

## Passive Transfer of HIV-1 Antibodies and Drug Resistant Virus during a Health Care Worker Accident: Implications for HCW Post-Exposure Management

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**Abstract: Problem statement:** We studied in detail a case in which a nurse caring for an HIV-infected child suffered a deep-laceration accident with contaminated blood. **Approach:** The patient had been treated with zidovudine (ZDV) and the nurse became infected despite prophylactic use of ZDV initiated 2 h after the accident. A reactive anti-HIV-1/2 EIA and an indeterminate western blot (gp120/160 reactivity) were obtained from the nurse on the day of the accident, suggesting pre-exposure infection. However, a negative western blot and positive DNA PCR were documented 10 days after the accident and seroconversion occurred an additional two weeks later. **Results:** Phylogenetic analyses of HIV-1 tat and C2-C4-gp120 env regions confirmed that the nurse infected by two different HIV-1 strains present in the child. Strains present in both subjects revealed multi-nucleoside resistant HIV-1. Dilutional serological studies using 10 HIV-infected patients' sera demonstrated that passive seroreactivity could occur with infusion of less than 1 uL of blood when highly sensitive assays are employed. **Conclusion:** This is the first well-documented case of passive HIV antibody detection after a percutaneous exposure. Reactive baseline serology should not be assumed to represent prior infection nor exclude prophylaxis. Transmission of drug-resistant HIV-1 corroborates the medical history and supports use of drug history and resistance testing to guide antiretroviral prophylaxis.

**Key words:** HIV-1, passive transfer of antibodies, health care work accident, bottleneck, transmission of resistant HIV-1

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### INTRODUCTION

The average risk of Health Care Worker (HCW) infection by the Human Immunodeficiency Virus (HIV) after a percutaneous exposure to contaminated blood is approximately 0.3%<sup>[1-3]</sup>. Factors linked to increased risk of infection after percutaneous exposure include whether the lesion is deep or caused by an instrument with visible blood and whether the source patient is at an advanced disease stage or has a high viral load<sup>[4,5]</sup>.

Case-control studies have demonstrated the efficacy of antiretroviral prophylactic therapy in

reducing the rate of HCW transmission<sup>[6-9]</sup>. Recently, reports of expanding transmission of HIV that is resistant to antiretroviral therapies<sup>[10-12]</sup> has further complicated management of HCW cases. Indeed, a case has been recently described of transmission of a drug-resistant HIV-1 strain after an occupational exposure despite post exposure prophylaxis with a combination antiretroviral regimen<sup>[6-9]</sup>. This has led to review and revision of post-exposure prophylaxis recommendations for exposed HCW to include multi-drug regimens and consideration of drug exposure and resistance profiles of source patients<sup>[4]</sup>.

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In this study we report a case of occupational exposure that occurred in the United States in 1993. As will be demonstrated, despite following all the recommendations suggested at the time<sup>[13]</sup>, blood exposure from a source patient infected this HCW with an antiretroviral drug-resistant HIV-1 strain. One of the factors that makes this case particularly important is that transient seroreactivity was documented by enzyme-linked immunoassay (third-generation HIV-1 and 2 EIA) and Western blot (indeterminate classification with gp120 +/- and gp160 1+bands) performed on the blood sample collected from the health professional 2 h after the exposure event. Thus, this case draws attention to the possibility of detecting passively transmitted antibodies if assays of extreme sensitivity are used. Such a finding could result in misdiagnosis of prior infection in the HCW and consequently, an inappropriate decision to discontinue post-exposure prophylactic therapy.

**Case description:** A nurse who was caring for an HIV-infected child was covering a blood collection tube when it broke. The glass went through her glove and the skin of the palm of the hand, causing a cut one millimeter deep and three centimeters long that was grossly contaminated with the patient's blood. The nurse promptly washed the lesion using iodated solution, water and soap. The contaminated blood had been collected from a three-year-old patient with documented HIV infection and clinical AIDS. The patient had been treated with ZDV 120 mg PO every 6 h during the 21 months preceding the accident. The viral load of the patient was not available, but the CD4 lymphocyte count measured three weeks earlier was 75 cells mm<sup>-3</sup>.

The written report of the accident indicates that during her first physical exam, the nurse did not show clinical abnormalities. She started prophylactic use of ZDV 2 h after the accident. A blood sample collected from the nurse on the same day of and subsequent to the accident was reactive for antibodies against HIV-1/2 tested using the Abbott® 3A77 EIA, a third-generation recombinant DNA antigen-sandwich EIA test. An indeterminate result (gp120 +/-, gp160 +) was obtained on the Western blot assay. Follow-up tests were requested and a Western blot of a sample collected 10 days after the accident was negative, but a DNA PCR from the same sample was positive. On the day these results were discussed with the nurse (13 days after the accident) she was reporting fatigue, malaise, loss of appetite, nausea and vomiting, symptoms which were

attributed to side effects of ZDV. It was decided to discontinue the use of ZDV at that time. Two weeks later the tests were compatible with complete seroconversion to HIV, with EIA reactivity and a positive Western blot.

The nurse was a white female in her thirties who had never had a blood transfusion. She denied use of injection drugs and reported that she had been celibate in the 6 years preceding the accident. Table 1 shows the progression of the diagnostic exams made on the nurse's samples.

Further study detailed below was done to investigate the genetic linkage between the HIV-1 population present in the nurse and the putative source patient and to study the level of inoculum required for producing passive anti-HIV reactivity.

## MATERIALS AND METHODS

Informed consent was obtained from the nurse and from the child's parents prior to access and study of the stored samples.

**Samples evaluated:** Peripheral Blood Mononuclear Cells (PBMC) collected from the nurse 20 days after the accident and serum from the child taken 10 days after the accident, were available for analysis. The samples were HLA typed through allele-specific PCR using radioactive markers<sup>[14]</sup> to verify that they belonged to different individuals. Aliquots of these samples were independently and simultaneously analyzed in three different institutes: Blood Centers of the Pacific (BCP), in San Francisco, California (specimens from the nurse and child), the University of Washington (UW) in Seattle (for specimens from the Nurse) and Children's Hospital and Medical Center (CHMC) in Seattle (for specimens from the child).

**Extraction of nucleic acids and PCR amplification of HIV-1 sequences:** For experiments done at BCP, DNA from mononuclear cells and plasma RNA were extracted using the QIAamp Blood and QIAamp Viral RNA kits (QIAGEN Inc, Santa Clarita, CA), respectively. The extracted RNA was reverse transcribed to generate a complementary DNA that could be amplified by PCR<sup>[15]</sup>. Experiments performed at the UW and CHMC used in-house rapid lysis<sup>[16]</sup> to obtain cellular DNA.

At each institute, fragments encompassing the V3 to V5 region of the env gene of HIV-1 were amplified using nested PCR resulting in an approximately 650

base pair fragment (HXB2 coordinates 7001-7647; GenBank access number K03455). The env primer sequences and PCR conditions used are published elsewhere<sup>[17]</sup>.

PCR products were amplified under conditions compatible with the end-point dilution technique described by<sup>[18,19]</sup> followed by DNA automated sequencing. At the BCP laboratories, Heteroduplex Mobility Analysis (HMA) was done using env (C2-V5) PCR products as described by<sup>[20]</sup> In a first experiment, env PCR products from the source patient were paired and analyzed by HMA. This procedure demonstrated the presence of two distinguishable virus populations among the patient's env amplicons. A representative PCR product from each of those two populations was used as a probe to trace similar sequences among all other env PCR products from both the child and the nurse using Heteroduplex Tracking Assay (HTA)<sup>[27]</sup>. After this initial screening, 13 env PCR products from the child and 17 from the nurse were selected for DNA sequencing. env PCR products independently generated at the UW and CHMC laboratories were DNA sequenced (13 from the child and 3 from the health care worker).

At the BCP laboratories, nested PCR of the same samples were performed to amplify the first exon of tat (HXB2 coordinates 5905-6133, 285 bp)<sup>[21]</sup> and the Reverse Transcriptase (RT) coding region of pol (HXB2 coordinates 2518-3320, 750 bp). The primers and PCR conditions used for RT amplification combined the procedure of Kozal *et al.*<sup>[22]</sup> for the first round and with that described by Frenkel *et al.*<sup>[23]</sup> for the second round.

**DNA or cDNA sequencing:** At BCP, sequencing was done using the alfexpress automated sequencer for the separation of the sequencing products obtained with the Thermo Sequenase kit (Amersham Pharmacia Biotech, Piscataway, NJ). At the UW and CHMC, reactions were done with commercially available kits (Dye Terminator Sequencing kit, Applied Bio Systems, Foster City, CA) and separated on an automatic ABI model 377. In all sequencing reactions, products labeled with fluorescent molecules were present either in the primers or in the terminators included with the kit reagents. All the sequences produced for the env region (GeneBank accession numbers: pending) were compared with those already available in HIV-1 genome databases (local, GenBank and the Los Alamos HIV sequence database)

using BLAST<sup>[24,25]</sup>. Phylogenetic reconstructions were created using maximum likelihood estimation<sup>[26]</sup> under HKY models of evolution<sup>[27]</sup> (model details are available from the authors upon request) with the computer program PAUP\* version 4.0b10<sup>[28]</sup>. Bootstrap support<sup>[29]</sup> of phylogenetic relationships were calculated using neighbor-joining trees. Only sequences generated at the BCP are presented in the alignment and phylogenetic analysis in this study, since the work generated at the UW and CHMC confirmed the overall results.

**Serum dilution studies:** Five-fold serial dilutions were prepared from the serum of 10 HIV seropositive patients (5 asymptomatic and 5 with clinical AIDS) at an interval of 1:5<sup>3</sup>-1:5<sup>12</sup> using HIV-1 seronegative normal donor serum as diluent. Each dilution was tested using (1) EIAs containing HIV-1 viral lysate antigen (Abbott Diagnostic Systems, Abbott Park, IL and Genetic Systems, Redmond, WA); (2) third-generation antigen-sandwich EIAs for detection of HIV-1 and -2 (Abbott and Ortho Diagnostic Systems, Raritan, NJ); (3) HIV-1 viral lysate Western blot (Ortho) and (4) recombinant immunoblot assay (RIBA) for HIV-1 and -2 (Chiron Corporation, Emeryville, CA).

## RESULTS

Table 1 shows the time course of samples evaluated from the nurse and child and summarizes results of the HIV serologic and viral nucleic acid amplification assays. Table 2 shows HLA typing that confirmed that the samples from the nurse (serum collected 10 days post exposure) and child (PBMC collected 20 days post accident) were genetically discordant and thus collected from different individuals.

**Relationship between viruses from the putative source patient, the child and the exposed HCW:**

**Env region sequence analysis:** A total of 30 PCR amplicons for the env gene were sequenced from DNA from the child's sample while 17 env gene sequences were derived from RNA from the nurse's sample. To control for the potential for sample mix-up or contamination, a search for similar sequences was done in three databases: Gen Bank (Rockville MD), the Los Alamos National Laboratory (LANL) HIV-AIDS database and a database of sequences determined within the laboratories at the UW and CHMC.

Table 1: Laboratory studies of the nurse's serial serum samples for direct (p24 antigen; RT-PCR; DNA PCR) or indirect (ELISA; Western blot) detection of HIV infection. The days are expressed relative to the date of the occupational accident

Day	-659	-576	-493	-434	0 (Day of accident)	10	21	24
HIV-1 viral lysate EIA	Neg	Neg	Neg		Neg			
HIV-1/2 recombinant antigen EIA				Neg	Pos			Pos
Western Blot					Ind	Neg	Ind	Pos
P24					-	-	+	+
gp41					-	-	-	+/-
P51					-	-	-	+/-
P66					-	-	-	+/-
Gp120					+/-	-	-	+/-
gp160					2+	-	+/-	+
HIV-RNA (RT-PCR)					Neg			
HIV DNA (qualitative PCR)						Pos	Pos	Pos
HIV RNA (quantitative PCR) mL <sup>-1</sup>								417,000 copies
P24 antigen EIA					Neg			49 pg mL <sup>-1</sup>

Table 2: Allele-specific PCR for the determination of HLA type in the nurse and patient samples

	DR 1	DR 2	DR 4	DR 7	DR 11	DQB 2	DQB 4	DQB 5	DQB 601	DQB 603	DQB 7	DQB 8
Nurse	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	NEG
Patient	POS	NEG	NEG	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG

No sequence was sufficiently close so as to suggest a specimen contamination<sup>[30]</sup>. The closest sequence found was ~10% divergent (hiv509env; GenBank accession number L48063), a clade B virus from South Africa. This finding was consistent with the distance to an epidemiologically unlinked infection. In contrast, sequences from the nurse and child were more closely related (Fig. 2), with inter subject distances ranging from zero to 9% (4% average; Confidence Interval (CI) of 2-5%). In addition, two distinct populations of sequences were found within the child's sample (mean distance between populations: 6.98%, range 6.47-7.53%).

As may be seen in the alignment shown in Fig. 1, in addition to the remarkable nucleotide similarity, certain sequences from the nurse and child also shared a pattern of deletions. As is usually the case, deletions and insertions of nucleotides were not considered in our evolutionary distance studies. However, they can, as shown here, confer a specific signature to groups of sequences. The same sequences that show deletions also show a difference in amino acid sequence at the crown of the V3 loop (GPGR for GPGK) and loss of the highly conserved N-linked glycosylation site sequon spanning the N-terminus of the V3 loop (bold on the alignment, Fig. 1). The phylogenetic tree (Fig. 2) showing sequences from these two individuals in the context of sequences from epidemiologically unlinked HIV from the GenBank database, show that both groups of sequences from both individuals form a unique cluster. All of the foregoing data indicate a close

relationship between the viruses in the two infections, consistent with direct transmission from the child to the nurse.

**Env region Heteroduplex Tracking Analysis (HTA):**

In addition to the V3-V5 amplicons sequenced at BCP (n = 30), other products of the env PCR were analyzed by HTA. Based on the sequencing data, the products identified as 11746055 and 11746073 (obtained from the child) were used as probes in the hybridization procedures, representing the NCT/GPGR and DCT/GPGK/Δ group sequences, respectively. With this approach, 12 additional sequences related to the NCT/GPGR (visualization of heteroduplexes when hybridized with 11746073, but not with 11746055) and 15 sequences related to the DCT/GPGK/Δ group (visualization of heteroduplexes when hybridized with 11746055 but not with 11746073) were identified among the PCR products from the nurse's sample. Interestingly, a low prevalence of sequences of the DCT/GPGK/Δ group among the products of the env PCR of the patient was also observed using the HTA procedure (33 related to NCT/GPGR and only one to DCT/GPGK/Δ). Combining sequencing and HTA results, the child presented 3 DCT/GPGK/Δ and 46 NCT/GPGR related sequences, compared to 24 and 46, respectively, from the nurse. Sequence 11760222 from the nurse was a recombinant between the two major populations present, defined by Boot Scan analysis,<sup>[31]</sup> presenting a 5' end related to the 11746055 and a 3' end related to 11746073 (data not shown).

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-----C2-----*-----V3-----*-----
#1174647 SLAEEVVIR SENFTNNAKT IIVHLNLSVA INCTRPNNNT RKGIHIGPGR AFYATGQIIG DIRQAHCNIS KEEMNKTLGQ IVTKLREKFG NNKTI
#11746218 .....
#1174669 .....
#11746220 .....
#11746209 .....
#1174655 .....
#1174659 .....
#1174676 .....V.....
#1174649 .....
#1174679 .....
#1174621 .....I.....E.....Y.....V.....R.....TK.D...E.....
#1174673 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#1174686 .....E..E..D.....K.....EG.....R.K....E...M.....-I.

#1176003 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#1176004 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#1176008 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760249 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760330 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760344 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760349 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760375 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760378 .....E..E..D.....K.....EG.....R.K....E...M.....-I.
#11760222 .....K..E..I.....K.....EKK.....I...K...-...
#11760302 .....E.....I.....N.....G.K.....I.....K...-...
#1176007 .....
#1176012 .....
#1176015 .....
#1176059 .....K.....
#1176058 .....
#11760305 .....

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(a)

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-----C3-----*-----V4-----*-----C4-----
#1174647 VFNSSSGGDP EIVTHSPNCG GEFFYCDSTK LFNSTWYENG TWIGSMDTEV NITLPCRIKQ IIMMWQEVGK AMYAPPRIQV IRCSSNITGL LLT
#11746218 .....
#1174669 .....K.....
#11746220 .....F.....K.P.....
#11746209 .....N.....
#1174655 .....N.....K.....
#1174659 .....N.....K.....
#1174676 .....N.....K.....Y.....
#1174649 .....N.....G.....N.....P.....
#1174679 .....MT.....K.N.....T...GE.....K...W.....
#1174621 I.K.....NT.....K.N.....T...GE.....K...W.....
#1174673 A.EP.A...M.....Q...K...-...N.E K.....G.....
#1174686 A.EP.A...M.....Q...K...-...N.E K.....G.....

#1176003 A.EP.A...M.....Q...K...-...N.E K.....P.....G.....
#1176004 A.EP.A...M.....Q...K...-...N.E K.....G.....
#1176008 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760249 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760330 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760344 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760349 A.EP.A...M.....Q...K...-...N.E K.....H.....G.....
#11760375 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760378 A.EP.A...M.....Q...K...-...N.E K.....G.....
#11760222 ..QP.....N..E.....-D...F...G.....K.....G.....
#11760302 .....K.....
#1176007 .....N.....K.....
#1176012 .....N.....K.....
#1176015 .....N.....K.....I.....
#1176059 .....N.....P.....K...SW.....
#1176058 .....N.....T.....
#11760305 .....

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(b)

Fig. 1a and b: Sequence alignment of amino acids encoded by amplicons obtained from the C2-V4 region of the child (identified by numbers starting with 11746) and the nurse (starting with 11760). Dots represent identities; dashes represent amino acids deletions. The GPGR motif at the crown of the V3 loop and the N-linked glycosylation site sequon are marked in bold (Panel a)

**Tat region sequence and HTA analysis:** The analysis of the sequences of the tat viral gene region, although limited to a reduced number of amplicons, resulted in a similar pattern (Fig. 3). Although several sequences in the databases had more than 90% identity to the test sequences, comparisons of tat sequences from the nurse and child demonstrated a much closer relationship

(genetic distance of 2%, CI = 1-3 vs. 9%, CI = 8-10%). These latter genetic distances are compatible with previous data from cases of proven direct epidemiological transmission<sup>[21]</sup>. Sequence 746086 was an outlier among this group (Fig. 3), suggesting the existence of two related yet distinct viral populations in the child as found in env.

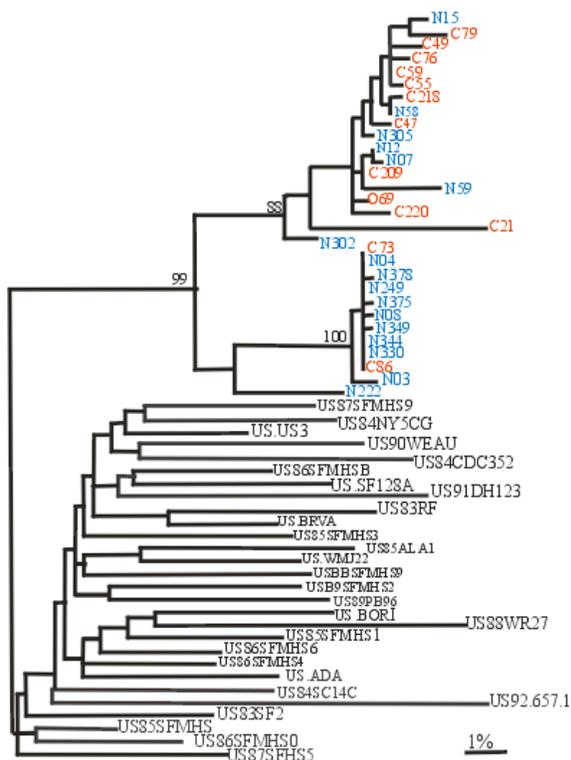


Fig. 2: Maximum likelihood phylogram (-LnL = 4221.0130) representing the reconstruction of the phylogenetic relationships between the env (V3-V5) sequences obtained from the nurse (blue), the child (red) and 28 sequences chosen from GenBank. Ten random sequence addition iterations were used. Scale bar represents 1% genetic distance (0.01 substitutions/site). Bootstrap values are shown at nodes with greater than 70% support

tat PCR products 760026 and 746086 were used as probes in an HTA<sup>[21]</sup> with all the other tat PCR products available from both subjects. An additional 4 amplicons derived from the nurse's sample were closely related to 746086 (only homoduplexes), but not to 760026 (only heteroduplexes). Conversely, two other tat amplicons available from the nurse were related to 760026. The child's sample, however, had the reciprocal pattern of representation of the two forms, with 3 out of 50 related to 746086, while the other 47 formed homoduplexes with 760026 (data not shown).

**Pol region sequence analysis:** Finally, analysis of the sequences of the Reverse Transcriptase region of the pol gene from nurse and child samples was performed.

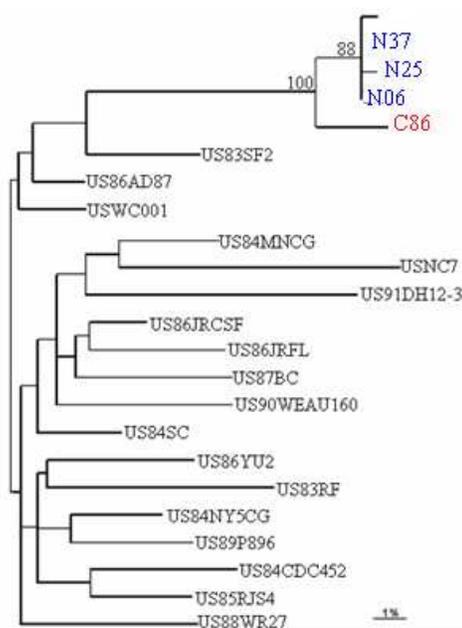


Fig. 3: Maximum likelihood phylogram (-LnL = 1229.0419) representing the reconstruction of the phylogenetic relationships between the tat sequences obtained from the nurse (blue), the child (red) and 18 sequences chosen from GenBank. Thirty-five random sequence addition iterations were used. Scale bar represents 1% genetic distance (0.01 substitutions/site). Bootstrap values are shown at nodes with greater than 70% support

There was, as expected for epidemiologically linked infections, near identity between the amplicons obtained from the two individuals (data not shown). They also demonstrated identical Nucleoside Associated Mutations<sup>[32]</sup>, consistent with the known use of ZDV by the child (M41L, D67N, T69D, K70R, H208Y and L214F)<sup>[33-38]</sup>.

**Serial dilution studies:** Figure 4 and 5 show the seroreactivity of serially diluted HIV positive plasma. As shown in Fig. 4a, the third generation HIV-1/HIV-2 EIAs (Abbott and Ortho) were the most sensitive assays by end-point-dilution titration analysis. Note that the Abbott 2nd-generation HIV-1 viral lysate EIA (3A11) failed to detect reactivity at dilutions exceeding  $10^3$ - $10^4$ , while the Abbott and Ortho 3rd generation HIV-1/2 recombinant antigen-based EIAs, detected seroreactivity in four of the 10 cases when samples were diluted  $\geq 10^5$ -fold. Western Blot reactions, although not considered positive on the dilutions that were reactive by 3rd generation EIAs ( $10^3$ - $10^4$ ),

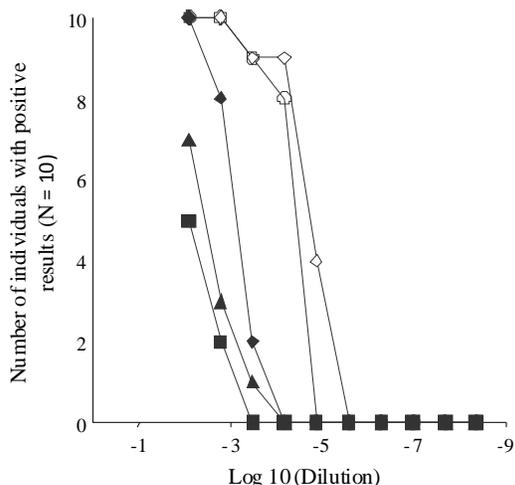
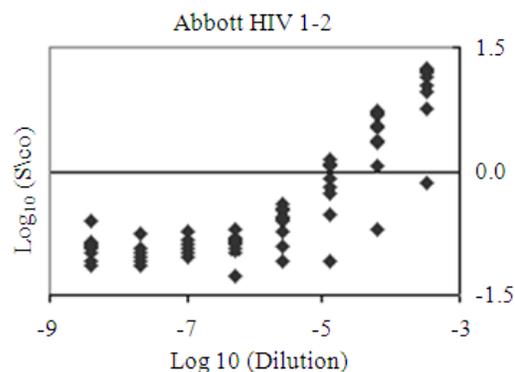


Fig. 4a: Five-fold serial dilutions were prepared with sera from 10 HIV-1 carriers (5 asymptomatic; 5 with clinical AIDS). The abscissa shows the dilution factor on a log<sub>10</sub> scale. The ordinate indicates the number of individuals with positive results on HIV-1 antibody tests. Open diamond: Abbott 3rd-generation HIV-1/2 combination EIA (3A77); open circle: Ortho 3rd-generation HIV-1/2 combination EIA; filled diamond: Abbott 2nd-gen HIV-1 EIA (3A11); filled triangle: Western blots; filled square: recombinant immunoblot assay (RIBA)-positive



Regression formula:  
 $Y = 5.659 + 1.528 * x + 0.087 * x^2$ ;  $R^2 = 0.88$   
 Log<sub>10</sub> (s\co) 95% confidence limits equal to zero  
 Indicates a dilution between  $10^{4623} - 10^{6694}$

Fig. 4c: Optical Density (OD) regression curve obtained from HIV-1 antibody detection of five-fold dilutions of 10 serum samples from HIV positive subjects (5 AIDS patients and 5 asymptomatic HIV-1 carriers) using third generation EIA (Abbott 3A77HIV-1/2, or COMB)

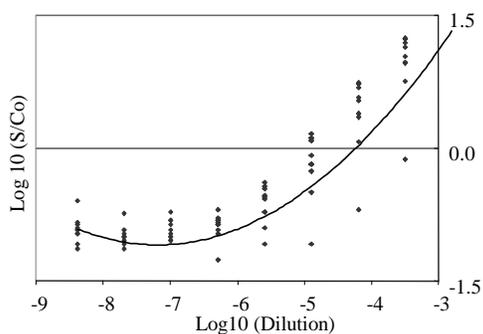


Fig. 4b: Optical density (OD) regression curve obtained from HIV-1 antibody detection of five-fold dilutions of 10 serum samples from HIV positive subjects (5 AIDS patients and 5 asymptomatic HIV-1 carriers) using 3rd-generation HIV-1/2 EIA (Abbott 3A77)

continued to show indeterminate results at dilutions up to  $10^8$  fold (Fig. 5) with an envelope-only band pattern similar to that in the nurse's post-exposure sample (Table 1).

The end-point dilution data allows estimation of the minimal amount of HIV seropositive blood that would have to be "diluted" in the intravascular system of an inoculated person so that a result above the limit of detection (cutoff) for the 3rd generation EIA test could be obtained (Fig. 4c). Considering the nurse's weight (66 kg) and hematocrit (38.9%) at the time of the accident, her estimated volumes of blood and plasma would be 4.6 and 2.8 liters, respectively<sup>[39]</sup>. Based on data obtained from Fig. 4b, showing that 4/10 seropositive individuals had a reactive EIA, we can speculate that one would be able to detect antibodies in some patients with a 1:1000,000 dilution. Taking into account that the estimated volume of nurse's plasma is 2.8 L, in a conservative estimate, it would take 28  $\mu$ L of transferred blood in order to result in a reactive EIA. It is known that one 30-gauge needle, 0.5-cm depth needle stick is able to transfer 7  $\mu$ L of blood<sup>[40]</sup>. According to the nature and the extent of the accident in the case here studied, we believe that more than 28  $\mu$ L could be transferred from the source patient to the nurse. The Western blot results at this same dilution level demonstrate gp120  $160^{-1}$  bands, compatible with the findings of the nurse's blood collected on the day of the accident.

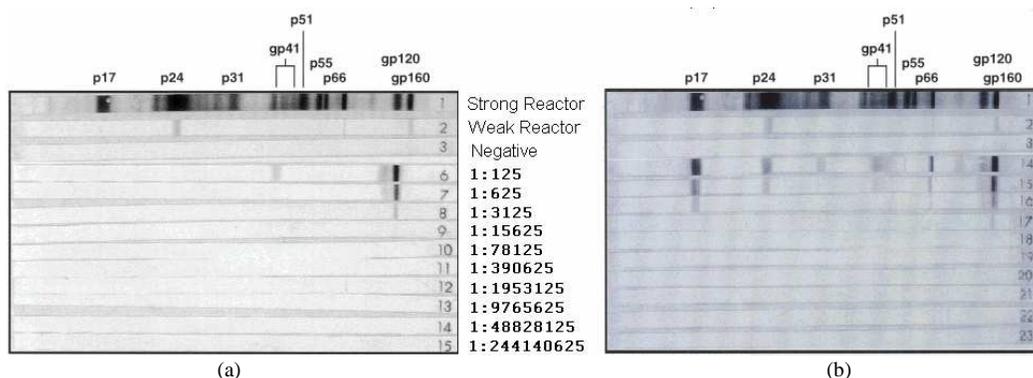


Fig. 5: Representative samples of Western blot HIV-1 positive patients' results in serially diluted samples (five-fold). The figure legend between the Western blot images indicates the presented dilution. (a): Asymptomatic HIV carrier; (b): AIDS patient

As a final verification, we evaluated whether the Western blot reactivity seen on the baseline exam (i.e., gp120 +/-; gp160 +) could have been a false-positive result restricted to the detection of reactivity against a previously defined non-specific epitope of the HIV-1 envelope protein. Sayre *et al.*<sup>[41]</sup> have demonstrated that in some non-infected blood donors with false positive Western blot results, the reactivity is restricted to a 20-amino acid sequence of gp41 that is shared with non-HIV antigens; if the donor's sample is pre-incubated with a solution containing this amino acid sequence, the Western blot reactivity is blocked. This procedure was done with the nurse's baseline sample and the result remained the same, i.e., the peptide did not reduce the gp120 or 160 band reactivity (data not shown). Thus, all of our evidence is consistent with the hypothesis that the Western blot reactivity observed in the nurse was specific to HIV envelope and attributable to transient detection of passive HIV antibodies. Unfortunately, we did not have any left over sample from the source patient in this case to perform serial dilution studies.

### DISCUSSION

The molecular genetic evidence shown in this case confirms that the child and the nurse had closely related viral populations, consistent with infections acquired from the same source or transmitted from one individual to the other. This, added to the evolving pattern of the serological and direct viral markers of HIV infection (PCR and p24 antigen) in the nurse's samples, confirmed that she acquired the infection from the child as a result of the accident and rejected the possibility of a pre-existing infection resulting in the seroreactivity of

the baseline sample.

It is interesting that the nurse became infected with the two different viral populations present in the child and the amount of genetic diversity within child's sequence and nurse's sequence is similar. Although it is recognized a genetic restriction within viral population during primary infection(bottleneck)<sup>[32,42-48]</sup>, in some cases, more genetically diverse populations can be transmitted<sup>[49,50]</sup>. This may have resulted from an unusually large inoculum given the results of the dilution series serology analysis, the baseline reactivity found in the nurse by Western blot and third-generation EIA is most compatible with detection of passively infused antibodies. Third-generation assays were developed to achieve extremely high sensitivity to allow detection of IgM and IgG early in seroconversion, decreasing the immunological window period<sup>[51-53]</sup>. These tests employ immunodominant HIV-1 and HIV-2 antigens produced through recombinant DNA or peptide synthesis techniques and generally use minimal sample dilutions (neat or 1:2, versus 1:100 to 1:400 for sec generation EIA) and therefore have substantially improved analytical sensitivity relative to first and second generation assays. Because of this, third generation assays are required in blood banks. However, they are not widely employed in U.S. clinical or public health laboratories due to their higher cost and the requirement for further discrimination of HIV-1 versus HIV-2 infection for seroreactive specimens. In this case, the nurse's post-exposure sample was sent to a local blood bank and subjected to a third-generation EIA test. Data from the literature do not usually include details of the diagnostic tests used for evaluation of baseline reactivity in HIV-exposed health professionals

in the U.S., although most probably used first and second generation EIA, since testing was likely performed in hospital or commercial reference laboratories. This may explain the lack of documentation of detection of passively acquired antibodies in previous reports following HCW accidents<sup>[54-57]</sup>.

Other studies have reported the detection of passively transferred antibodies in cases of post-transfusion hepatitis C<sup>[58,59]</sup> and HIV<sup>[60]</sup>, which is not surprising given the large volume of plasma contained in blood components (20-250 mL). Our finding of baseline antibody reactivity against HIV in ultra-sensitive tests such as third generation EIA indicated that a moderate amount of contaminated blood entered the circulation of the exposed individual at the time of the accident, rather than indicating pre-existing infection. Whether such passive antibodies are indicative of high-level exposure and predictive of transmission warrants further study. In any event, these results indicate that special care must be taken in interpreting baseline tests at the time of making decisions involving initiation or continuation of prophylactic therapy following high-risk HCW exposures.

This case is also relevant in that it documents the transmission of a virus resistant to multiple nucleoside analogs from a patient to a health care worker after an occupational injury. Not surprisingly, the virus from the source patient had genotypic evidence for resistance not only to ZDV (M41L, D67N, K70R), but also to DDC, DDI (T69D) and with partial genotypic resistance to D4T and Abacavir (M41L, D67N, K70R) (<http://hivdb.stanford.edu>, [www.ablnetworks.com](http://www.ablnetworks.com)). Although the patient had been treated for a long period of time with ZDV immunotherapy, it is currently known that the Nucleoside Analog Mutations selected by this drug can confer varying degrees of reduced susceptibility (cross resistance) to all nucleoside and nucleotide analogues reverse transcriptase inhibitors tested to-date<sup>[61]</sup>. These data and other showing that sources or potential source in occupational accidents involving health care works have antiretroviral resistant HIV<sup>[62]</sup>, support guidelines recommending the use of HAART for post exposure prophylaxis in health care workers. The transmission of a virus containing genetic markers of resistance from an antiretroviral experienced patient also supports guidelines that take into consideration the medical history of the patient and anti-viral response profiles of the source patients' virus

when deciding on antiretroviral prophylaxis in post-exposure settings<sup>[4]</sup>. Although the turnaround time for resistance testing (a few days to 2-3 weeks) makes it infeasible to guide initial post-exposure antiretroviral prophylaxis, it may be used to expeditiously adjust the drugs according to the resistance profile of the exposure source. Furthermore, prophylactic antiretroviral should preferentially choose a combination of reverse transcriptase inhibitors, since protease inhibitors will act after the cell is already infected.

## CONCLUSION

This is the first well-documented case of passive HIV antibody detection after a percutaneous exposure. Reactive baseline serology should therefore not be assumed to represent prior infection nor exclude prophylaxis. Transmission of drug-resistant HIV-1 corroborates the medical history and supports use of drug history and resistance testing to guide antiretroviral prophylaxis.

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