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## Prevalence of HIV P24 Antigen; A Sensitive Marker among Seronegative Antibody Blood Donors in Some Hospitals Within Kaduna Metropolis, Nigeria

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### Abstract

*Detecting and monitoring HIV infection is crucial for effective disease management. The p24 antigen serves as an early viral marker in HIV diagnosis. Despite advancements in diagnosis and monitoring, there is a need for a comprehensive assessment of p24 antigen prevalence in HIV cases. Therefore, this study aims to ascertain the prevalence of p24 antigen among a diverse population of blood donors in Kaduna metropolis. The study recruited 261 blood donors aged between 18 and 55 from various blood bank units in the metropolis. Initial HIV status determination utilized the Immune-Chromatographic Determine® HIV rapid test kit, followed by re-screening with UnigoldRecombigen HIV 1 and 2, boasting 99.70% specificity and 100% sensitivity. HIGHTOP (HIV) ELISA test kit was employed to screen HIV antibody-negative blood donors for HIV p24 antigen. Additionally, demographic factors like gender, residency, age, and marital status were taken into account. Results indicated p24 antigen detection in 9 out of 261 blood samples, yielding a prevalence of 3.5%. Conversely, 252 samples tested negative for the antigen, resulting in a prevalence of 96.5%. The study's findings support the assertion that HIV p24 antigen serves as a sensitive marker, advocating for its integration into routine blood donor screening within Kaduna metropolis to enhance HIV infection detection rates.*

**Keywords:** Blood donor, Human Immunodeficiency virus (HIV), HIV p24 antigen, antibody, Immune-Chromatographic Determine® HIV rapid test kit, HIGHTOP (HIV) ELISA

### INTRODUCTION

The Human Immunodeficiency Virus (HIV) continues to pose a significant global health threat (UNAID, 2020). It targets immune cells, particularly T cell lymphocytes and other white blood cells with CD4 receptors on their surfaces. Monocytes can also be infected by HIV, which enters these cells by binding with the CD4 receptor (Kou et al., 2009; Carvahó et al., 2018). This leads to the deterioration of CD4+ T-cells, crucial components of the immune system, ultimately weakening the body's ability to combat infections (Kou et al., 2009).

While HIV can be transmitted through bodily fluids like breast milk, semen, and vaginal secretions (WHO, 2020), the primary modes of transmission are sexual intercourse and blood transfusion (Moukoko et al., 2014). Blood poses a higher risk of transmission due to its elevated HIV viral load compared to other transmission routes (Donegan et al., 1990). Unsafe blood screening practices have been reported in various countries, especially in sub-Saharan Africa, where unsafe blood transfusion accounts for 5-10% of HIV infections (Yooda et al., 2019).

In Nigeria, limited national blood transfusion services, inadequate infrastructure, untrained personnel, and financial constraints contribute to significant concerns regarding blood safety (Adekunle et al., 2016).

Efforts to detect HIV infection have seen considerable progress. Early onset infection, known as Acute HIV infection, can be identified using HIV nucleic acids or the P24 antigen, potentially reducing the risk of transmitting the virus during the window period. While HIV RNA PCR is the most effective method for identifying acute infections in antibody-negative individuals, it is expensive and often unavailable in resource-constrained settings (Patel et al., 2010). In Nigeria, HIV testing in blood donors predominantly employs rapid antibody detection kits (3rd generation tests), which are cost-effective and easy to administer but may not detect individuals in the early stages of infection (Japhet et al., 2016). The p24 antigen, a protein covering the viral capsid, can be detected before HIV antibodies during acute infection, especially after the initial burst of virus replication.

This is associated with high levels of viremia, making the person highly infectious but potentially antibody negative (Lefrère *et al.*, 2011). Even in individuals with suppressed viremia, p24 antigen has been detected, showing an inverse relationship with CD4+ T helper cell concentrations and a direct correlation with activated CD8+ T cytotoxic cell subsets (Gao *et al.*, 2014). HIV-negative blood, screened using antibody detection alone, cannot guarantee freedom from HIV infection (Weber, 2006). This study aims to detect the p24 core antigen in blood donors previously screened as HIV-negative by antibody-based tests.

## MATERIALS AND METHODS

### Study area

The research was conducted at Barau Dikko Teaching Hospital, Yusuf Dantsoho Memorial Hospital, and Kawo General Hospital, all located within Kaduna Metropolis, Nigeria. The rapid HIV diagnostic tests were performed in the Medical Microbiology Laboratory of Kaduna State University.

### Ethical approval

Ethical approval for the study was obtained from the Ministry of health Kaduna State (Reference number: MOH/ADM/744/VOL.1/1124: NHMREC/17/03/2018).

### Study population

The study's population comprised individuals donating blood at the blood bank units of Barau Dikko Teaching Hospital, Yusuf Dantsoho Memorial Hospital, and the Laboratory of Kawo General Hospital in Kaduna. This encompassed voluntary, family replacement, and paid donors, totaling 261 participants, of which 223 were male (85.4%) and 38 were female (14.6%), within the age range specified. All participants provided their consent prior to inclusion in the study.

Inclusion criteria encompassed both male and female blood donors aged 18 to 55, weighing a minimum of  $\geq 50$ kg, without known medical conditions, and meeting specified hemoglobin levels (12.5mg/dl for males and 12.0mg/dl for females). Additionally, individuals not currently on any medication at the blood donation centers were included. Excluded were individuals requiring immediate rehydration during or after donation, those with recent surgeries or blood transfusions within the last 6 months, as well as females who were menstruating, breastfeeding, or pregnant.

The sample size was calculated using Cochran's formula for cross-sectional description study

(Cochran *et al.*, 1977) with a previous prevalence rate of 5.9 by Japhet *et al.* (2016).

$$N = \frac{Z^2 pq}{d^2}$$

Where N = Number of samples

$$Z = 95\% \text{ Confidence Interval (CI)} = 1.96$$

P= Percentage of existing prevalence = 5.9% (0.059). (Japhet, *et al.*, 2016)

$$Q = (1-P) = 1-0.059$$

$$D = \text{error margin } 5\% (0.05) = 0.0025$$

Inputting the figures into the sample size formula,

$$N = \frac{(1.96)^2 * (0.059) * (1-0.059)}{(0.05)^2} = 85.32$$

However, 261 subjects were recruited for the study. 261 subjects were recruited contrary to the calculated value because the research is a prevalence study which requires higher sample size to achieve higher prevalence possibility. Furthermore, the Elisa kit contains 96 samples, 6 of which are control samples, leaving only 90 samples left for the P24 antigen test. As 3 Elisa test kits were used, the total number of samples used was 270, out of which 9 were invalid.

### Methodology

As per the manufacturer's instructions, blood donors initially screened negative for HIV antibody using the Determine test kit were subsequently screened for HIV p24 antigen using HIGHTOP (HIV) ELISA. In summary, negative controls (R3), HIV-1 Ab positive controls (R4), and HIV Ag positive controls (R5) were placed in their respective wells. To each well, 125 microliters of conjugate 1 were added, followed by 75 microliters each of the specimen, HIV Ag positive control, and HIV Ab positive control. The mixture was thoroughly mixed.

After an hour of incubation at 37°C with the plate sealed tightly with provided adhesive film, the contents of all wells were aspirated, and the plate was washed five times with washing solution, soaking for approximately 30 seconds each time. The plate was resealed, and a second incubation of 30 minutes at room temperature was conducted after adding 100 microliters of conjugate 2 into each well. Following the same washing procedure, 80 microliters of the prepared substrate solution were swiftly added into each well.

The reaction was allowed to develop for 30 minutes at room temperature in the dark without using adhesive film. Subsequently, 100 microliters of stopping solution were added.

Within 30 minutes of stopping the reaction, the pink color of the substrate either disappeared (indicating negative samples) or changed (indicating positive samples) from blue to yellow. The data were read using a 450 nm filter microplate reader, and the values were accurately computed. The HIGHTOP (HIV) ELISA, a 4th generation kit, detects both HIV antibodies and the p24 antigen.

**STATISTICAL ANALYSIS**

The data analysis was conducted using the SPSS 20 software package. Cross tabulations were employed to explore statistical associations between variables in the study results, and the chi-square test was utilized to assess proportional relationships between groups, with a significance threshold set at  $p < 0.05$  (95% confidence interval).

**RESULTS**

A total of 261 blood donors participated in this study across various hospitals in Kaduna metropolis, Kaduna. All 261 blood samples tested negative for HIV using both the Determine test kit and Unigold Recombigen test kit. However, HIV p24 antigen was detected in 9 out of the 261 blood samples, with the remaining 252 samples testing negative for the antigen. This results in a total prevalence of 3.5% for positive markers and 96.5% for negative markers. Figure 1 illustrates the comparison of HIV reactivity using the Determine test kit and HIGHTOP (HIV) ELISA TEST KIT, while Table 1 provides an overview of the socio-demographic characteristics of the blood donors involved in this research.

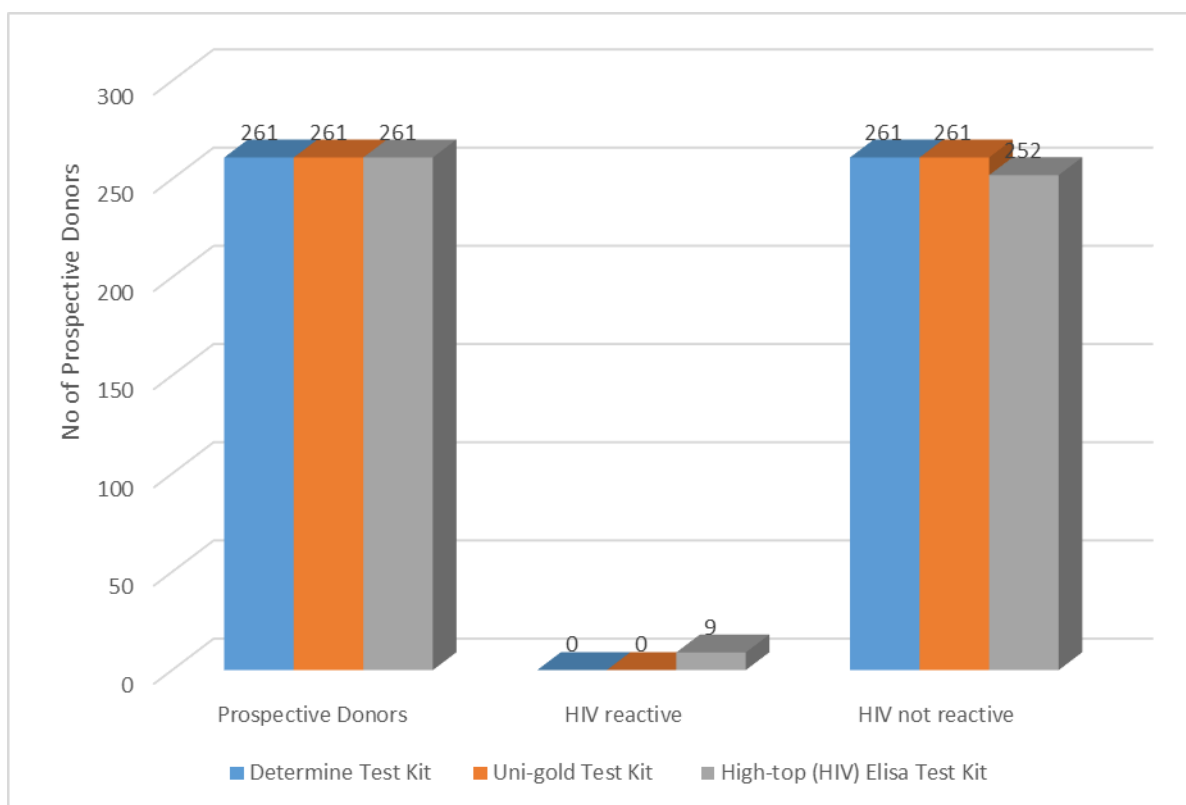


Figure 1.0: Prevalence of HIV p24 antigen using Determine test kit, Unigold test kit and High-top (HIV) Elisa test kit.

**Prevalence of P24 positivity among blood donors by gender**

The study included 223 males and 38 females. Among males, 6 tested positive for the p24 antigen, accounting for 2.3% of the male participants. Similarly, 3 females tested

positive for the antigen, constituting 1.2% of the female participants. Additionally, the chi-square test indicated no significant relationship between HIV prevalence and gender ( $\chi^2=0.441$ ,  $p>0.05$ ), as shown in Table 1.

**Table 1: Prevalence of P24 positivity among blood donors by gender**

Demographic factor	Categories	Number tested (%)	HIV positive (%)	x <sup>2</sup>	Alpha value
Gender	Male	223(85.4)	6(2.3)	0.441	0.623
	Female	38(14.6)	3(1.2)		
Total		261(100)	9(3.5)		

Note: \*p < 0.005 = Significant; \*p > 0.05 = Not significant

**Prevalence of P24 positivity among blood donors by age**

The majority of blood donors tested fell within the 26-35-year age group (40.9%), surpassing other age categories. Furthermore, the study revealed that HIV prevalence was highest among individuals aged 26-35 years (1.9%),

followed by those aged 36-45 years (0.8%). Conversely, the age groups 18-25 years and 46-55 years both demonstrated the lowest HIV prevalence at 0.4%. The results from the chi-square test indicated no significant relationship between age group and HIV prevalence (x<sup>2</sup>=1.342, p>0.05), as detailed in [Table 2](#).

**Table 2: Prevalence of P24 positivity among blood donors by age**

Demographic factor	Categories	Number tested (%)	HIV positive (%)	x <sup>2</sup>	Alpha value
Age group	18-25 years	64(24.5)	1(0.4)	1.342	0.722
	26-35 years	107(40.9)	5(1.9)		
	36-45 years	65(24.9)	2(0.8)		
	46-55 years	25(9.7)	1(0.4)		
Total		261(100)	9(3.5)		

Note: \*p < 0.005 = Significant; \*p > 0.05 = Not significant

**4.4 Prevalence of P24 positivity among blood donors by marital status**

Out of the 261 blood donors examined, 117 (44.8%) were single, 111 (42.5%) were married, 10 (3.8%) were divorced, 4 (1.5%) were separated, and 19 (7.4%) were widowed. Among these groups, single donors had the highest HIV prevalence both in terms of absolute number (5 cases) and percentage (1.9%), followed by

married individuals with 3 cases (1.1%), and widowed individuals with 1 case (0.5%). Conversely, both divorced and separated individuals showed no HIV prevalence. Furthermore, the chi-square analysis presented in [Table 3](#) demonstrated no significant relationship between marital status and HIV prevalence (x<sup>2</sup>=1.571, p>0.05), as outlined in [Table 3](#).

**Table 3: Prevalence of P24 positivity among blood donors by marital status**

Demographic factor	Categories	Number tested (%)	HIV positive (%)	x <sup>2</sup>	Alpha value
Marital Status					
Single		117(44.8)	5(1.9)	1.571	0.815
Married		111(42.5)	3(1.2)		
Divorced		10(3.8)	0(0)		
Separated		4(1.5)	0(0)		
Widowed		19(7.4)	1(0.4)		
Total		261(100)	9(3.5)		

Note: \*p < 0.005 = Significant; \*p > 0.05 = Not significant

**DISCUSSION**

The study revealed a 3.5% prevalence of HIV p24 antigen among seronegative prospective blood donors in Kaduna metropolis, Nigeria, detected in nine out of 261 blood samples. This prevalence rate is lower compared to earlier studies conducted by [Japhet et al. \(2016\)](#) at 5.9% and 5.8% reported by [Osaro et](#)

relatively low prevalence may be attributed to cultural practices and individual behaviors within the study population. The presence of p24 antigen indicates a potential risk of HIV transmission through blood transfusion. [al. \(2014\)](#), but aligns with the 3.3% prevalence reported by [Kwaru et al. \(2002\)](#).

The higher HIV prevalence observed among male donors may be attributed to their higher participation in blood donation activities, as supported by previous studies. Additionally, factors like lower levels of haemoglobin in females and a higher rate of adverse reactions to blood donation in women could contribute. Conditions such as anaemia, pregnancy, breastfeeding, or recent childbirth may also play a role. Religious and cultural beliefs, along with male dominance in the region, may further influence this gender bias towards HIV prevalence. The permissibility of multiple wives and concubines for men, contrasted with strict norms regarding extramarital affairs for women, could be a contributing factor. Additionally, practices associated with homosexuality and anal sex may play a role. In terms of residency, urban settlers exhibited a higher prevalence of HIV among blood donors compared to rural settlers. Risky behaviors such as tattooing, piercings, and multiple sexual encounters in urban areas could contribute to this disparity. These findings align with previous research by Adoga *et al.* (2010). However, chi-square results indicated no significant relationship between residency and HIV prevalence.

Regarding marital status, single blood donors showed a higher HIV prevalence compared to married donors. This aligns with previous studies suggesting that singles may engage in riskier sexual behaviors with multiple partners. Married individuals tend to practice greater

fidelity and have fewer sexual partners, thus reducing their risk of HIV transmission. HIV prevalence was also observed in the widowed group, which is consistent with research indicating that widows face numerous challenges that may increase their vulnerability to HIV infection. This study found no significant differences in relation to marital status.

## CONCLUSION

The findings of the study revealed a 3.5% detection rate of HIV p24 antigen, signifying a potential risk of HIV infection through blood transfusion. The study underscores the critical importance of employing highly sensitive laboratory tests for HIV screening, particularly those capable of identifying HIV p24 antigen, as part of the blood screening process before certification for transfusion.

To mitigate the possibility of HIV transmission through blood transfusion, it is imperative to raise awareness among the population about the significance of voluntary blood donation. Public enlightenment programs should be actively promoted, utilizing various channels including media, traditional and religious leaders, government officials, and organized civil society. Additionally, targeted campaigns should be conducted to educate the youth about the risks of HIV infection, given that young people tend to donate blood more frequently in developing economies compared to advanced nations.

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