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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
最近、SENウイルス（SENV）とよばれる新規のDNAウイルスが発見され、2種のSENV変異体（SENV-DおよびSENV-H）と非A−E型肝炎との間の強い関連性が報告された。小児におけるSENV感染の特徴を明らかにするために、PCR法を用いて非A−C型肝炎小児、輸血歴のある小児、あるいは輸血歴も肝疾患歴もない小児（コントロール群）、および妊婦における血清SENV DNAの検出率を調査した。SENV-Dは劇症肝炎の60%、急性肝炎の5%、慢性肝炎の11%、コントロール群の13%、妊婦の15%で検出された。SENV-Hは、劇症肝炎では検出されず、急性肝炎では5%、慢性肝炎では検出されず、コントロール群の2%、妊婦の12%で検出された。急性あるいは慢性肝炎とコントロール群の間においてSENV-Dに対する有意差はみられなかったが、劇症肝炎におけるSENV-D検出率はコントロール群より有意に高かった（P<0.05）。いずれかの肝炎とコントロール群の間におけるSENV-Hの有意差はみられなかったが、妊婦におけるSENV-H検出率はコントロール群より有意に高かった（P<0.05）。SENV-D、SENV-Hとも急性あるいは慢性肝炎には関与しなかったが、SENV-Dは劇症肝炎の危険因子となり得るかもしれない。	クロスエイトM250 クロスエイトM500 クロスエイトM1000 血液を原料とすることに由来する感染症伝播等 理論的なvCJD等の伝播のリスク

報告企業の意見	今後の対応
SENウイルス変異体（SENV-D）が劇症肝炎の危険因子となり得る可能性を示唆する報告である。	これまで、本製剤によるSENウイルス感染の報告はない。本製剤の製造工程には、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つ以上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、本製剤の安全性は確保されており、特別の対応を必要としないが、今後も情報の収集に努める。

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Prevalence of SEN virus among children in Japan

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Abstract

Recently, a novel deoxyribonucleic acid (DNA) virus, designated SEN virus (SENV), was discovered and strong associations between the two SENV variants (SENV-D and SENV-H) and non-A to E hepatitis were reported. To clarify the character of SENV infection in children, we investigated the detection rates of serum SENV DNA by polymerase chain reaction (PCR) among children with non-A to C hepatitis, with histories of transfusions, with neither histories of transfusions nor liver diseases (control), and among pregnant women. SENV-D was detected in 60% of fulminant hepatitis, 5% of acute hepatitis, 11% of chronic hepatitis, 13% of controls, and 15% of pregnant women. SENV-H was detected in none of fulminant hepatitis, 5% of acute hepatitis, none of chronic hepatitis, 2% of controls, and 12% of pregnant women. No significant difference was found for SENV-D between acute or chronic hepatitis and controls, however SENV-D detection rate in fulminant hepatitis was significantly higher than that in controls ($P < 0.05$). No significant difference was found for SENV-H between any hepatitis and controls, however SENV-H detection rate in pregnant women was significantly higher than that in controls ($P < 0.05$). Neither SENV-D nor SENV-H was associated with acute or chronic hepatitis; however, SENV-D might be a risk factor of fulminant hepatitis.
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Keywords: SEN virus; Non-A to C hepatitis; Fulminant hepatitis; Polymerase chain reaction

Introduction

Five viruses are responsible for the liver inflammation known as hepatitis; they are designated as hepatitis viruses A through E. However, additional pathogens have also long been suspected to cause the disease, since many people with hepatitis test were tested negative for all of the known hepatitis viruses. The cause is presently unknown in 10–20% of acute hepatitis cases, 30% of chronic hepatitis cases, and 50–60% of fulminant hepatitis cases (Alter, 1999). The pathogens responsible for most cases of fulminant hepatitis in children are unknown (Miyake et al., 1998). The newly defined GB virus-C and TT virus (TTV) have been associated with non-A to E hepatitis (Simons et al., 1995; Linnen et al., 1996; Nishizawa et al., 1997). Although the pathogenicity of some strains of TTV is believed to cause cases of hepatitis in adults (Itoh et al., 1999; Okamoto and Mayumi, 2001) and in children (Sugiyama et al., 2000; Okamura et al., 2000; Tajiri et al., 2001), other studies have failed to confirm an etiologic association between these viruses and human diseases (Alter et al., 1997; Naoumov et al., 1998).

Recently, a novel deoxyribonucleic acid (DNA) virus, designated as the SEN (the initials of the patient from which the virus was recovered) virus (SENV), was discovered in the serum of an intravenous drug abuser infected with human immunodeficiency virus (Primi et al., 2000). The SENV has approximately 3800 nucleotides and belongs to the family Circoviridae, the genus *Anellovirus*, a group of small, single-stranded, non-enveloped circular DNA viruses such as the TTV and a TTV-like mini virus (Hino, 2002). These circular single-strand DNA viruses may have evolved from a common ancestor (Tanaka et al., 2001).

To date, eight different SENV variants (designated as SENV-A through SENV-H) have been described. A recent study showed a strong association between infection with two SENV variants (SENV-D and SENV-H) and

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transfusion-associated non-A to E hepatitis (Umemura et al., 2001). However, more recent studies found only a weak association between SENV infection and liver diseases (Mikuni et al., 2002; Wong et al., 2002; Yoshida et al., 2002). The clinical relevance of SENV infection remains controversial. Many reports have demonstrated a prevalence of SENV infection in adult patients, but few reports have demonstrated a prevalence of SENV infection in children. Therefore, to clarify the character of SENV infection in children, we investigated the detection rates of serum SENV-D and SENV-H DNA by polymerase chain reaction (PCR) among Japanese children with non-A to C hepatitis, among Japanese children with histories of blood transfusions and among Japanese pregnant women. We also examined children with neither liver diseases nor histories of blood transfusions, as the control group.

2. Materials and methods

2.1. Subjects

We studied 140 children who visited the pediatric clinic at Nagoya City University Medical School or related hospitals. Of the 34 children with non-A to C hepatitis, 5 were classified into the fulminant hepatitis group (one boy and four girls; mean age ± S.D., 7.7 ± 3.5 years; mean alanine aminotransferase (ALT) levels±S.D., 1484±475 IU/l), 20 were classified into the acute hepatitis group (11 boys and 9 girls; mean age, 4.0 ± 4.1 years; mean ALT levels, 273 ± 488 IU/l), and nine were classified into the chronic hepatitis group (three boys and six girls; mean age, 2.1±2.8 years; mean ALT levels, 298±426 IU/l). Another 46 children had histories of blood transfusions (30 boys and 16 girls; mean age, 6.8±4.8 years; mean ALT levels, 66±136 IU/l). In these cases, at least 1 month had passed between the first transfusion and blood sampling. In the blood transfusion group, all children were negative for anti-human immunodeficiency virus antibody (HIVAb) and 13 of the 46 children tested positive for anti-hepatitis C virus antibody (HCVAb). On the other hand, no children in the other groups were HCVAb-positive. Sixty children with neither histories of blood transfusions nor liver diseases were studied as the control group (33 boys and 27 girls; mean age, 4.7 ± 3.7 years; mean ALT levels, 19 ± 23 IU/l). Informed consents for the analysis of viral infections were obtained from the mothers of all children. The adult group consisted of 60 pregnant Japanese women (mean age, 27.8 ± 2.8 years) who visited Nagoya City University Medical School or the Suzuki hospital. All of these women had no histories of blood transfusions and were negative for HIVAb, hepatitis B virus surface antigen (HBsAg), and HCVAb. The levels of HIVAb, HBsAg, and HCVAb were measured using commercially available enzyme immunoassays, radioimmunoassays, and second-generation enzyme-linked immunosorbent assays.

2.2. Detection of SENV DNA

All blood samples were separated by centrifugation. The sera were stored at −30 °C until SENV DNA analysis. The DNA was extracted from 200 μl of serum using a commercial kit (QIAamp DNA blood mini kit; Qiagen, Tokyo, Japan), according to the manufacturer's instructions. The sample volume was then brought to 50 μl with solution buffer. For PCR analysis, a 2 μl sample of each DNA preparation was amplified in a 20 μl reaction volume containing 0.5 U *Taq* polymerase (Takara, Otsu, Japan), 125 μM of each deoxyribonucleoside triphosphate, 0.5 μM of each primer pair, 50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM Tris–HCl (pH 8.3). The SENV DNA was detected by modified PCR methods using strain-specific primers, as described elsewhere (Umemura et al., 2001). For SENV-D detection, primer 1 (sense primer 5′-GTAACTTTGCGGTCAACTGCC-3′) (nucleotides 1322–1342 of AX 025730 in the GenBank) and primer 2 (antisense primer, 5′-CCTCGGTTKSAAAKGTYTGATAGT-3′ (K = G or T, S = C or G, Y = C or T)) (nucleotides 1521–1544) were used. Amplification by PCR consisted of 55 cycles (denaturing at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1.5 min) followed by the extension reaction at 72 °C for 7 min in a commercial cycling system (GeneAmp PCR System 9700; Applied Biosystems, Chiba, Japan). For SENV-H detection, primer 3 (sense primer 5′-GGTGCCCCTWGTYAGTTGGCGGTT-3′ (W = A or T)) (nucleotides 1271–1294 of AX 025838) and the same antisense primer 2 (nucleotides 1477–1500) were used. Amplification by PCR consisted of 55 cycles (denaturing at 94 °C for 1 min, annealing at 62 °C for 1 min, and extension at 72 °C for 1.5 min). The amplification products (223-bp fragments for SENV-D and 230 bp fragments for SENV-H) were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and visualized using an ultraviolet transilluminator. The bands then underwent DNA sequencing by the following method.

To determine the assay sensitivity, a dilution series was performed with plasmid DNA containing an SENV PCR target insert. We determined that 55 cycles of PCR amplification followed by electrophoresis and ethidium bromide staining was 10 times more sensitive than 40 cycles of PCR amplification followed by Southern blotting with horseradish peroxidase probes.

2.3. DNA sequencing

The amplified products were extracted from the agarose gel and purified using a commercial kit (QIAquick gel extraction kit; Qiagen), according to the manufacturer's instructions. The purified products were directly sequenced in both directions by a fluorescent dye terminator cycle system, using primers 1, 2, and 3 as templates with a commercial DNA sequencing kit (Applied Biosystems) and an autoanalyzer (ABI PRISM 310 Genetic Analyzer; Applied

Biosystems), according to the manufacturer's instructions. In the present study, we used the PCR-direct sequencing method. When mixed fluorescent waves were observed (at 1–3 points in a few cases), we determined the type of nucleotide with higher fluorescent intensities. The nucleotide sequences, excluding the primer sequences (178 bp fragments for SENV-D and 182 bp fragments for SENV-H), were compared with the consensus SENV sequence (the accession numbers in the GenBank are AX 025730 for SENV-D and AX 025838 for SENV-H). A homology search was performed using the GENETYX-MAC computer software package, version 8.0 (Software Development Co., Tokyo, Japan), and a phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using ODEN software, version 2.0 (Ina, 1994).

2.4. Statistics

Statistical analysis was performed using Fisher's exact test. But age and ALT levels were compared between groups using Mann–Whitney's *U*-test. Probability values less than 0.05 were considered statistically significant. Data were reported as mean ± S.D.

3. Results

The prevalence of SENV DNA in different patient groups is shown in Table 1. Among all groups, the range of the SENV-D detection rate was 5–60% and the range of the SENV-H detection rate was 0–12%. The detection range of both SENV-D and SENV-H DNA was 0–2%; both were found in only one out of the 46 children with a history of blood transfusion and in one out of the 60 pregnant women. None had concomitant HCV and SENV-D infections and only one child had concomitant HCV and SENV-H infections. No significant difference was found in the SENV-D detection rate between the control group and the groups with various types of liver disease. However, the prevalence of SENV-D in the fulminant hepatitis group was significantly higher than that in the control group, in the acute hepatitis group, and in the group of pregnant women ($P < 0.05$). All five patients in the fulminant hepatitis group had non-A to C hepatitis and were negative for GB virus C, Cytomegalovirus (one patient was not examined) (Miyake et al., 1998), and hepatitis E virus ribonucleic acid by reverse transcription PCR (Goto, submitted). In two of these five patients, TTV genotype 1 was detected (Sugiyama et al., 2000). Therefore, only one patient had a mixed infection of SENV-D and genotype 1 of TTV. No significant difference was found in the SENV-H detection rate between the control group and the groups with various types of liver disease. However, a significant difference in SENV-H detection rate was found between the control group and the group of pregnant women ($P < 0.05$).

No significant differences were found between the SENV-D positive children and the SENV-D negative children in sex (13 boys and eight girls versus 65 boys and 54 girls; $P = 0.35$), age (4.9 ± 3.7 years versus 5.2 ± 4.4 years; $P = 0.85$), ALT levels (286 ± 632 IU/l versus 121 ± 308 IU/l; $P = 0.93$), or history of blood transfusions (38% versus 32%; $P = 0.79$). Similarly, no significant differences were found between the SENV-H positive children and the SENV-H negative children in sex (four boys and one girl versus 74 boys and 61 girls; $P = 0.26$), age (3.2 ± 4.1 years versus 5.2 ± 4.3 years; $P = 0.30$), ALT levels (154 ± 302 IU/l versus 145 ± 376 IU/l; $P = 0.83$), or history of blood transfusions (60% versus 32%; $P = 0.20$).

The range of nucleotide sequence homologies between the consensus sequence and each group were 83.1–89.9% among SENV-D DNA samples and 75.4–82.4% among SENV-H DNA samples. All nucleotide sequences showed a difference of >10% from those of the consensus sequence. Conversely, the range of nucleotide sequence homologies in each group was 80.9–100% among SENV-D DNA samples and 75.3–100% among SENV-H DNA samples. An evolutionary tree-diagram was constructed from all sequences obtained using the neighbor-joining method (Fig. 1). The consensus sequence of SENV-C was classified into the cluster of SENV-H, as previously reported (Wilson et al., 2001). The SENV-H DNA samples consisted of two clusters in the present study; however, no specific strains were found for patients with hepatitis, control subjects, or pregnant women.

Table 1
Prevalence of infection with SEN virus (SENV) variants D and H DNA in different subjects groups in Japan

Group	Number of subjects	Subjects with infection (%)		
		SENV-D	SENV-H	SENV-D and SENV-H
Fulminant hepatitis	5	3 (60%)[a,b,c]	0(0%)	0(0%)
Acute hepatitis	20	1 (5%)	1 (5%)	0 (0%)
Chronic hepatitis	9	1 (11%)	0 (0%)	0 (0%)
Blood transfusion	46	8 (17%)	3 (7%)	1 (2%)
Pregnant women	60	9 (15%)	7 (12%)[a]	1 (2%)
Control	60	8 (13%)	1 (2%)	0 (0%)

[a] $P < 0.05$ compared with the control (compared using Fisher's exact test).
[b] $P < 0.05$ compared with pregnant women (compared using Fisher's exact test).
[c] $P < 0.05$ compared with patient with acute hepatitis (compared using Fisher's exact test).

4. Discussion

Whether the pathogenicity of SENV could cause hepatitis in adults remains debated. In this study, we examined serum samples from only one time point for the presence of SENV DNA. Therefore, we were unable to determine whether the SENV viremia observed in these patients was transient or persistent. Nevertheless, in this study, the rate of SENV-D

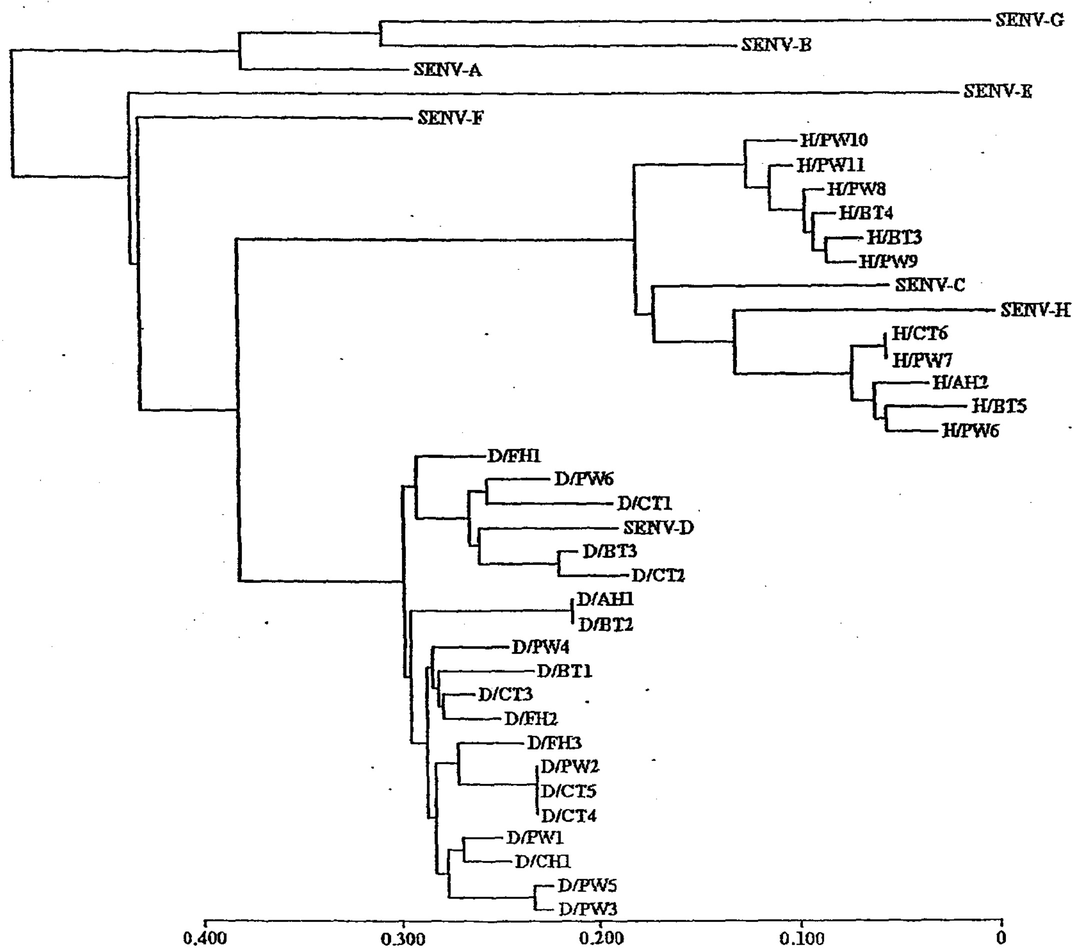

Fig. 1. Phylogenetic analysis of the SEN virus (SENV). A tree was constructed using the neighbor-joining method. The horizontal axis shows the number of nucleotide substitutions per site. FH1-3, fulminant hepatitis; AH1 and 2, acute hepatitis; CH1, chronic hepatitis; BT1-5, blood transfusion; PW1–11, pregnant women; CT1–6, control. PW6 and BT3 were both positive for SENV-D and SENV-H. Reference sequences obtained from GenBank (accession number): SENV-A (AX025667), SENV-B (AX025677), SENV-C (AX025718), SENV-D (AX025730), SENV-E (AX025761), SENV-F (AX025822), SENV-G (AX025830), and SENV-H (AX025838).

or SENV-H among children in the acute hepatitis group was 5%, not significantly higher than that among children in the control group. In addition, no significant difference in ALT levels was found between SENV DNA-positive children and SENV DNA-negative children. These results suggest that SENV-D and SENV-H do not contribute to acute hepatitis in children. However, three of five patients with fulminant hepatitis were positive for SENV-D, and this prevalence was significantly higher than that of the control group. This suggests that SENV-D might be a risk factor of cryptogenic fulminant hepatitis in children. However, because the number of fulminant hepatitis cases in this study is small, further investigation is needed. In the present study, we analyzed the sequences of SENV-D and SENV-H DNA and obtained strains specific to Japan. A phylogenetic tree showed no disease-specific strains among them. Homology studies revealed that all SENV-D or SENV-H strains in Japan differed by more than 10% from the consensus sequence (from strains found in Italy).

In this study, no significant difference was found in the SENV-H detection rate between the control group and the groups with various types of liver disease. However, the prevalence of SENV-H in the group of pregnant women (the control group of adults) was significantly higher than that in the control group of children. Conversely, no significant difference was found in the SENV-D detection rate between the group of pregnant women and the control group of children. These results suggest that SENV-H might be acquired later in life and differently from SENV-D.

In previous reports, the detection rates of SENV-D and SENV-H were 7% and 3% in blood donors in Japan (Shibata et al., 2001), 2% and 15% in healthy adults in Taiwan (Kao et al., 2002), and 15% and 24% in Canadian Inuit individuals (Wong et al., 2002). In the present study, the detection rates of SENV-D and SENV-H were 15% and 12% in pregnant Japanese women. These prevalence rates are higher than those reported for Japanese adults (Shibata et al., 2001). The difference might be in the different assay systems used or in

some immunologic tolerance during pregnancy that might activate latent virus.

Initially, the infection route of SENV was thought to be parenteral (Wilson et al., 2001). However, SENV-D and SENV-H DNAs were detected in 2% of eligible United States blood donors (Mushahwar, 2000), 15% of healthy persons in Taiwan (Kao et al., 2002), 20% of healthy persons in Japan (Umemura et al., 2000), and more frequently in patients with hepatocellular carcinoma who are infected with HBV and HCV (Umemura et al., 2000; Momosaki et al., 2000). Furthermore, another method that uses other primers to detect SENV DNA revealed that the prevalence of SENV was about 75% in healthy adults (Yoshida et al., 2002). Therefore, the primary route of infection is probably not parenteral. However, the high prevalence previously reported was the total prevalence of SENV-A, SENV-B, SENV-C, SENV-D, and SENV-H DNAs, and the prevalence of each variant was not reported. Our data indicate that SENV infection patterns may differ with each variant of this virus. One report suggested that mother-to-infant transmission was one possible route of transmitting SENV to children (Pirovano et al., 2002). In the present study, the rates of SENV-D and SENV-H among the blood transfusion group were higher than those among the control group, but the differences were not statistically significant. This suggests that blood transfusion is not a major transmission route of SENV-D or SENV-H infection in children.

In conclusion, neither SENV-D nor SENV-H was associated with acute or chronic hepatitis or transfusions; however, SENV-D might be a risk factor of fulminant hepatitis in children.

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No. 23

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2004.5.6	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	aaBB Association Bulletin. 2004.4.15	公表国
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）			米国

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
米国血液銀行協会（aaBB）は、2004年度に予測されるウエストナイルウイルス（WNV）の流行対策に関して、協会公報で情報提供を行った。aaBBに設置されたWNV対策委員会の会議で、WNV検査のデータを検討した。2003年のWNV流行シーズン中には約860万の供血血液に対して、ミニプールNAT（MP-NAT）および個別NAT（ID-NAT）を組み合わせた検査が行われている。一次判定で陽性の2,749件中1,000件が陽性と確定された。CDCは輸血によるWNV感染が疑われる23症例を検討し、6症例が確定された。流行地域ではWNV RNA検出にID-NAT実施が有用であるかもしれないが、ID-NATは、装置、試薬、更には熟練した技術者を確保できるかがその成否に影響する。血液事業施設がID-NATを実施する際には、次の点を考慮することが望まれる。ID-NATは早期に行うほど効果的である。ID-NATを開始する統計学的に意味のある「トリガー」を明らかにすることが妥当である。ID-NATの中止はデータに基づいた手順にのっとるべきで、特にID-NATトリガー基準が合致しなくなった際には、MP-NATを再開すべきである。ID-NATが適切に実施されたとしても、すべての感染した供血血液を検出するのは不可能である。	クロスエイトM250 クロスエイトM500 クロスエイトM1000 血液を原料とすることに由来する感染症伝播等 理論的なvCJD等の伝播のリスク

報告企業の意見	今後の対応
米国において、WNVの流行に対して行われたミニプールNATと個別NAT（ID-NAT）の結果についての報告である。ID-NATが適切に実施されたとしても、すべての感染供血血液を検出するのは不可能である。	これまで、本製剤によるWNV感染の報告はない。本製剤の製造工程には、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つ以上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、本製剤の安全性は確保されており、特別の対応を必要としないが、今後も情報の収集に努める。なお、平成14年11月5日の薬事・食品衛生審議会血液事業部会において「現在行われているウイルスの不活化処理により安全性が確保されているものと考えられる」との意見が取り纏められている。

ASSOCIATION BULLETIN

#04-03

Date: April 15, 2004

To: AABB Members

From: Kathleen Sazama, MD, JD - President
Karen Shoos Lipton, JD - Chief Executive Officer

Re: Update on WNV-Related Activities and Considerations, 2004; A Summary of the WNV Task Force Meeting

Summary:

This bulletin is intended to provide information to members about actions that may be taken during the anticipated West Nile virus (WNV) outbreak in the year 2004. The information is not intended to establish a formal standard, or a standard of practice. It does provide an overview of discussions held at the WNV Task Force meeting on February 11, 2004. The outcome of these discussions may be useful in providing a framework as members develop plans for their donation testing programs.

Background:

On February 11, 2004, AABB coordinated a meeting of the WNV Task Force, which includes representatives from the national blood organizations, test kit manufacturers, the Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH) to review data from (WNV) testing. During the 2003 season, approximately 8.6 million donations were tested through a combination of minipool (MP-NAT) and individual donation nucleic acid tests (ID-NAT). Confirmation testing of 2,749 initial reactive units resulted in approximately 1,000 confirmed positive units and over 1,700 confirmed negative units. During 2003, CDC investigated 23 cases of possible transfusion-associated transmissions of WNV illness. To date, six of the 23 cases have been confirmed, 11 have been classified as non-cases, and the remaining six are either inconclusive or the investigations are still open. ID-NAT was performed in some locations, either through retrospective research protocols or prospective testing programs. Both types of ID-NAT resulted in the detection of some additional NAT-positive samples, relative to MP testing. Transfused components from at least two such donations were associated with transfusion-transmitted WNV infection. Further studies to define the infectivity of low titer West Nile viremic samples are planned or are under way.

Participants at the meeting stressed the value of reporting results of WNV RNA testing to state and/or local public health departments and recommended that this practice be maintained in 2004. (See Association Bulletin #03-08.)

It is reasonable to anticipate that there will be an outbreak of WNV infection in the United States in 2004, but it is impossible to predict its magnitude or geographic locations. However, as of

April 7, 2004, infected birds or mammals had been reported in seven states, including Florida and its bordering states and in Texas, California, and New York (see http://www.cdc.gov/ncidod/dvbid/westnile/surv&control04Maps.htm). For planning purposes, it is prudent to assume that the overall magnitude of the outbreak in 2004 will be similar to that seen in 2002 and 2003. In such a circumstance, ID-NAT for WNV RNA might have additional value in areas of high incidence. However, it is recognized that the extent to which such testing is achievable depends upon the availability of adequate resources, especially equipment, reagents, and, most critically, trained technologists.

Blood establishments intending to implement ID-NAT may want to include the following considerations in their planning processes:

1. ID-NAT is likely to be most effective if it is initiated as early as possible, during the emergence of the epidemic phase of infection.

2. Accordingly, it may be appropriate to develop a statistically meaningful "trigger" for initiation of ID-NAT. Such a trigger should reflect local conditions and may include considerations of the achievable level of ID-NAT. For example, centers may choose to convert from MP-NAT to ID-NAT based on the frequency of positive MPs that contain individually identified reactive donations. In addition to the frequency of positive MPs, the absolute number of individual WNV-reactive donations identified from MP testing within a predetermined geographic area is important. Although the ratio of donations having low viral loads (detectable by ID-NAT only) versus those with higher viral loads (detectable by MP-NAT) will vary as the epidemic moves through a given area, the value of accumulating the absolute number of cases is to verify whether the epidemic is real in a given geographic area. Some studies have suggested that with every four WNV RNA positive donations detected by minipools, there is one donation that would require ID-NAT for identification. A frequency of 1:1,000 or higher coupled with two to four WNV RNA positive donations from the predetermined geographic area was used by some centers in 2003.

   Blood establishments should, therefore, have processes for timely monitoring of the results of testing, as well as the number of donations collected in a specific geographic area. Establishments that send their testing to another facility will need to have extensive discussions with the contract laboratory before the WNV season to ensure the feasibility of the desired thresholds.

3. There should be a well-defined data-based process for cessation of ID-NAT. Specifically, when the ID-NAT trigger criteria are no longer met, the blood establishment would resume MP testing.

4. Consideration should be given to the manufacture of frozen components during the time of ID-NAT. If inventory permits, blood establishments may elect to convert their production of Fresh Frozen Plasma to recovered plasma during the times that ID-NAT is performed for a given area. This would allow the maximum protection in areas that have the highest risk of a WNV transfusion transmission.

5. It should be recognized that even with ID-NAT in place, it is possible that not all potentially infectious donations may be detected.

During 2004, the AABB will continue to host conference calls every other week or weekly as needed to monitor the WNV outbreak and assist in coordinating testing practice and the collection of results of blood donation testing for WNV RNA.