

# Transfusion of HIV-infected blood products despite highly sensitive nucleic acid testing

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**BACKGROUND:** In France, the risk of HIV transmission by transfusion was reduced by implementing pooled nucleic acid testing (NAT) in 2001 and individual NAT in 2010. We report here the first case in France of transfusion of human immunodeficiency virus (HIV)-infected blood donated during HIV pre-ramp-up phase that tested individual NAT negative.

**METHODS:** Blood donations are screened for HIV antibodies and HIV RNA (ProcleixUltra, Grifols; limit of detection at 95%, 23 copies/mL). When a repeat donor tests positive for HIV, a repository sample from the previous donation is tested with the Cobas Taqman HIV-1 test (CTM, Roche; limit of detection at 95%, 17 copies/mL).

**RESULTS:** In August 2017, a 57-year-old male repeat donor was screened positive for HIV antibodies and RNA (plasma viral load, 11,599 copies/mL). The previous donation had tested negative with Ultra in March 2017 but was positive with an unquantifiable plasma viral load when tested with CTM. Sequencing showed no mismatch between Ultra primers/probes and the target sequence. HIV transmission was excluded by lookback studies in the recipient of platelets, which had been pathogen reduced, but not in the recipient of RBCs due to premature death.

**CONCLUSION:** This case demonstrates that the risk of contaminated donations due to the early HIV infection phase going undetected by highly sensitive NAT is real but exceptional. The absence of transmission to the platelets recipient could be due to the very low viral inoculum and/or to the efficacy of the viral inactivation. This case also highlights the additional value of a systematic donation archiving and the importance of donor education and predonation selection.

In most countries, the screening of blood donors for the three major blood-borne agents, human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C virus, relies on serologic methods. Additionally, nucleic acid testing (NAT) has been implemented for its ability to detect early seronegative infections and reduce the window period.<sup>1,2</sup> The window period duration depends therefore on the type of test used. Regarding HIV, serology assays yield a window period between 17 and 19 days<sup>3</sup> and HIV NAT between 5 and 9 days,<sup>3-7</sup> depending on assays and individual or pooled formats.

**Abbreviations:** CTM = Cobas Taqman HIV-1 kit; FFP = fresh frozen plasma; HIV = human immunodeficiency virus; ID-NAT = individual nucleic acid testing; LOD<sub>95</sub> = limit of detection at 95%; MP-NAT = pooled format nucleic acid testing; NAT = nucleic acid testing; PC = platelet concentrate; pVL = plasma viral load; RT-PCR = reverse transcription polymerase chain reaction.

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In countries where NAT is performed to ensure the safety of blood transfusion, the risk of missing HIV-infected donations has been described in two situations, potentially leading to transfusion-transmitted infections.

The first situation is the inadequacy between the primers and probes used in NAT assays and the sequence of viruses found in blood donors.<sup>8-11</sup> Indeed, due to multiple cross-species transmission events of simian immunodeficiency viruses to humans<sup>12</sup> and a continuous genetic drift due to a high error rate during replication,<sup>13</sup> HIV exhibits a very broad genetic diversity. Nucleotide divergence reaches 70% between HIV types 1 and 2 (HIV-1 and HIV-2)<sup>14</sup> and 50% between HIV-1 groups (M, N, O, and P) in the envelope gene.<sup>15</sup> This diversity is enhanced by frequent recombination events<sup>16,17</sup> that allow shuffling of genomic regions and increase the complexity of HIV viral populations. Currently, HIV molecular epidemiology is largely dominated by HIV-1/M subtype C (48% of infections globally), essentially present in southern Africa, the Middle East, and India, followed by subtypes A (12%) and B (11%), which are the major subtypes in Europe and the Americas.<sup>18</sup> Interestingly, HIV-1 recombinants, circulating and unique recombinant forms, now representing 20% of infections, have a growing genetic complexity, with molecular patterns involving sometimes up to seven “pure” subtypes.<sup>19</sup> Moreover, although HIV-2 and HIV-1 minor variants are mostly found in West and West Central Africa, respectively, they have also spread to a minor extent to Europe and North America. Consequently, this high genetic diversity has an impact on serologic<sup>20,21</sup> and molecular assays<sup>20,22</sup> used in diagnosis and blood screening. In this context, NAT development and implementation are very challenging, especially in regions where the pandemic HIV-1 group M cocirculate with HIV-2<sup>23,24</sup> or HIV-1 minor variants.<sup>15</sup> To address this issue, it has been proposed to target multiple conserved regions,<sup>11</sup> as implemented in two NAT assays Cobas TS is indeed based on PCR, Ultrio is based on TMA (Cobas TaqScreen MPX test, version 2.0; Roche [LTR and gag] and ProcleixUltrio assay; Grifols [LTR and pol]).<sup>25</sup>

The second and most frequent situation leading to infected but HIV NAT-negative blood products corresponds to blood donations undertaken during the very early phase of infection before NAT becomes positive.<sup>9-11,27-31,36</sup> This window period is all the longer when NAT is performed in a pooled format (MP-NAT), which reduces the assay sensitivity. Several studies have shown that the use of individual NAT (ID-NAT) would have detected the virus and prevented the transfusion of HIV-infected blood units.<sup>9-11,27-31,36</sup> In France, MP-NAT was introduced in 2001, with eight (Procleix HIV-1/hepatitis C virus assay, Gen-Prob/Chiron) or 24 (Cobas AmpliScreen/Amplicor, Roche) donations per pool. ID-NAT (ProcleixUltrio, Grifols) was then progressively implemented from 2010 to 2013.<sup>37</sup>

Thanks to dramatic progress in NAT sensitivity, the risk of facing undetected HIV-positive donations, at least in

high-income countries, is currently exceptional. However, we report here the first case, in France, of a blood donation collected during the HIV pre-ramp-up phase that went undetected by ID-NAT with subsequent transfusion of products derived from infected blood.

## MATERIAL AND METHODS

### Blood donation testing

In France, the national blood service (Etablissement Français du Sang) and to a lesser extent the Army Blood Service (Centre de Transfusion Sanguine des Armées) are in charge of collecting donations, performing blood screening, and preparing and delivering blood products. All blood donations are currently screened for HIV antibodies (PRISM anti-HIV-1/2 O assay, Abbott, in mainland France; ARCHITECT HIV Ag/Ab Combo, Abbott, in overseas territories; and Gen-screens ULTRA HIV Ag-Ab, Biorad, in the Centre de Transfusion Sanguine des Armées) and HIV-RNA (ID-NAT, ProcleixUltrio assay). The 95% limit of detection (LOD<sub>95</sub>) of the ProcleixUltrio assay is 39 IU/mL (23 copies/mL) for HIV-1/M subtype B, according to the manufacturer. Probit analysis performed on 24 replicates of the HIV-1 RNA World Health Organization standard 98/750 (subtype B) yielded a value of 33 IU/mL (19 copies/mL), and the same analysis on a panel of different HIV genotypes (HIV-1/M subtype A-B, CRF01 and HIV-1/O) gave LOD<sub>95</sub> between 18 and 48 IU/mL or 10 to 28 copies/mL.<sup>38</sup> In the case of HIV-positive samples, western blotting, and HIV-discriminatory NAT are performed to confirm HIV infection.

### Role of the French National Reference Centre for infectious risks in transfusion

The French National Reference Centre for Infectious Risks in Transfusion collects a large volume of all positive donations to complete investigations regarding viral markers. Regarding HIV, a confirmation assay (western blot) and a recent infection assay,<sup>39</sup> which allows dating infections as more or less than six months, are systematically performed for HIV-1 antibody-positive donations. Plasma viral load (pVL) is also determined using the Cobas TaqMan (CTM) HIV test 2.0 assay (Roche; LOD<sub>95</sub>, 17 copies/mL; limit of quantification, LOQ<sub>95</sub>, 34 copies/mL). Finally, HIV strains are sequenced in three genomic regions (see the molecular analyses section).

### Hemovigilance procedures

The French hemovigilance system, under the supervision of the French Drug Agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé), mandates reporting any dysfunction in the transfusion chain and involves many partners.

Regarding infectious risks, the Etablissement Français du Sang is in charge of coordinating investigations in case of a donor's or a recipient's seroconversion (lookback studies). Private and public health facilities notify any secondary effect

that might arise during or after transfusion, including seroconversion for a viral marker. Finally, the French National Reference Centre for Infectious Risks in Transfusion is involved in lookback studies, by analyzing repository samples of previous donations, archived for three years: pVL is quantified, and when positive, HIV sequencing is performed to assess the relationship between the different donations and between donors and recipients.

**Molecular analyses**

Reverse transcription polymerase chain reaction (RT-PCR) and sequencing were performed in *pol* (protease and reverse transcriptase) and *env* (gp41) genes to genotype HIV strains. RT-PCRs were performed as follows: viral RNA was extracted from 200 µL of plasma using a viral RNA mini kit (QIAmp, Qiagen), eluted in 60 µL of elution buffer, and 10 µL of extract was used in a 50-µL RT-PCR reaction, using a RT-PCR system (SuperScriptIII One-Step RT-PCR System with Platinum Taq DNA polymerase, Invitrogen), 20 pmol of each primer (Table 1), 0.2 mM of each deoxynucleotide, and 2.0 mM MgSO<sub>4</sub>. Two microliters of amplification product were used in a 50-µL nested PCR using HotStar Taq Master Mix (Qiagen), 20 pmol of each primer (Table 1), and 1.0 mM MgCl<sub>2</sub>. (RT-)PCRs were run on a thermal cycler (Gene Amp 9700, PerkinElmer), and cycling conditions are described in Table 1. A full-genome amplification was also conducted on the control sample, as previously described.<sup>40</sup> Amplicons were sequenced at GATC, with primers used for nested PCR, and sequences were aligned with a set of HIV-1/M sequences from the Los Alamos HIV database<sup>19</sup> using computer software (MEGA 7).<sup>41</sup> HIV-1/M subtypes were determined using Los Alamos HIV BLAST<sup>19</sup> and phylogeny. Phylogenetic trees were computed, using MEGA 7, with the neighbor-joining Kimura 2-parameter method, and 1000 bootstrap replicates to assess the reliability of the branching order. The nucleotide sequences resulting from this study have been submitted to the GenBank database under the accession numbers MH663503 and MH663504.

**RESULTS**

In France, from 2011 to 2016, a total of 57 HIV-1 seroconversions were identified in blood donors where 1) prior donations had been screened by ID-NAT (53 of 57) or eight-donation minipools (4 of 57) and 2) index donations were collected within 3 years after prior donations (duration of the repository plasma bank turnover). Hemovigilance lookback studies were conducted on 54 cases where the prior sample could be tested with the CTM assay, which yielded negative results.

In August 2017, a 57-year-old male repeat blood donor was tested HIV positive, with a full western blot profile and a pVL of 11,599 copies/mL (CTM). He had given blood five times over the two previous years. Previous (N-1) donation had screened negative for anti-HIV Ab and HIV-RNA (Procleix

**TABLE 1. Primers and PCR conditions used to sequence HIV strains at the National Reference Centre**

Target region	Step	Primers	Primer sequence (5'-3')	Fragment size (bp)	Cycling conditions		
					Annealing (°C)	Elongation time	Cycles
PR	RT-PCR	S prot 1 (FW)	TAATTTTGGGAAGATCTGGCCTTC	653	55	30	35
		AS prot 1 (RV)	GCAAACTACTGGAGTATTGTATGGATTTTCAGG				
	Nested PCR	S prot 2 seq (FW)	TCAGAGCAGACCAGGCCAACAGCCCCA	515	55	30	30
		AS prot 2 seq (RV)	AATGCTTTTATTTTTTCTTCTGTCAATGGC				
RT	RT-PCR	S rt 1 (FW)	AGTAGGACCTACACCTGTCA	941	55	30	35
		AS rt 1 (RV)	CTGTTAGTGCCTTGGTTCCTCT				
	Nested PCR	S rt 2 (FW)	TTGGTTGCACITTAATAATTTCCCATTAGTCCTATT	805	55	30	30
		AS rt 2 (RV)	CCTACTAATCTGTATGTCAATTGACAGTCCAGCT				
gp41	RT-PCR	1S (FW)	TGGAGAGGAGATATGAGG	730	55	30	35
		T20AS1 (RV)	GTGAGTATCCCTGCCCTAACTCTAT				
	Nested PCR	T20S1 (FW)	GAGGACAAITGGAGAAGTGAATT	655	55	30	30
		2AS (RV)	CTACCAAGCCTCCCTACTATC				

PCR = polymerase chain reaction; RT-PCR = reverse transcription polymerase chain reaction.

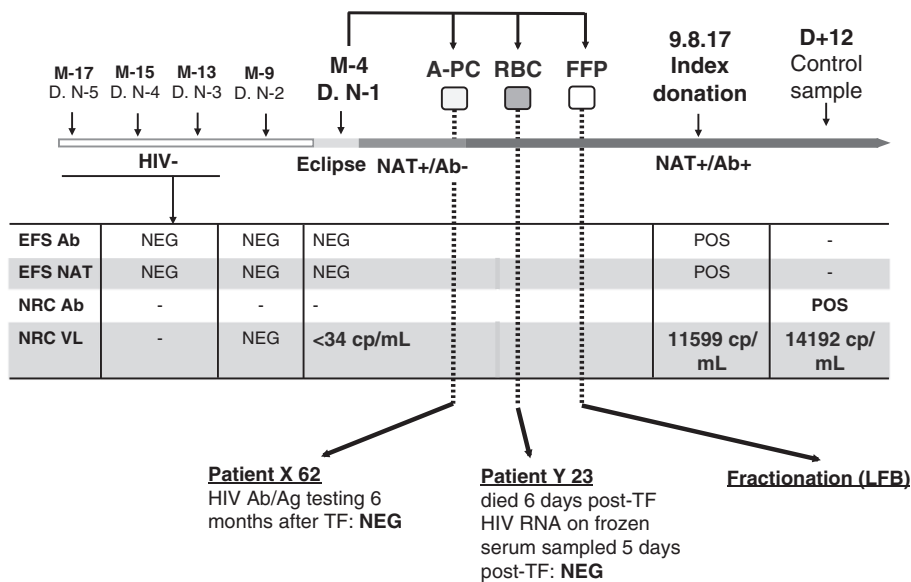
Ultrio S/CO = 0.05; positive >1) in March 2017, but was positive when tested in the archived sample with CTM, showing a pVL below the quantification level (34 copies/mL) (Fig. 1). Only the gp41 region yielded a positive amplification on the N-1 archived sample, while the three target regions were amplified in the control sample. The N-1 sequence showed a 99.8% similarity with the sequence found in a control sample collected 12 days after the positive donation (1 of 539 nucleotides showing an ambiguity in the sequence of control sample; Fig. 2). The strain near full-length genome was sequenced from the control sample and was entirely related to HIV-1/M subtype B. From donation N-1, three blood products had been prepared: an RBC unit, a buffy coat contributing to a whole blood pooled platelet concentrate (PC) in additive solution and a fresh frozen plasma unit (FFP). The residual donor plasma content in the RBC unit and PC was approximately of 20 mL. The RBC unit had been transfused to a 23-year-old recipient of an allogenic bone marrow transplant who died of graft-versus-host disease six days after transfusion. The detection of HIV RNA performed on a plasma sample collected five days after transfusion was negative (Fig. 1). The whole blood pooled PC had undergone pathogen reduction (amotosalen/UVA treatment, INTERCEPT Blood System, Cerus) and had been transfused to a 62-year-old woman with acute myeloblastic leukemia. HIV serology (fourth generation enzyme immunoassay, antigen/antibody combo) performed on the patient six months after the transfusion was negative. Finally, the FFP had entered a manufacturing plasma pool for fractionation by the Laboratoire Français du Fractionnement et des

Biotechnologies (Fig. 1). No withdrawal measure was judged necessary given the very low HIV viral titer in the blood donation and the various pathogen reduction processes implemented during the plasma fractionation process. Despite the patient recall and personal anamnesis, no risk factor could be identified. The donor's partner was tested negative for HIV. No mismatches were found between the primers/probes used in the ProcleixUltrio assay (LTR and *pol*) and the virus sequence, precluding a false-negative result by misalignment of the viral strain with test probes and primers (Grifols, private communication).

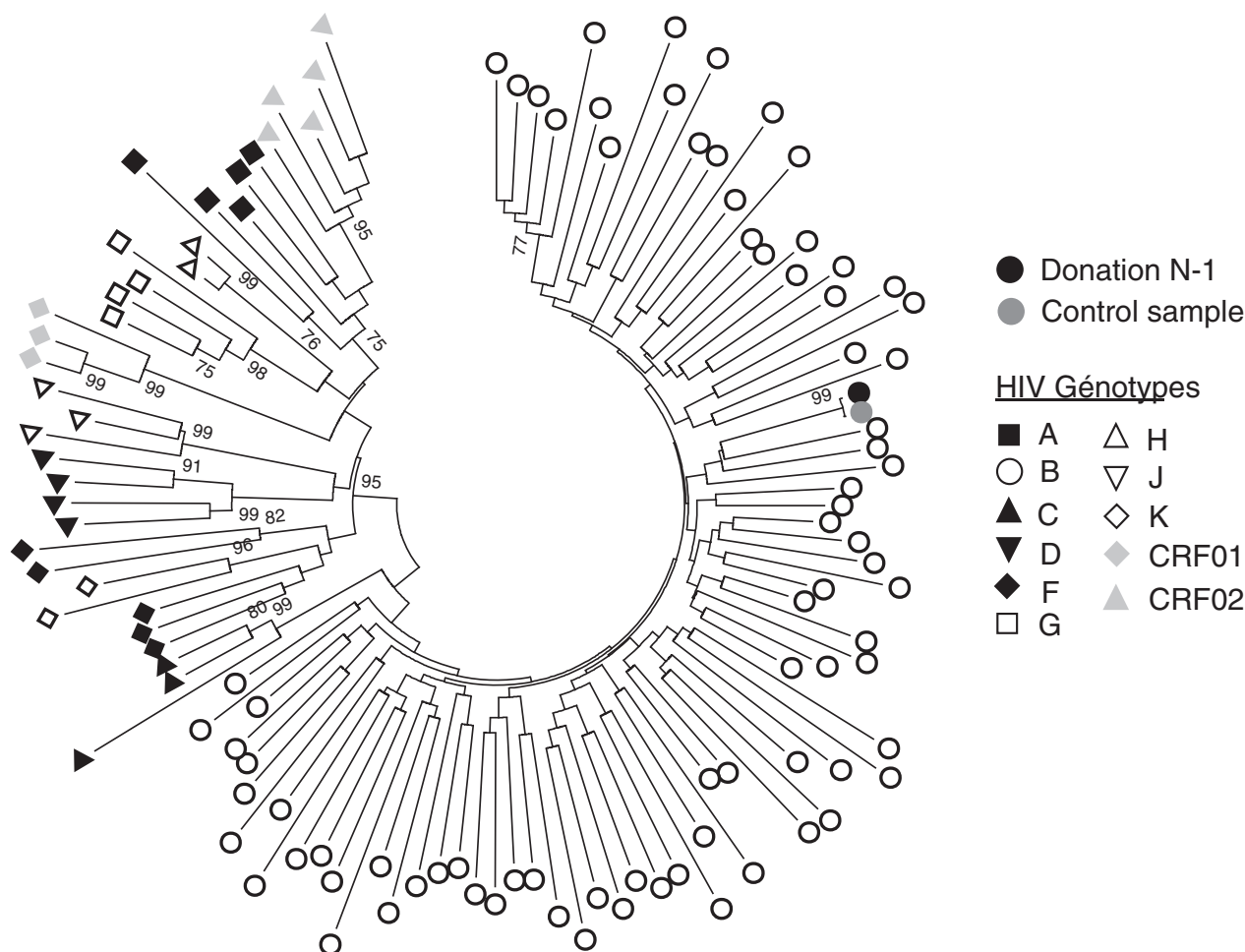
### DISCUSSION

To our knowledge, the case we describe here is the first description of a blood donation collected during HIV pre-ramp-up phase in France, since implementation of ID-NAT, without evidence of HIV transmission to the recipients.

Between 2000 and 2018, more than 20 HIV-infected blood components that tested HIV antibody and NAT negative, were reported worldwide, with or without transmission to the recipients.<sup>8,10,11,26-36,42</sup> These cases correspond to two particular situations: 1) the presence of polymorphism in HIV strain, leading to mismatches between primers/probes and the viral sequence,<sup>8,10</sup> and 2) failure to detect HIV RNA when NAT is performed in minipools.<sup>8,10,11,26-36</sup> In some cases, the convergence of both situations dramatically reduced the NAT sensitivity, yielding false-negative results.<sup>8,10</sup> Only one recent case, described in South Africa, showed an ID-NAT



**Fig. 1. Sequence of events.** The six blood donations and the control samples are set against the different phases of HIV infection. The results of the blood screening (EFS) and of the subsequent testing (NRC) are shown as a table below the timeline. Blood components prepared with the index donation and the corresponding recipients are also indicated. Ab = antibodies; Ab/Ag = fourth-generation EIA; A-PC = amotosalen/UV-A -treated whole blood pooled platelets concentrate; M-15: 15 months before index donation, D. N-2 = next-to-last donation before index donation; NEG = negative; POS = positive; TF = transfusion.



**Fig. 2.** Neighbor-joining trees in *gp41* with a large panel of subtype B sequences ( $n = 80$ ). Sequences were retrieved from the Los Alamos HIV sequence databases (<http://www.hiv.lanl.gov>). Phylogenetic analyses were performed using MEGA software. Evolutionary distances were computed using the Kimura two-parameter method with 1000 bootstrap replicates to assess the reliability of the branching order. Bootstrap values are shown only when significant ( $>70$ ). Donation N-1, control sample and the different HIV-1/M subtypes are indicated with forms and gray-scale code.

failure in detecting HIV RNA.<sup>42</sup> In the case we describe here, the strain sequenced from the blood donor belongs to HIV-1/M subtype B, the most prevalent subtype in France in the general population and in blood donors (67% of strains found in blood donors).<sup>37</sup> In addition, no mismatch was observed between the primers and probes used in the NAT assay and their target sequence in the strain genetic sequence. Therefore, the NAT failure is very likely due to the extremely low pVL in the donor. Due to the limited volume of the repository sample, we could not perform repeated tests of CTM to estimate the pVL in N-1 donation. Nevertheless, given that Ultrio  $LOD_{50}$  has been estimated to 3.3 copies/mL,<sup>43</sup> N-1 pVL should be around this value or below.

This case is also consistent with HIV residual risk in blood transfusion in France, which is currently estimated at 1 in 5.9 million donations, that is, one donation every 2 years.

Although the last HIV seroconversion of a blood recipient was notified in 2002, hemovigilance data are not inconsistent with the estimation of the residual risk, when considering underreporting (e.g., no systematic testing for seroconversion and premature death of recipients) and the fact that contamination through infected blood products does not occur systematically, as shown by the differential transmission of HIV when several blood products are prepared from the same infected donation.<sup>26,34,36</sup>

This very low value of residual risk can be explained by the very low incidence of HIV infection among blood donors ( $0.68/10^5$  over 2015–2017; Josiane Pillonel, personal communication). This relies on the low HIV incidence in the French general population ( $17/10^5$  person-year in 2008)<sup>44</sup> and the effectiveness of pre-donation procedures. In 2017, of 2.9 million blood donations, 21 (0.07%) were positive, of which two were antibody negative/NAT positive.

These findings underscore the very low yield of HIV NAT. Nevertheless, the HIV viral loads of NAT-positive/antibody-negative blood donations in France range from 1.53 to 6.44 log copies/mL, with a median of 4.47 log copies/mL, and two-thirds of the donations with viral loads above 3 log copies/mL<sup>37</sup> (updated with 2016–2017 data). Such viral loads are probably contagious, as they are much higher than those observed in most cases of transfusion-transmitted HIV infections.<sup>26–30,33–36</sup> The introduction of NAT could have therefore prevented the occurrence of HIV infection in transfusion recipients. In a study conducted on macaques, although plasma from the very early eclipse phase was shown to be non-infectious, plasma from the pre-ramp-up or early window period phase (NAT positive/antibody negative) was shown to be relatively contagious—a total of 250 copies or 125 virions negative, data compatible with viral loads going undetected by MP-NAT.<sup>45</sup> Interestingly, three studies previously reporting HIV breakthrough transmission despite a negative MP-NAT showed that the viral load in the donation could have resulted in a negative ID-NAT as well.<sup>33–35</sup> Namely, in the report of Salles and collaborators,<sup>33</sup> the probit analysis of the contaminated donation gave an estimated pVL between 3.7 and 7.5 IU/mL (2.2 and 4.5 copies/mL), probably around the one found in the N-1 donation we describe here. Importantly, in Salles's report, the RBC unit transmitted HIV infection.

Also, in addition to the viral load, several factors may influence the infectivity of a blood component and can be divided into three categories<sup>46</sup> 1) the viral properties, including the viral genotype, the stage of infection, and the presence of neutralizing antibodies in the component; 2) the transfusion factors, namely, the storage of the blood components (here the PC was transfused as recommended by the French policy, i.e., within five days after donation) and the use of PRT; and 3) the recipient factors, including the lack of receptor or, in the case of HIV, coreceptor), their degree of immunocompetence, and their blood volume. In our report, at least three factors may have contributed to the absence of HIV transmission in the PC recipient: 1) the extremely low total number of virions, probably close to 66 copies or 33 virions (3.3 copies/mL [Ultra LOD<sub>50</sub>] × 20 mL (residual volume of plasma)); 2) the low plasma content in the PC; and 3) pathogen reduction procedure applied to the PC. As already mentioned, several studies reported a differential transmission of HIV to recipients of blood products prepared with the same blood donation.<sup>26,34,36</sup> In cases with low viral loads, the component that transmitted HIV was the one with the greater plasma volume (PC without additive solution or FFP),<sup>34,36</sup> allowing reaching the minimum infectious dose. Importantly, pathogen reduction technologies, currently in development for whole blood and RBCs, can contribute to further mitigate the risk of transfusion-transmitted infections, in particular in cases where target viruses go undetected by MP- or ID-NAT.

Finally, further factors could contribute to NAT-positive/antibody-negative or even NAT-negative/antibody-negative HIV-infected donations resulting from low viral loads, as the early initiation of antiretroviral treatments or a preexposure

prophylaxis breakthrough. These situations lead to an abolished or a delayed seroconversion, as an immediate control of viral replication prevents the stimulation of the immune system.<sup>47</sup> Although they may increase the residual risk of infected blood components, the current ID-NAT high sensitivity probably allows limiting the risk to those cases with very low viral loads and therefore limited infectivity.

In conclusion, our study shows that highly sensitive NAT can miss HIV-1-positive donations in the very early phases of infection and underscores the usefulness of repository donor samples that allow detecting those rare infectious events, which might become more prevalent in the preexposure prophylaxis era.

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## CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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