：1,250，3,366 [IU/L]）。HEV RNAは，数ヶ月以内に消失することが確認され，HEVウイルス血症は献血から最長55日間持続した。NAT陽性献血者の76%（59/78）は，献血前に動物内臓を食べていた。

結論：北海道の献血者の約1/8,300はHEV RNA陽性で，多くは無症候性であったがウイルス血症は数ヶ月間持続する。これら献血者は，動物の内臓の摂取による人畜共通性食物媒介感染の可能が高い。

報告企業の意見	今後の対応
2005～2007年に北海道で実施したプールNATによるHEV RNAスクリーニングの結果、献血者の約1/8,300はHEV RNA陽性であった。ほとんどの献血者は動物内臓を摂取しており、無症候性であったがウイルス血症は数ヶ月間持続したとの報告である。HEVは脂質膜のないRNAウイルスである。これまで、本剤によるHEV感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、今後もHEV感染の実態に関する情報の収集及び安全対策に努める。日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。加えて、北海道における輸血後HEV感染症報告を受け、試験的に北海道では本報告のベースとなった研究的NATを行うなど安全対策を実施している。

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

(5)

negative. Self-trigger sites had fewer TPs (1) than primary and neighbor sites (21 and 11 respectively); primary and self-trigger sites yielded more FPs (10 and 4) than the neighbor trigger (2 FPs), $p < 0.0001$. 75% of centers (6 of 8) using primary trigger criteria had ID-yields versus 67% (8 of 12) using neighbor triggers, and 8% (1 of 12) using self triggers. At 57 centers that did not trigger, 17 (30%) had at least 1-PVD identified by MP. FPs occurred more frequently with ID vs MP ($p < 0.0001$); FP rates did not differ between automated (Tigris) and semi-automated (eSAS) testing, $p < 0.2792$. **Conclusions:** These data demonstrate that the recommended minimal AABB trigger criteria of 2-PVDs and a rate of 1:1000 missed viremic donors; therefore it is reasonable to adopt more stringent triggers for the 2008 season, including elimination of the rate criterion and triggering on 1 PVD for regions adjacent to centers which have already triggered. However, self triggering prior to the detection of any PVDs had very limited yield and required a significant proportion of testing capacity.

TABLE 1. WNV Procleix Assay Test Results: June–November 2007

Test Format	Negative		Initial Postives		False Positives		True Positives	
	#	%	#	%	#	%	#	%
MP-NAT	1,143,590	93.88572	103	0.008	5	0.00041	129	0.0106
ID-NAT	74,273	6.097617	100	0.008	35	0.00287	34	0.0028
Total	1,217,863	NA	203		40		163	

Note: MP-NAT true positives include ID-tested donations, positive at 1:16 (MP) dilution.

Disclosure of Conflict of Interest

Joan Dunn Williams, Gene Robertson, Sally Caglioti, Robert Williams, Michael P. Busch, Randall Spizman, Steven Kleinman: Nothing to Disclose

SP156

Effectiveness of Single Unit Testing in Detecting West Nile Virus in Viremic Donations

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Background: A Canadian blood agency has tested all donations for West Nile Virus (WNV) in pools of 6 since July 1, 2003. There are strategies in place to test donations for WNV by Single Unit testing (SUT) following the identification of one positive donation found through Minipool testing (MP) or when human cases within the previous 2 weeks were identified in the population of a health region at a rate of greater than 1 in 1000 in rural areas or greater than 1 in 2500 in urban areas. A study was undertaken to determine the effectiveness of SUT in 2006 and 2007. **Methods:** Plasma was available from 50 donations (4 from 2006 and 46 from 2007) identified as WNV positive by SUT and confirmed by an alternate WNV NAT assay and/or by the presence of WNV IgM and/or IgG antibodies. Master 1 in 6 dilutions of each donation were prepared with 4.5 mL of donor sample plus 22.5 mL of Normal Human Plasma (NHP) as diluent to mimic MP. Each of 2 WNV testing laboratories was sent 3 replicates of each dilution from the 50 donations and 3 replicates of NHP as controls. All replicates were labelled as "blind" samples for each testing site. Testing was performed with the Roche cobas TaqScreen West Nile Virus Test, for use with the cobas s 201 System. **Results:** WNV was consistently detected in MP for 46% of the samples as 23 of 50 donations were MP positive for all 6 replicates. WNV was not consistently detected in MP in 54% of the samples – 12 of 50 donations (24%) were MP negative in 1 to 5 replicates and 15 of 50 donations (30%) were MP negative for all 6 replicates. All NHP controls were MP negative. When IgG and/or IgM WNV antibodies were present, the samples were less likely to be MP positive. The 3 donor samples that were negative by alternate WNV NAT but had detectable WNV IgG and IgM antibodies were negative by MP. **Conclusion:** WNV SUT has proven to be an effective strategy to detect WNV viremic donors through the infectious season. MP testing is still not sensitive enough to detect all potentially infectious donations.

No. MP Replicates Positive	No. Donations	Alternate NAT			WNV IgG and/or IgM Antibodies		
		Pos	Neg	Neg	Pos	Equiv.	NT
All (6)	23	23	0	16	2	0	5
Some (1-5)	12	12	0	2	7	2	1
None (0)	15	12	3	1	13	0	1
Total	50	47	3	19	22	2	7

Equiv. = Equivocal NT = Not Tested

Disclosure of Conflict of Interest

Gordon Hawes, Margaret Fearon, Jamie Brown: Nothing to Disclose
Edna Zuber: Roche Molecular Systems – Board NewGen – No honoraria or financial support
Nicholas Dibdin: Not Specified

SP157

Evaluation of NS1 Antigen Detection of Dengue Virus in Healthy Blood Donors During a Dengue Outbreak in Martinique

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Background: A dengue virus type 2 (DENV-2) outbreak occured in Martinique from Sptember 2007 to January 2008. Among an insular population of 400,000 inhabitants, 17,990 people were infected (5%) according to the dengue vigilance network. Since the first care in blood transfusion remains the viral safety, it was decided by the "Etablissement français du Sang" (EFS) to evaluate the validity of NS1 antigen (Ag) detection in blood donations as screening assay. **Methods:** The presence of NS1 Ag was detected by the Platelia dengue NS1 Ag kit purchased from Bio-Rad Company. The performance of ELISA was evaluated with, as reference test, RT-PCR using serotype-specific primers. Three studies were conducted to evaluate NS1 Ag detection. A first retrospective study included 136 blood samples coming from a clinic serum library and known as RT-PCR positive for dengue virus (DENV-1: 2; DENV-2: 125; DENV-3: 3; DENV-4: 6). All these samples were tested for the presence of NS Ag. A second prospective studies consisted of 110 blood samples from patients consulting, during dengue outbreak, for severe febrile syndrome compatible with dengue infection. On each of the second series NS1 Ag was carried out in comparison with RT-PCR technique. The third study was a prospective screening for NS1 Ag and dengue genomic material on 561 blood samples from healthy blood donors. This last investigation was performed during the epidemical peak of dengue outbreak. **Results:** In the first series, NS1 Ag was found positive in 83/136 (61%) samples positive for dengue virus with RT-PCR. No false positive (NS1 Ag+/RT-PCR-) were observed. In the second prospective study, one half of the samples (55/110) were negative for dengue markers (NS1 Ag and RT-PCR). The other half was positive in RT-PCR for DENV-2. Among these positive samples, 36/55 (65%) reacted with the NS1 Ag assay. In the last prospective investigation in healthy blood donors, one sample was found positive as well for the NS1 Ag as for the DENV-2 RT-PCR (1/561, or 1.8 per thousand). The donor concerned was asymptomatic before and after (1 week) his blood donation. In the mean time, we have performed NS1 Ag detection as screening test for all blood donors during dengue outbreak and we have found 6 sera positive for NS1 Ag among the 6,904 tested donations (1,5 per thousand). All the six donors concerned were asymtomatic. **Conclusions:** In comparison with RT-PCR technique, NS1 Ag assay showed sensitivity around 60-65%. According to these results, dengue NS1 Ag detection did not totally fit the gold standard in transfusion screening. Our first evaluation concerning incidence of dengue virus in healthy blood donors are preliminary results. More specific studies with accurate epidemiological tools will follow.

Disclosure of Conflict of Interest

Michel Rits, Raymond Cesaire: Nothing to Disclose
Najioullah Fatiha, Pascale Richard: Not Specified

SP158

HEV Infection Among Blood Donors in Hokkaido, Japan

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Background: Several cases of transfusion-transmission of HEV have been recognized in industrialized countries including Japan. However, little is known about the situation of the HEV infection among blood donors. On the other hand, zoonotic food-borne route is regarded as a main route of HEV infection in Japan, which causes sporadic cases of hepatitis E. **Methods:** Blood donors were screened for the presence of HEV RNA by pooled NAT from 2005 to 2007 in Hokkaido. Look-back and follow-up studies were carried out for the NAT-positive donors with HEV RNA (real-time RT-PCR) and anti-HEV antibodies (ELISA). For look-back, the samples at previous donations were used. HEV genotype was determined by direct sequencing of PCR products of partial regions within ORF1 and/or ORF2. Questionnaire survey on eating history before the donation was also conducted for the NAT-positive donors. **Results:** Out of 834,843 donors, 100 of HEV NAT-positive donors were detected. Male/female, average age and genotype 3/4 were 72/28, 41.0 ± 12.5 and 92/6, respectively. In 74 HEV positive donors, no anti-HEV was detected and in 20 donors, IgM anti-HEV was detected at the donation. Thirty-nine positive donors had histories of previous donations within 6 months and no HEV marker was detected in the samples of such previous donations. None of donors showed clinical sign of hepatitis at the donation. Out of 23 NAT-positive donors who could be followed up more than twice within a month after the donation, 13 showed elevation of ALT level higher than 60 IU/L. The ALT elevation was transient in 11 donors. However, two of the 13 developed hepatitis E and their peak ALT levels were 1250 and 3366 IU/L, respectively. HEV RNA of all the 23 donors was confirmed to disappear within a few months. HEV viremia persisted up to 55 days at the longest after the HEV-positive donation. In 3 donors, IgG anti-HEV became undetectable after 1 to 1.5 year after donations. Most of NAT-positive donors (59/78, 76%) had histories of eating animal viscera before their donations. **Conclusion:** About 1/8300 of blood donors in Hokkaido were HEV RNA-positive. Most of them were in their early phase of HEV infection at donation and remained asymptomatic, although HEV viremia persists for a few months. They are likely to be infected via zoonotic food-borne route by eating animal viscera.

Disclosure of Conflict of Interest

Hisami Ikeda, Keiji Matsubayashi, Hidekatsu Sakata, Hiromi Takeda, Emi Kon, Shinichiro Sato, Toshiaki Kato, Ikuma Abe, Hino Satoru, Kenji Tadokoro: Nothing to Disclose

SP159

Switching to Single-unit Testing: Importance of an in-house Test for Blood Donor West Nile Virus Testing

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Background: West Nile Virus (WNV) nucleic acid testing (NAT) is routinely done in mini-pool format. Single-donor testing is used for mini-pool resolution, when there are not enough samples to prepare a mini-pool or in situations of high incidence of WNV infection in a given area. Since the summer of 2004, Hema-Quebec has performed single-unit testing on blood donors from areas with high WNV activity. The decision to switch from mini-pool to individual donor testing is based on the identification of a positive donor sample by the testing laboratory. This report describes the contribution of a previously described in a previous AABB meeting (San Diego, 2003) in-house assay to the management of the decision-making process concerning the switch from mini-pools to single-donor testing. **Methods:** Routine screening of blood donations is performed by our testing laboratory in mini-pools of 6 donors using the Cobas TaqScreen WNV NAT assay (Roche Molecular Systems). An in-house confirmatory WNV NAT was designed by our Operational Research unit with specific DNA primers distinct from those used in the Roche Molecular Systems testing kit. In-house kits were produced within a Good Manufacturing Practices environment and their use was approved by Health Canada. Stability and sensitivity were monitored monthly and results were reviewed by quality assurance. WNV-positive samples were sent to the research testing unit for confirmation and test results were returned to the Medical Director within 24 hours. **Results:** During summers of 2004 to 2007, 499,681 blood donors were tested and 10 mini-pools were positive with the WNV assay. After resolution, samples from 2 mini-pools were all negative and 8 samples were found positive. Of these, 7 were tested with the in-house assay. Two samples were confirmed positive while 5 came out negative for WNV. None of the 5 unconfirmed donors have developed antibodies to WNV on follow-up, whereas the two confirmed by our in-house assay were also confirmed by seroconversion with an immunological assay. **Conclusion:** Single-donor testing has a major impact on resources in the blood testing laboratory. Decisions based on false-positive screening test results could lead to substantial costs. The rapid availability of confirmatory results through a close collaboration between Research and Operations contributes to well-informed decisions by Operations management.

Disclosure of Conflict of Interest

Isabel Chateauneuf, Marie-Claire Chevrier, Louis Thibault, Gilles Delage, Cindy Castilloux, Marie-Eve Nolin, Matthieu Guerin, Brigitte Caron, France Bornier, Maryse St-Louis: Nothing to Disclose

SP160

The Role of Platelet Bound Antibodies on Thrombocytopenia in Acute Dengue Virus Infection

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Background: Dengue is an endemic-epidemic mosquito-borne viral disease, caused by the dengue virus (DV) with an increasing incidence in the world-wide distribution. This disease may have unusual complications such as hepatic damage, cardiomyopathy, encephalopathy and severe hemorrhagic manifestations. Even patients with mild symptoms may present thrombocytopenia and the exact mechanism for the low platelet count has not yet been established. The mechanisms proposed are; transient marrow suppression, platelet aggregation to endothelial cells targeted by DV, hemophagocytosis and platelet immune destruction with dengue antibody complexes. The aim of the present study was to identify the prevalence of thrombocytopenia and evaluate a possible correlation to platelet bound antibodies on acutely DV infected (ADI) patients during the 2007 spring outbreak. **Methods:** 47 ADI patients were included (49% female, 51% male; median age: 38.5 years, range: 17-69 y). Platelet counts were performed in an automated counter. Sera were evaluated by flow cytometric assay to investigate the presence of platelet bound IgG or IgM antibodies in patients and in a group of 50 non-transfused group O male blood donors as a control group. A positive result was defined as a fluorescence ≥2 standard deviation (sd) from negative control and inconclusive result as a fluorescence ≥1 sd, <2 sd from negative control. **Results:** Positive IgG or IgM tests were significantly lower in the control group compared to patients (64% x 23.4%, $P = 0.00013$, $x = 14.58$).. The prevalence of thrombocytopenia found among patients was 68.1%. No correlation was found between thrombocytopenia and IgG or IgM tests among patients. Nevertheless, a significantly higher prevalence of positive tests was found in thrombocytopenic patients, when compared to controls (40.6% x 22.0%, $P = 0.002$, $x = 5.65$). The results are summarized in the table below. **Conclusions:** The results of this study confirm that thrombocytopenia is a frequent finding (68%) in ADI patients. Platelet bound antibodies are also frequent in these patients (~45%). These antibodies may have a role on thrombocytopenia as they have higher prevalence in thrombocytopenic ADI (~41%) than in controls (22%), but other mechanisms are probably involved since non-thrombocytopenic patients also have a high prevalence of these antibodies. Study granted by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – São Paulo State Research Support Foundation.)

Platelet Bound Antibody	Acute Dengue Patients			Controls
	Plt ≤ 150 × 10^3/L N = 32 (68.1%)	Plt > 150 × 10^3/L N = 15 (31.9%)	Total N = 47	N = 50
IgG/M Negative	9 (28.1%)	2 (13.3%)	11 (23.4%)	32 (64.0%)
IgG/M Inconclusive	10 (31.3%)	5 (33.3%)	15 (31.9%)	6 (16.0%)
IgG/M Positive	13 (40.6%)*	8 (53.4%)*	21 (44.7%)*	11 (22.0%)**

* $P = 0.002$, $x^2 = 5.65$; ** $P = 0.00013$, $x^2 = 14.58$

Disclosure of Conflict of Interest

Rodrigo Angerami, Vagner Castro, Maria L Barjas-Castro: Nothing to Disclose
Fernanda Rossi, Joyce Annichino-Bizzacchi, Brigina Kemp, Manângela Resende, Vania del Guercio, Luiz Silva: Not Specified

TTID 1: Testing Issues (Virology)

SP161

Development of a Parvovirus B19 DNA Assay and Systems Software for Plasma Screening

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Background: Recently the FDA asked manufacturers of derivatives to include "in-process" screening of recovered plasma for high titer Parvovirus