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	厚生労働省処理欄
一般的名称	新鮮凍結血漿、赤血球		研究報告の公表状況	Transfusion (United States) Jun2004, 44 (6) p929-33.	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
米国においてミニプールNAT実施後2番目のウインドウ期間中の献血者からのHIV伝播の報告である。 前回献血から2ヵ月後の献血時スクリーニング（ミニプールNAT、ELISA、EIA）においてHIV陽性を示した。この献血者は健康状態が良好な18歳の女性であり、献血を拒否するようなリスク因子は認められなかった。 前回の献血に由来する血液製剤の投与を2人が受けていた。受血者Aは内出血のため新鮮凍結血漿（FFP）の投与を、受血者Bは手術のため赤血球（RBC）の投与を受けており、EIA法およびウェスタンブロット法によるHIV検査を行ったところ、2人ともHIVに感染していた。 米国においては、何重もの安全対策がHIVや他の血液伝染性病原体が伝播されるリスクを低減するための広範で費用のかかる方策がとられているにも関わらず、ウインドウ期間中の伝播が非常に稀ではあるが起こっている。 プール検体での検査がHIV RNAを検出できなかったことは、個別検査の実施が正当化されるのではないかとして例証されているが、個別NATは、一部の地域では評価されているが、利用したとしてもHIV検出のためのウインドウ期をわずかしか短縮しそうもない。例えば、360万人の献血者を対象とした欧州研究において、ミニプールNATにより見逃されたHIV陽性献血はなかった。これは、個別検査を行った場合、費用は増大するにもかかわらず、輸血の安全性がわずかにしか高まらないことを示唆している。	

報告企業の意見	今後の対応
ウインドウ期間は検査方法の進歩等により、短縮されている。更に血漿分画製剤はウイルスを不活化するための複数の工程が設けられているが、引き続き動向に留意していきたい。	今後もHIV感染リスク等について情報収集に努めていく。

Window-period human immunodeficiency virus transmission to two recipients by an adolescent blood donor

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BACKGROUND: Pooled NAT and donor screening have reduced the diagnostic window period for HIV in the blood donor population to approximately 10 to 15 days. This report describes two cases of transfusion-acquired HIV infection and verification of transmission from the donor to the recipients, and attempts to identify how the 18-year-old donor acquired her infection.

STUDY DESIGN AND METHODS: After a repeat donor had a positive HIV test result, two recipients of the donor's previous donation were identified and tested. The donor and recipients were interviewed and blood samples were obtained for HIV DNA sequencing and phylogenetic analysis.

RESULTS: The two recipients had positive HIV test results. Phylogenetic analysis showed a high genetic similarity among the viruses (bootstrap 100%), consistent with transmission from the donor to the recipients. Four of five men with whom the donor had sexual contact during the critical time period when infection most likely occurred were located and tested; results were negative for HIV.

CONCLUSIONS: Pooled NAT of blood donations has not eliminated the window period for HIV identification during seroconversion.

Before the implementation of pooled NAT, estimates of the risk for transfusion-transmitted HIV in the United States varied from 1 in 450,000 to 660,000[1,2] for donors with a history of prior blood donation (repeat donors). Since implementation of pooled NAT, this risk has been estimated to be 1 in 2,135,000.[3] In first-time donors, the residual risk of transfusion-transmitted HIV is estimated to be 1 in 1.2 million[4] to 1 in 1.8 million[5] in the United States. NAT has been evaluated through an investigational new drug application with FDA since March 1999[6] and has been used in addition to the standard tests for HIV including HIV-1/2 EIA and p24 antigen during the blood donation screening process. NAT has further reduced the window period during which HIV is not detectable to approximately 10 to 15 days after the date of infection.[7,8] Despite these improvements in the detection of HIV in the blood donor population, the risk for transmission, although very small, is not zero. Therefore, when HIV transmission occurs, it is essential that it be aggressively and systematically investigated by public health agencies to identify preventable errors and elucidate novel or emerging epidemiologic dynamics that may be amenable to intervention. This report describes two cases of transfusion-acquired HIV infection, verification of transmission from the donor to the recipients, and attempts to identify how the 18-year-old female donor acquired her infection.

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CASE REPORT

In July 2002, the CDC and FDA were notified that a repeat blood donor had positive HIV test results during the donor-screening process in May. The donor was an 18-year-old female high school student in good health. Screening revealed no risk factors that would preclude blood donation. This was the fifth time she had given blood in the past 8 months during high school blood drives. Her first donation was on September 12, 2001, in response to the September 11 terrorist attacks.

Look-back studies indicated that the donor had given blood in March 2002 and that two persons had received products from that donation. Recipient A had received FFP for internal bleeding; Recipient B had received RBCs after surgery. The two recipients were notified and tested for HIV; both were HIV infected, according to EIA and Western blot. The March donation, however, had screened negative for HIV.

MATERIALS AND METHODS

We therefore conducted a laboratory and epidemiologic investigation to document that the infections in the transfusion recipients were acquired from blood products that had tested negative for HIV and to determine the route of infection for the female donor of these products.

As part of routine screening, blood samples from the May donation were tested using the General-Probe 16 minipool NAT (General-Probe, San Diego CA), HIV-1 p24 Antigen ELISA Test System (Coulter Corporation, Miami, FL), and Genetic Systems HIV-1/HIV-2 Peptide EIA (Bio-Rad Laboratories, Redmond, WA). All tests produced positive results for HIV. No residual blood samples from the donor's March donation were available for testing. The March donation had been initially tested for HIV using the General-Probe 16 minipool NAT, HIV antigen and HIV 1/2 EIA assays, and all results were negative. FBS contacted the FDA since General-Probe NAT had been licensed in February 2002[6] and reagents for the investigational new drug were still in use during a 6-month transition period. FDA, in turn, contacted CDC.

Verification of transmission from donor to recipients

In July 2002, the donor and recipients signed a CDC Institutional Review Board-approved informed consent and were interviewed by Florida health department staff using a questionnaire for HIV risk factors developed by CDC. Blood samples for DNA analyses were obtained at the time of interview.

CDC and FDA collaborated to perform a comprehensive laboratory DNA analysis of HIV strains from the donor and Recipients A and B. CDC did sequencing and genetic analysis using preparations of PBMNCs obtained from the donor and two recipients by the Florida Department of Health the week of July 22-26, 2002. Plasma samples from the donor and Recipient B and serum from Recipient A were drawn by FBS on July 17, 2002, during the initial look-back investigation and notification and were sent to the FDA.

Nucleic acid was extracted and subsequently amplified and sequenced independently by CDC and FDA with primers and amplification protocols provided by CDC. The p17 region (402 bp) of the *gag* gene and the C2V3C3 region (454 bp) of the envelope gene of HIV were amplified and sequenced from the donor and recipients by CDC and FDA.

The PBMNC specimens sent to the CDC were processed for DNA extraction with a kit (QIAamp DNA Blood mini kits, Qiagen, Valencia, CA). Amplification of extracted DNA was accomplished with Platinum Taq PCR Supermix (Invitrogen, Carlsbad, CA) with nested primers encompassing the p17 gene (402 bp) of the *gag* region[9] and the C2V3C3 region (454 bp) of the envelope gene.[10,11] Thermocycling conditions were as follows: 2 minutes at 94°, followed by 35 cycles of 30 seconds at 94°, 30 seconds at 55°, and 1 minute at 72° and a final extension for 5 minutes at 72°. Secondary PCR amplicons were purified with kits (QIAquick PCR purification kits, Qiagen) and subsequently sequenced with the nested amplification primers using a sequencing kit (BigDye Terminator Cycle Sequencing kit, Applied Biosystems, Foster City, CA) and an automated DNA sequencer (ABI 373).

All FDA specimens were processed for RNA isolation with TRIZOL LS Reagent (Invitrogene, Carlsbad, CA). RT-PCR and first-step amplification of extracted RNA was accomplished with Access RT-PCR system (Promega, Madison, WI). Nested PCR was carried out using Platinum Taq PCR Supermix. Both amplifications were made using pairs of primers encompassing the p17 gene (402 bp) of the *gag* region[9] and the C2V3C3 region (454 bp) of the envelope gene.[10,11] Thermocycling conditions were as follows: 45 minutes at 48°, 2 minutes at 94°, followed by 40 cycles of 30 seconds at 94°, 1 minute at 55°, and 2 minutes at 68° and a final extension for 7 minutes at 68° for the RT-PCR and first step of amplification, and 2 minutes at 94°, followed by 35 cycles of 30 seconds at 94°, 30 seconds at 55°, and 1 minute at 72° and a final extension for 5 minutes at 72° for the second amplification. Secondary PCR amplicons were purified using a kit (High Pure PCR Product Purification Kit, Roche, Indianapolis, IN) and subsequently sequenced with the nested amplification primers, using a sequencing kit (BigDye Terminator Cycle

Sequencing kit) and an automated DNA sequencer (ABI 310).

For the C2V3C3 region, CDC sequenced one PCR amplified product from the donor, one from Recipient A and four from Recipient B. FDA sequenced two PCR-amplified products from the donor, one from Recipient A and two from Recipient B. For the p17 gene of *gag* CDC sequenced one PCR-amplified product from the donor, one from Recipient A, and two from Recipient B. FDA sequenced two PCR-amplified products from the donor and two from each of the recipients. The 11 (combined CDC and FDA) C2V3C3 sequences were aligned and nucleotide differences genetically analyzed, as were the 10 p17 sequences.

These gene region sequences were included in separate alignments including contemporary HIV sequences originating from several US cities (p17) or other US strains available from the HIV sequence database (C2V3C3). Phylogenetic tree construction utilizing the neighbor-joining and bootstrapping methods contained within PAUP[12] was then used to assess relatedness of the potential donor and recipient sequences in combination with other US strains.

Attempts to identify how the donor acquired her infection

The donor was interviewed on four different occasions in an effort to name and locate all sex partners she had had during the past year as well as to ascertain any other possible risk factors for HIV.

RESULTS

Verification of transmission from donor to recipients

Interviews with the two recipients revealed no other apparent risk factors for HIV. Partners of each of the two infected recipients were tested and were negative for HIV. Laboratory analysis revealed that 8 of the 11 C2V3C3 sequences from donor and recipients generated from CDC and FDA laboratories were an exact match, and that two of the donor sequences had five nucleotide differences (1.1%) from the matching eight sequences. One sequence from Recipient B had 12 differences (2.6%) from the eight that were identical.

For the p17 sequences from both CDC and FDA laboratories, 7 of the 10 were an exact match for donor and recipients. Two of the donor sequences and one of the recipient sequences had one base substitution compared with the seven identical sequences (0.2%). Phylogenetic analyses demonstrated that the donor and recipient sequences formed a monophyletic clade (100% bootstrap support) distinct from contemporary US sequences in both the C2V3C3 (Fig. 1) and p17 regions (Fig. 2). The small numbers of nucleotide differences seen between the donor and the recipient HIV sequences in the C2V3C3 region of the *env*, which is the most variable gene, as well as their close association observed in phylogenetic tree analysis, together with the epidemiologic investigation, support the hypothesis of transmission from the donor to both recipients.

Attempts to identify how the donor acquired her infection

The donor denied any history of injection drug use and did not recall any symptoms that would indicate an acute retroviral illness. She reported having had vaginal intercourse with a total of five men. No anal intercourse was reported. Four of the five partners were located and tested for HIV; test results were negative. Despite extensive

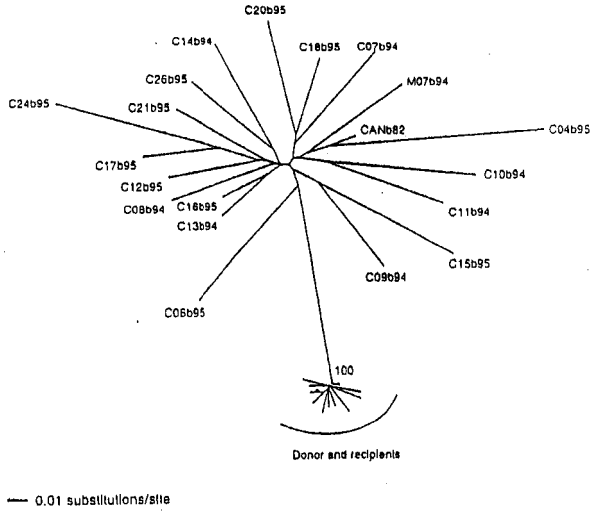

Fig. 1. Phylogenetic relationship of the 11 HIV sequences (C2V3C3 region of the *env* gene) derived from the donor and two recipients, and 20 HIV database subtype B strains. Implementation of the neighbor-joining method with bootstrapping (2000 replicates) was done in PAUP.[12] The number at the node indicates the percentage of bootstrap values.

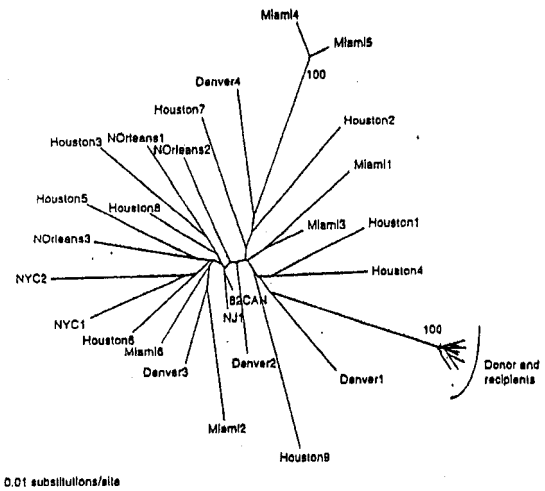

Fig. 2. Phylogenetic relationship of the 10 HIV sequences (p17 gene of the *gag* region) derived from the donor and two recipients, and 25 HIV subtype B sequences originating from several US cities (and one subtype B sequence [82CAN] from Canada used as an outgroup). Implementation of the neighbor-joining method with bootstrapping (2000 replicates) was done in PAUP.[12] The numbers at the nodes indicate the percentage of bootstrap values.

efforts, the one remaining identified partner could not be found. Most significantly, the untested sex partner had contact with the donor within the known 10-day marker negative period, when undetected infection in the donor must have been present. The donor and infected recipients were counseled and referred for medical care.

DISCUSSION

The public has little tolerance for any risk for HIV transmission from the blood supply.[13] In the United States, multiple layers of safety reduce the risk for transmission of HIV and other bloodborne pathogens; these layers include voluntary blood donation rather than donation for monetary gain, education and screening of blood donors, and laboratory testing of donated blood.[14] However, despite these extensive and costly measures, transmission during the window period does occur, although very rarely.

This is the second report of transmission of HIV from a blood donor during the window period since the implementation of minipooled NAT in the United States.[15] Unfortunately, there was no blood remaining from the March donation to test using a single-unit assay. The failure of pooled assays to detect HIV RNA has been cited as possible justification for implementing single-unit testing.[16] Single-unit NAT, although being evaluated in some areas, is not generally available due to its limited automation but would likely result in only a modest shortening of the window period for detection of HIV.[8] For example, a European study of 3.6 million blood donations found that no donation positive for HIV was missed by minipool NAT. This finding suggests that single-unit testing would add little, if any, significant benefit to transfusion safety, while incurring significant increase in cost.[17]

In the case described here, the young woman did not know that she was infected and did not know the HIV status of any of her partners. All located partners were less than age 25 years. She did not consider herself to be at increased risk for HIV from heterosexual transmission and did not defer herself as a donor. It has been suggested that if donor deferral criteria were instituted to exclude persons with multiple heterosexual partners, the effect on transfusion safety would be relatively minor.[18] Such criteria would most likely result in the exclusion of a large number of potential donors who were not infected with HIV. Further, our investigation suggests that adolescent donors may not know they are at increased risk from heterosexual transmission and would not self-defer from donation. Finally, the systematic and expanded questioning on four different occasions that prompted this donor to recall all of her partners would not be possible in a blood donation setting.

It is estimated that approximately 54 percent of women reported with AIDS in 1998 acquired their HIV through sex with a man for whom risk factors for HIV were not known, including those in the no-identified risk category who are also estimated to have been infected through heterosexual sex.[19] The inability to identify the donor's source of exposure represents a continued challenge in investigation of cases of heterosexual transmission for which HIV status of casual partners is very often not known, and young people do not perceive themselves to fall into a group at high risk for infection.

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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 2004 年 4 月 13 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	First report of human immunodeficiency virus transmission via an RNA-screened blood donation Delwart, E.L. et al., Vox Sanguinis, 2004;86:171-177	公表国 米国	
販売名（企業名）						

研究報告の概要

1999 年，アメリカ国内の血液バンクは，HIV ウイルスおよび C 型肝炎ウイルス（HCV）の感染防止を目的として，血液製剤用の献血ミニプールの核酸増幅検査（MP-NAT）を開始した。しかしながら，セロコンバージョン前の血液サンプル中に存在するウイルス RNA が微量（150 コピー/mL 以下）の場合，MP-NAT では適切に検知できない可能性があり，当論文でも，MP-NAT 検査陰性の血液を輸血した結果，HIV 感染した症例を紹介している。一方，個別に核酸増幅検査(ID-NAT)した場合，ウイルス性 RNA を確実に検知することが可能であることから，血液製剤の安全性を更に高めるためにも，MP-NAT の精度向上および ID-NAT の制度化（ランニングコストの抑制・処理能力の向上）が提案・検討されている。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
弊社の血漿分画製剤では，HCV および HIV のウィンドウピリオドを考慮して，供血者から採取した血漿をすぐにプール血漿とはせずに採取日から 60 日間以上保存し，ドナーが再来したときに再検査を実施している。また，血漿プールでは HCV および HIV に対する NAT 検査を実施し，製造工程においてもこれらウイルスの不活化処理が行われており，感染を防止するための十分な安全対策が講じられていると考える。	現時点で，具体的な安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

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Vox Sanguinis (2004) 86, 171–177

ORIGINAL PAPER

First report of human immunodeficiency virus transmission via an RNA-screened blood donation

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Vox Sanguinis

Background and Objectives Blood banks in the USA have recently introduced minipool nucleic acid amplification testing (MP-NAT) of blood products to reduce the transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) by transfusions. However, MP-NAT is limited in its ability to detect preseroconversion samples with very low viral RNA loads.

Materials and Methods To determine whether a red blood cell unit, from an MP-NAT-negative donation, transmitted HIV when transfused to a patient, we compared the viral sequences from the blood donor and recipient. The implicated donation was also tested by commercially available NAT assays at a range of dilution factors to determine whether the infectious unit could have been detected using individual-donation NAT (ID-NAT).

Results Phylogenetic linkage of HIV sequences in the blood donor and recipient confirmed the transmission of HIV by blood transfusion, the first such case identified since introduction of MP-NAT screening in 1999. Viral RNA was reliably detected by ID-NAT, but only inconsistently detected by MP-NAT.

Conclusions Even following the introduction of MP-NAT, a preseroconversion donation with a viral load of ≤ 150 copies of RNA/ml went undetected and resulted in an HIV transmission. Implementation of ID-NAT will further reduce such rare transmissions, but at a considerable cost per infectious unit interdicted.

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Key words: blood donation, HIV, nucleic acid testing, transfusion.

Introduction

In 1999, United States (US) blood banks implemented nucleic acid amplification testing (NAT) of blood donations to reduce human immunodeficiency virus (HIV) and hepatitis C virus (HCV) transmissions by infectious, preseroconversion window-period units [1,2]. Owing to the limited throughput capacity of semiautomated testing equipment, NAT was initially implemented and is currently performed primarily using minipools comprising 16–24 units of plasma (MP-NAT). Although NAT screening has not yet been formally mandated, over 37 million blood donations were screened in the US from mid-1999 to April 2002 (the most recent period for which national NAT-yield data has been compiled) [3]. This has resulted in the detection of 12 HIV (1 : 3·1 million) and > 170 HCV (1 : 270 000), RNA-positive, antibody-negative donations [2].

Prior to the introduction of routine MP-NAT, a publication reported HIV transmission via a window-phase donation,

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from a donor in Singapore [4]. The recovered plasma from this donation was subsequently shown to contain 5–39 HIV RNA molecules/ml, and dilutional studies were performed which demonstrated that this unit would have been missed by MP-NAT, but probably would have been detected by individual-donor NAT (ID-NAT) assays that are now employed for screening in the US [4]. We report the first observed case of HIV transmission by a blood product that was tested prospectively by MP-NAT prior to transfusion.

Materials and methods

Donor and recipient follow-up and consent procedures

Following documentation of HIV seroconversion of a repeat whole-blood donor, the donor was notified of his HIV infection status, interviewed and the medical history and testing records from prior donations reviewed. Hospitals issued with blood components from prior donations were notified according to HIV 'look-back' procedures specified by the Food and Drug Administration (FDA). The hospital transfusion service medical directors and transfusing physicians then traced and, if alive, notified and offered HIV testing to exposed recipients. The NAT screening programme at the South Texas Blood and Tissue Center was reviewed and approved by a Committee for Human Research and the FDA (IND #BB-IND 8361). The implicated donor and infected recipient each signed informed consent documents to permit testing.

Donor screening procedures

The donor underwent all routine medical history and laboratory screening procedures, as required by the FDA, including enzyme immunoassay (EIA) screening for HIV antibody and p24 antigen (Abbott Laboratories, Abbott Park, IL). Investigational MP-NAT (developed and performed at the South Texas Blood and Tissue Center, San Antonio, TX) was performed on acid-citrate-dextrose (ACD)-anticoagulated whole-blood samples, taken from the blood bag after collection and mixing, which was then centrifuged to yield ACD plasma (≈ 20% dilution by liquid anticoagulant). The frozen recovered plasma component from the implicated donation, which was retrieved for viral load assessment and to evaluate the impact of pooling on HIV RNA detection, had this same dilution factor. This plasma component had been prepared from ACD whole blood by centrifugation and was frozen at a temperature of equal to or less than −30 °C within 15 h of phlebotomy, according to the standard operating procedures of the blood centre. The plasma was subsequently thawed and rapidly refrozen (at −70 °C) twice prior to viral load and dilutional NAT testing.

NAT assay procedures

The non-commercial HIV NAT assay performed on the implicated donation involved the processing of 1·8 ml of plasma from 24-member minipools (75 µl per donation) that had been prepared using an automatic pipetting system. Samples were treated with proteinase K and sodium dodecyl sulphate (SDS) to lyse the viral particles, followed by guanidine isothiocyanate and Triton-X-100 extraction of nucleic acids. Nucleic acids were isolated using silica beads in the presence of a chaotropic salt. HIV RNA amplification used primers to the highly conserved gag gene and a one-step reverse transcription-polymerase chain reaction (RT-PCR). Amplicons were resolved using agarose-gel electrophoresis with ethidium bromide staining. Bovine viral diarrhoea virus, used as an assay internal control, was added directly to each pool sample and extracted and assayed in parallel using the RT-PCR assay. The analytical sensitivity of this assay was established as part of pre-IND studies by serial dilution of the FDA's HIV RNA Standard Panel member no. 3 (which contains 25 000 copies of HIV RNA/ml) to a concentration of 100 HIV RNA copies/ml. Nine replicates of this dilution were evaluated by the HIV assay's procedure in three different test runs. All nine replicates tested positive for HIV RNA, supporting a 95% sensitivity of at least 100 copies/ml, as required by the Center for Biologic Evaluation and Research (CBER) for HIV NAT (FDA IND # BB-IND 8361). Commercial NAT assays were performed by the assay manufacturers on coded panels that included controls, the implicated plasma donation, and replicate aliquots of 1 : 8, 1 : 16 and 1 : 24 dilutions (for the two whole-blood NAT assays) or a 1 : 512 dilution (for the source plasma NAT assay) of implicated plasma from the recovered plasma component prepared using HIV-negative donor plasma. The assays were performed by the National Genetics Institute, Los Angeles, CA (NGI Ultraqual™ and Superquant™ Assays); GenProbe Incorporated, San Diego, CA (Chiron Procleix™); and Roche Molecular Systems, Pleasanton, CA (HIV Ampliscreen™). Detailed methodology and validation data for these assays were as published previously [5–7].

HIV sequencing and phylogenetic analyses

The blood donation collected 3 months after the implicated donation had a plasma HIV viral load of 19 800 RNA copies/ml (COBAS Amplicor HIV-1 Version 1·5; Roche Molecular Systems, Pleasant, CA, USA). The follow-up donation was used, in a nested RT-PCR, to amplify HIV-1 gag p17 [8] and env V3 [9] regions. A recipient blood sample collected 47 weeks following the transfusion, and after the patient initiated antiretroviral therapy, had a viral load of < 50 copies/ml; we therefore extracted genomic DNA from purified peripheral blood mononuclear cells (PBMCs) obtained at that time point

and amplified the same two regions of HIV by nested PCR. Nucleic acids were extracted and PCR tubes were set up on different days for the two different subjects using a pre-amplification room free of amplified HIV products. Two to four independent nested PCR products (amplicons) were generated from both subjects at both loci and the amplicons were directly sequenced using an automated DNA sequencer (GenBank accession numbers: AY062239–AY062249). The maximum-likelihood (ML) phylogenetic analysis was performed on Nirvana, a 2048-processor cluster of SGI Origin 2000 systems (Silicon Graphics, Mountain View, CA, USA), and on a dual-processor Pentium III computer provided by the Advanced Computing Laboratory, Los Alamos National Laboratory (Los Alamos, NM). The ML analysis was performed using versions of fastDNAml [10,11] and DNArates (G. J. Olsen, S. Pracht, and R. Overbeek, unpublished data [http://geta.life.uiuc.edu/~gary/programs/DNArates.html]), modified as described previously [12]. Maximum parsimony trees were constructed using PAUP 3·2 [13]. Neighbour-joining phylogenetic trees were constructed with the PHYLIP programs DNADIST (using the maximum-likelihood f84 model of base substitutions, transition : transversion ratio 1·7 for gag and 1·5 for env) and NEIGHBOUR (using subtypes A and D as outgroups to the subtype B clade) (http://evolution.genetics.washington.edu/phylip.html) [10]. One-hundred bootstrap replicates were performed with the PHYLIP seqboot and consense programs. Donor and recipient sequences were compared against the entire HIV-1 sequence database using the BLAST program at the HIV Database (http://hiv-web.lanl.gov/content/hiv-db/BASIC_BLAST/basic_blast.html) to identify the sequences with the greatest similarity to donor and recipient sequences.

Results

Seroconversion of a recent blood donor

A whole-blood donation (in late August 2000) from a 32-year-old male who did not admit to any disqualifying risk behaviour, was tested for antibodies to HIV, p24 antigen, and HIV and HCV RNA using a locally developed, non-commercial NAT assay on a pool of 24 donations. The donation sample was negative in all assays and therefore released for transfusion. The same donor gave whole blood again in December 2000. This donation tested positive for HIV RNA by MP-NAT (resolved to the individual donation) and antibody assays (EIA repeat reactive; Western Blot positive with p24 and gp120/160 bands only, consistent with recent seroconversion), but was negative for p24 antigen and all other viral markers. Following notification and counselling, the donor again denied all deferrable HIV risk behaviours and had no history of an acute infection.

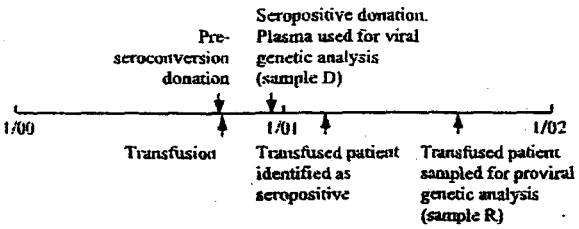

Fig. 1 Timeline of samples analysed and mentioned in the text.

Identification of a newly infected blood-product recipient

Lookback investigation of this donor's previous donations identified a patient who had been transfused in early September with red blood cells (RBCs) prepared from the late August donation (a platelet component was prepared but not transfused, and a recovered plasma component was prepared and shipped to Europe for pooling and fractionation). The patient also received eight other RBC units and six units of fresh-frozen plasma during this hospitalization; none of the donors of these units subsequently tested positive for HIV antibody, antigen or RNA. In early February 2001, the transfused patient, who denied all other HIV risk factors, was tested and found to be HIV seropositive. His viral load was 29 716 copies/ml, and an antiviral regimen, consisting of efavirenz, d4T and 3TC, was started. As no frozen sample had been retained from the recipient's initial diagnostic sample, he was resampled 47 weeks post-transfusion, at which time he remained asymptomatic and had a plasma HIV viral load of < 50 copies/ml. Figure 1 shows the timeline of donations, transfusion and sample acquisition.

Genetic linkage of HIV in the blood donor and recipient

In order to test whether the RBC recipient was infected with HIV in the transfused blood product, the gag p17 and env V3 regions from the blood donor and recipient viruses were sequenced. All seven independently generated, directly sequenced, PCR amplicons from the 536-bp p17 region (four from the donor and three from the transfused patient) were identical. The most closely related p17 sequence in the Los Alamos HIV database differed by 26 mutations, or > 4·8%. Two independent PCR amplicons were also generated from each subject from the more rapidly evolving env V3 region. The 513-bp region amplicons were identical within individuals and differed between donor and recipient by 13 substitutions (2·5%). The most closely related sequences in the Los Alamos HIV database differed by 43 and 40 substitutions from the blood donor and recipient, respectively (> 7·7%). The low level of sequence difference between the env

sequences is consistent with divergent viral evolution having occurred between the transfusion and collection of the samples used for genetic analysis (fig 1) [8]. Except for one mixed base in one p17 amplicon, all sequencing electrophoregrams from both loci showed homogenous viral populations without minor variant nucleotide base peaks. Phylogenetic analyses using three methods (maximum likelihood, neighbour-joining, and maximum parsimony) [10,12,13] all showed that the donor and recipient sequences formed a monophyletic clade to the exclusion of all other subtype B sequences. One-hundred bootstrap replicates of the data set, analysed by both the maximum likelihood (Fig. 2) and neighbour-joining methods (data not shown), showed 100% bootstrap support for the donor–recipient clade in the gag and env trees. The phylogenetic analyses are therefore consistent with HIV transmission as a result of the RBC transfusion from the implicated donor.

Inconsistent detection of the infectious unit using the current 16–24 sample MP-NAT

In order to test the ability of commercial NAT assays to detect such an infectious donation, frozen plasma from the implicated donation was retested neat (without further dilution) and using dilutions consistent with minipool sizes currently used in MP-NAT screening. Three different assays were evaluated. The only currently licensed NAT assay for commercial source plasma donations (National Genetic Institute, Los Angeles, CA) was HIV RNA negative in the standard 512-member pool size used for pooled source plasma. A quantitative assay performed using undiluted plasma at the National Genetic Institute measured a viral load of 150 RNA copies/ml (180 copies/ml after adjustment for 20% dilution in ACD plasma). Using the two commercially available assays for whole-blood donations [both under IND at the time of testing], a significant fraction of replicate testing at 1 : 16 and 1 : 24 dilutions (representing the current blood-bank pool sizes) failed to yield detectable HIV RNA. The same assays were positive using undiluted plasma (Table 1).

Table 1 Results of testing of plasma from the implicated transmitting donation by two commercially available whole-blood nucleic acid amplification testing (NAT) systems (Chiron/GenProbe Procleix and Roche HIV Ampliscreen)

Dilution of recovered plasma	NAT positive/replicates		
	Assay 1	Assay 2	Total
Undiluted	1/1	3/3	4/4 (100%)
1/8	3/3	8/9	11/12 (92%)
1/16	3/3	5/9	8/12 (67%)
1/24	1/3	2/9	3/12 (25%)
Negative control	0/3	0/9	0/12 (0%)

A 13-member coded panel was evaluated, which included undiluted plasma and triplicate aliquots representing dilutions consistent with currently used minipool NAT screening. Assay 1 used the entire 0·5 ml of each panel member to generate a single result per aliquot, whereas for assay 2 the plasma extract was divided into three parts and triplicate human immunodeficiency virus (HIV) RNA assays were performed.

Discussion

These results demonstrate that very low-level HIV viraemia during the preseroconversion window period is infectious by blood transfusion, and confirm that, as predicted, a very small risk of transfusion-associated HIV transmission remains, even after screening with MP-NAT. It is important, however, to place this case report in context. During the 3-year period following implementation of MP-NAT screening in the USA, more than 35 million units of donated blood have been screened for HIV and HCV RNA by MP-NAT, and more than 50 million MP-NAT-negative blood components have been transfused into > 20 million patients. The present case of HIV transmission by an MP-NAT-negative unit, collected mid-2000, was the first MP-NAT breakthrough infection to be documented in the USA, with two additional HIV breakthrough cases (one of which infected two recipients and the other one recipient) and one HCV breakthrough case subsequently observed in 2001 and 2002 [14] (Minutes of the FDA Blood Products Advisory Committee, September 12, 2002) [15]. One case of HCV transmission, missed by MP-NAT, was also recently reported in Germany [16], and one case of HIV transmission has been reported in France [17]. Although these cases are disturbing, the rarity with which they have been observed confirms that the residual risk of viral infections from transfusions is extremely small, and represents remarkable progress relative to the risks for HIV and HCV transmission that exceeded 1 in 1000 prior to the introduction of antibody screening in the 1980s [18,19] and risks in the range of 1 in 100 000 in the mid-1990s prior to MP-NAT [20].

The rapid introduction of MP-NAT over the past several years in the USA and many other developed countries thus represents a significant advance in applying molecular technologies to improve blood safety. At least 98% of US blood collections are now tested using 16–24 member minipool NAT for HIV and HCV RNA. Most other developed countries have also implemented MP-NAT screening of whole-blood donations using pool sizes ranging from four to 96 donations per pool [21]. Application of sample pooling strategies, as opposed to individual-donation screening, was required to expeditiously introduce NAT owing to the limited capacity of specially designed testing facilities and semiautomated instrumentation, and limited availability of reagents

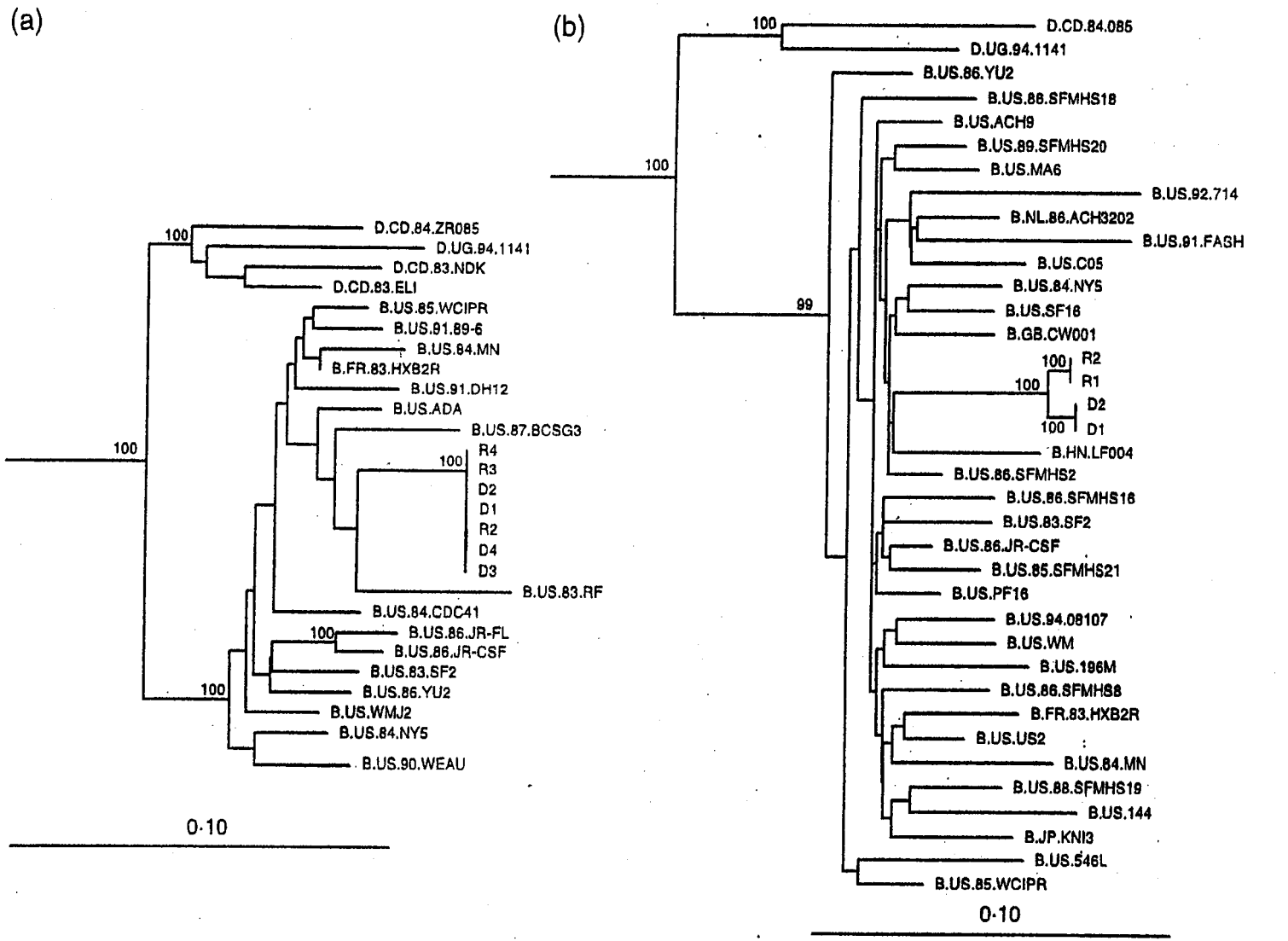

Fig. 2 Maximum likelihood tree with nodes supported by greater than 70% of bootstrap replicates noted. (a) Gag p17. (b) Env V3. Sequences are from the blood donor (D) and recipient (R) and the most closely related subtype B controls from the human immunodeficiency virus (HIV) database. Subtype D HIV-1 variants are used as outlier sequences. Horizontal distances are based on phylogenetic distance. The scale bar 0·1 represents the maximum-likelihood estimate of 0·1 base substitutions per site.

and personnel trained to perform high-volume and rapid turnaround-time NAT screening [1]. The only blood programs in the USA that are currently performing routine ID-NAT are the Department of Defense (DOD) blood banks, which implemented ID-NAT in 1999, and the National Institutes of Health (NIH) Blood Bank and Oklahoma Blood Institute (OBI), which initially used MP-NAT but converted to ID-NAT in mid-2002. These programs collect and test relatively small numbers of blood units (< 20 000 units per year per site at the DOD and NIH programs and ≈ 150 000 donations per year at the OBI), compared to the majority of NAT screening laboratories which process 200 000 to > 1·5 million donations per year.

It is anticipated that improvements in existing sample extraction and amplification and detection systems, and future development and the FDA licensure of fully automated, high-throughput NAT-screening instruments, will enable the universal evolution from MP-NAT to ID-NAT screening over the next few years, as well as implementation of NAT screening for hepatitis B virus, West Nile virus and other viral agents [1]. Anticipating this transition, the FDA recently approved one NAT screening system (Chiron/GenProbe Procleix Assay) for either minipool or individual-donation screening for HIV and HCV RNA. However, the FDA has not recommended that ID-NAT be implemented in preference to MP-NAT at the present time, and the FDA Blood Products Advisory Committee, which reviewed the MP-NAT breakthrough cases at its September 2002 meeting, endorsed MP-NAT as the current standard of practice pending development and licensure of automated ID-NAT platforms [1].

It is possible that additional cases of HIV and HCV transmission have occurred since MP-NAT screening was introduced but have not been detected, as not all infected donors return to allow identification of seroconversion which triggers recipient lookback, and transfused patients are not routinely monitored for transfusion-transmitted infections. Based on incidence window-period models, it is estimated that as many as one per 2–3 million units (four to six donations per year in the US) are given by very recently infected donors with low-level viraemia that would test ID-NAT positive, but MP-NAT negative, for HIV or HCV [1]. These projections, and the cases of confirmed breakthrough transmissions, support the potential value of moving from MP-NAT to ID-NAT. However, there are multiple factors that must be weighed into that decision, including the logistics of increasing test throughput 16–24-fold, and the incremental cost and cost-effectiveness of ID-NAT vs. MP-NAT.

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				2004.7.30	該当なし	

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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)		Vox Sang. 2004; 87(1): 44-45.	ドイツ

研究報告の概要

ドイツにおいて供血血液に対するHIV-1 NATで、HIV-1グループOを検出できなかった事例が報告された。ケニア人女性との性交渉による感染症リスクがある37歳男性ドナーの供血血液は、6プールで実施したHIV-1 NATで陰性を示した。HIV-1/2抗体検査の結果が陽性であり、塩基配列解析からHIV-1のグループOであることが判明した。欧州ではHIV-1グループM サブタイプBが圧倒的に多いが、世界各地への旅行や移住が盛んになるにつれ、サブタイプB以外のHIV-1感染症が増加しつつある。グループMの検出を目的に設定されたNATによって、確実にHIV-1グループOを検出できるというわけではない。そのため、グループOに対する感度を増強した第三世代アッセイの抗体検査が必要となる。本事例から、流行地域以外においてもHIV-1変異株のサーベイランスを継続することの必要性が改めて強調される結果となった。

使用上の注意記載状況・その他参考事項等

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
ドイツにおけるHIV-1 NATでは検出できなかったHIV-1グループOの供血血液に関する報告である。本邦では、HIV-1グループOは発生していない。	これまで、本製剤によるHIV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。また最終製品についてHIV-NAT陰性であることを確認していることから、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。

(9)

Vox Sanguinis (2004) 87, 44–45

LETTERS

Vox Sanguinis

First human immunodeficiency virus-1 group O infection in a European blood donor

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From 1 May 2004, nucleic acid amplification testing (NAT) for human immunodeficiency virus-1 (HIV-1) will be mandatory in Germany for all blood donations [1]. We report the failure of standard HIV-1 NAT to detect HIV-1 group O infection in a German blood donor.

At his first donation (28 August 2003) a 37-year-old Caucasian man failed to report specific risks for transfusion-transmitted diseases. On retrospective questioning, however, he admitted to unprotected sexual contact with a Kenyan woman 9 years earlier, which would have led to permanent exclusion from blood donation.

The initial negative HIV-1 NAT was performed in a six-sample pool using an in-house-method approved by the Paul Ehrlich Institute (the regulatory authority in Germany). Results of additional tests performed owing to a positive result in the HIV1/2 antibody test are summarized in Table 1. Sequence analysis revealed HIV-1 group O infection (Fig. 1).

In Europe, HIV-1 group M subtype B is predominant, while the prevalence of non-subtype B HIV-1 infections is increasing as a result of global travel and migration [2]. The residual risk of transfusion-transmitted HIV-1 infection in Germany is low (< 1 : 1 000 000) [3] and will be further reduced by obligatory

Table 1 Results of sequential testing for human immunodeficiency virus (HIV) infection

Markers of HIV infection	Result	Assay	Manufacturer
Anti HIV-1/2	Positive	AxSYM HIV 1/2 gO[a]	Abbott Diagnostika
	Positive	Murex HIV 1-2-O	Abbott Diagnostika
Anti HIV-1/2-IgG/p24	Positive	Vidas HIV DUO	bioMerieux
HIV-1 antigen (p24)	Negative	Elecsys HIV Ag	Roche Diagnostics
HIV-1-IgG Immunoblot	Positive[b]	New LAV I	Bio-Rad Laboratories
HIV-2-IgG Immunoblot	Negative[c]	New LAV II	Bio-Rad Laboratories
HIV-1 NAT	Negative	Taqman RT-PCR[a]	In house
	Negative	Cobas AmpliScreen HIV-1 Test, v1-5	Roche Diagnostics
	Negative	Versant HIV-1 RNA 3-0 Assay (bDNA)	Bayer Health Care
	Positive	p31 (Integrase) RT-PCR	In house

[a] Assay used for donation screening.

[b] Strong staining of p24/25, gp160; faint staining of p34, p40, p55.

[c] Staining of p26.

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donor     :aattacaaaaacaaatttttaaaattcaaaaatttcaggtctattacagagacagcagag  694
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
98CMA010:aattacaaaaacaaatttttaaaattcaaaaatttcaggtctattacagagacagcagag
donor     :atcctatttggaaaggaccggcacagctcctgtggaaaggtgagggagcagtagtcatac  754
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
98CMA010:atcctatttggaaaggaccggcacagctcctgtggaaaggtgagggagcagtagtcatac
donor     :aagataaaggagacattaaggtagtaccaagaaggaaggcaaaaataattagacattatg  814
           ||||||||||*|||||||||||||||||||||||||||||*|||||||||||||||||||
98CMA010:aagataaaggtgacattaaggtagtaccaagaaggaaggcagaaataattagacattatg
donor     :gaaaacagatggcag 829
           ||||||||||||||||
98CMA010:gaaaacagatggcag
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Fig. 1 Sequence analysis. Donor human immunodeficiency virus (HIV-1) sequence (bases 635–829 of the integrase gene) aligned to the known HIV-1 group O isolate, 98CMA010, from Cameroon (GenBank accession no. AF422215; numbering according to HXB2 integrase). Alignment was performed using HIV BLAST search; HIV subtyping was performed using the BLAST HIV-1 subtyping tool.

implementation of HIV-1 NAT. Owing to the genetic diversity of HIV-1, NAT methods designed to detect group M are not always reliable in the diagnosis of group O infections. Therefore, antibody testing using 'third-generation' assays, whose sensitivity has been enhanced to group O, remains indispensable. The question of whether or not to include group O in HIV-1 RNA testing should not be answered on the basis of only one case. However, this case emphasizes the need for continuous surveillance of HIV-1 variants outside endemic areas.

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