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# Detection of HIV antigen and cDNA among antibody-negative blood samples in Nigeria

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**Summary** In developing countries as many as 50% of patients for whom a transfusion is indicated are at risk of dying immediately if transfusion is withheld. It is therefore important that blood transfusion is made as safe as possible. This study was designed to assess the safety of blood transfusion in two large blood banks in Ibadan, Nigeria. Aliquots of 250 samples already screened and passed as negative for HIV-1 and -2 were collected from each of the blood banks. Samples were tested for the presence of HIV-1 antigen (ELAVIA Ag I) and the antigen-positive samples tested for the presence of specific HIV-1 antibodies by Western blot (BioRad, France). All antigen-positive samples were also subjected to PCR. HIV-1 antigen was detected in 6 (1.2%) of the 500 samples, of which 4 (0.8%) and 3 (0.6%) were Western blot-indeterminate and PCR-positive, respectively. Transfusion of HIV-contaminated blood may be contributing significantly to the spread of the virus in Nigeria. There is therefore an urgent need for an organized blood-banking system with facilities for more sensitive assays for the detection of HIV in blood to prevent transmission through transfusion.


## 1. Introduction

The effectiveness of HIV intervention programmes, especially prevention of transmission through blood transfusion, depends largely on the accuracy of diagnosis of the infection. Blood transfusion is the most efficient mode of transmission of the virus. Reports have shown that over 90% of recipients of seropositive blood subsequently became infected (Simonsen et al., 1999). In developed countries, the rate of HIV transmission through transfusion has substantially reduced since 1985, when HIV antibody testing became available to screen blood and blood products for transfusion (Donegan et al., 2003). However, this is not the case in Africa, where blood transfusion is very common (Lee and Allain, 2004), even when it may not be medically indicated. According to Simonsen et al. (1999), injections are given when oral medicines would suffice and transfusion when more benign therapy should be preferred.

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Although data on HIV transmission by blood transfusion in Africa are very scarce, several factors, including variability of the virus, facilities, cost and competence of laboratory personnel (Lee and Allain, 2004; Olaleye, 2006), are some of the problems of HIV diagnosis that may enhance this mode of transfusion in the region. Multiple subtypes and circulating recombinant forms are known to circulate in Nigeria (McCutchan et al., 1999; Ojesina et al., 2006), and the quality of HIV testing varies with the level of health care facilities, whether private, secondary or tertiary. In addition, recent reports have indicated that the use of contaminated syringes and needles as well as false-negative HIV-testing results may be contributing to the large number of infections in many developing countries (Odaibo et al., 1998, 2004; Simonsen et al., 1999). We assessed the safety of blood units for transfusion in two large blood banks in Nigeria.

## 2. Materials and methods

This study was carried out using samples from two large blood banks (blood bank I and blood bank II) in Ibadan, the capital of Oyo State, Nigeria, from March to October 2005. The two blood banks used the same procedures: a single ELISA for testing of blood units (GeneScreen Plus test kit; BioRad, Paris, France).

Aliquots of 250 whole blood samples (10 ml) from every fifth blood unit previously tested and recorded as HIV-1- and HIV-2-negative with the GeneScreen Plus ELISA were collected from each of the blood banks and transported in an isolated specimen box containing ice packs to the Department of Virology, University College Hospital, Ibadan, for further analysis. The samples were centrifuged and plasma separated in aliquots immediately in the laboratory on arrival. Both plasma and packed cells were stored at −20 °C until analysed.

### 2.1. HIV-1 antigen capture assay

All the plasma samples were tested for the presence of HIV-1 antigen using a commercial sandwich-type ELISA for p24 antigen detection (ELAVIA Ag I; Sanofi Diagnostics Pasteur, Marnes La Coquette, France). Briefly, each plasma sample was treated with a mild acid solution-ICS (ELAVIA Ag I immune complex dissociation reagent) to dissociate any antigen/antibody complexes. The treated samples were then transferred to reaction plates and the test performed according to the manufacturer's instructions.

### 2.2. Western blot analysis

All HIV-1 antigen-positive plasma samples were further tested for the presence of specific HIV-1 antibodies using a commercially available Western blot kit, LAV BLOT 1 (Bio-Rad, Paris, France). Samples and reagents were brought to room temperature and the test performed according to the manufacturer's instructions. Briefly, samples were added onto Western blot strips, and after washing, incubation with conjugate and colour development solution, the reaction was stopped by washing three times with distilled water. The presence of antibody to at least one envelope protein of HIV-1 was considered to be positive; the presence of any band that did not meet the criteria for positivity was taken as indeterminate; and no band at all was interpreted as negative.

### 2.3. DNA extraction and PCR analysis

DNA was extracted from the stored packed cells of the HIV-1 antigen-positive samples using the Qiagen Miniprep genomic DNA extraction kit (Qiagen Inc., Valencia, CA, USA) and then subjected to PCR. A set of primers [H1P4327 (TAAGACAGCAGTACAAATGGCAG) and H1P5155as (CTCTGTGGCCCCTGGTCTTCT); supplied by the Institute of Tropical Medicine, Antwerp, Belgium was used to amplify a region of the HIV-1 pol gene. The PCR cycling protocol was 2 min at 94 °C for denaturation, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s and then 72 °C for 7 min for final extension. The PCR product was subjected to gel electrophoresis in 2% agarose gel and viewed under UV light.

## 3. Results

In total, 500 HIV antibody-negative samples from the two blood banks (250 samples from each) were analysed (Table 1), and 6 (1.2%) were found to be positive for HIV-1 antigen by the antigen-capture ELISA. Further testing of the antigen-positive samples by Western blot showed that none was positive and 4 (0.8%) were indeterminate. However, 3 (0.6%) of the samples were found to be positive for HIV-1 antigen by PCR. The two Western blot-negative samples were also negative by PCR, whereas three of the Western blot-indeterminate samples were positive by PCR (Table 2).

**Table 1** Detection of HIV-1 antigen and cDNA (by PCR) in seronegative blood samples for transfusion in Ibadan, Nigeria

| Site | No. samples (antibody-negative) | HIV-1 Ag | Analysis of HIV-1 Ag +ve samples | | | |
|---|---|---|---|---|---|---|
| | | | Western blot | | | PCR |
| | | No. (%) +ve | No. (%) +ve | No. (%) indeterminate | No. (%) −ve | No. (%) +ve |
| Blood bank I | 250 | 4 (1.6) | 0 | 3 (1.2) | 1 (0.4) | 2 (0.8) |
| Blood bank II | 250 | 2 (0.8) | 0 | 1 (0.4) | 1 (0.4) | 1 (0.4) |
| Total | 500 | 6 (1.2) | 0 | 4 (0.8) | 2 (0.4) | 3 (0.6) |

**Table 2** Western blot and PCR reactivity pattern of HIV-1 antigen-positive samples

| Sample ID no. | HIV-1 Ag | Western blot | PCR |
|---|---|---|---|
| 001 | +ve | Indeterminate | +ve |
| 002 | +ve | Indeterminate | −ve |
| 003 | +ve | −ve | −ve |
| 004 | +ve | Indeterminate | +ve |
| 005 | +ve | −ve | −ve |
| 006 | +ve | Indeterminate | +ve |

## 4. Discussion

HIV infection resulting from blood transfusion of infected blood has been documented since the first case report in 1981 (Curran et al., 1985). The results of this study show that HIV-1 antigens were detected in 6 (1.2%) of 500 antibody-negative blood units, which was further confirmed by PCR in 3 (0.6%) of the samples. Similar observations have been reported in other parts of the world (Stramer et al., 2004). Failure to detect HIV antibodies in blood units donated by HIV-infected people who have not seroconverted has been documented (Nelson et al., 1999; Sitas et al., 1994). Significant changes in the antigenic structure of HIV may affect the sensitivity of serological tests, a likely scenario when circulation of multiple subtypes of the virus takes place, as is the case in Nigeria (McCutchan et al., 1999; Odaibo et al., 2006; Ojesina et al., 2006; Peeters et al., 2003).

The rate of unidentified HIV-1-positive blood units found in this study is considered to be high compared with reported cases in developed countries. For instance, only 6 of 8710 (0.07%) Western blot-indeterminate samples and 0 of 7562 Western blot-negative samples were confirmed positive for HIV RNA in the United States (Stramer, 2004). The findings in this study indicate that transfusion of HIV-contaminated blood may be contributing to the spread of the virus in Nigeria and that there is a need for urgent and necessary action by the relevant health authorities to prevent transfusion-associated transmission. The inclusion of P24 antigen testing and the nucleic acid amplification test, which detects HIV-1 RNA in minipools, has been shown to further reduce the risk of transfusing HIV-contaminated blood in the United States (Stramer, 2004).

It is interesting that none of the six antigen-positive samples was confirmed by Western blot, although four were indeterminate. This may indicate that the donors were recently infected with low levels of antibody or that they were infected with virus variants of the epitopes used for the antigen base of the assay kit. The reduced sensitivity of commercial diagnostic assays is particularly problematic in regions where multiple HIV-1 non-B subtypes and recombinants are predominant, as in Nigeria (Horal et al., 1991; Peeters et al., 2003). Thus there is a need for continuous monitoring of the performance of diagnostic assays, especially with a view to incorporating the structures of new subtypes/variants in the antigenic base. Although the indeterminate results may be due to the presence of other related, but different, retroviruses in the area, amplification of the pol gene fragment of HIV from three of the four indeterminate samples confirmed the presence of the virus in some of the blood units.

The inability to amplify HIV cDNA from three (50%) of the antigen-positive samples may be due to primer mismatch caused by variation in the sequence of the virus. Apart from the cost and lack of facilities, sequence diversity of HIV has been a constant challenge for the application of PCR in African countries, where multiple genetic types and subtypes co-circulate (Fonjungo et al., 2000; Peeters et al., 2003). Nevertheless, PCR is a potentially valuable tool to diagnose HIV in donated blood and thus reduce the risk of transmission through blood transfusion.

It is also worthy of note that the blood banks where samples were collected for this study are in an urban setting where facilities and manpower can be considered close to adequate. The role of blood transfusion in semi-urban and rural communities, where there are little or no facilities available for pre-transfusion HIV screening, is likely to be much more significant. Thus it is necessary to put in place centralized blood-banking systems by state or region, where facilities for more sensitive assays for detection of HIV antibody/antigen in blood units for transfusion can be provided and to ensure proper monitoring through links or networking with HIV reference laboratories in Nigeria as well as other African countries.

## Authors contributions

GNO and DOO designed the study protocol, carried out antigen detection and molecular analysis and interpreted the data; AT and YAA carried out sample collection and screening of blood units at the respective blood banks; GNO drafted the manuscript and DOO revised it. All authors read and approved the final manuscript. DOO is guarantor of the paper.

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**Conflicts of interest:** None declared.

**Ethical approval:** University of Ibadan/University College Hospital Ethical Review Committee, Ibadan, Nigeria.

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