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## Original article

# HIV screening among prospective blood donors who are negative for conventional screening methods in referral hospitals in Kebbi State Nigeria

Mohammed Bashar Danlami <sup>\*1,2</sup>, Aisha Abdullahi <sup>2</sup>, Basiru Aliyu <sup>1</sup>, Tajudeen Yahaya <sup>3</sup>, Zaharaddin Muhammad Kalgo <sup>1</sup>, Shuaibu Bala Manga <sup>4</sup>

1- Department of Microbiology, Faculty of Science, Federal University Birnin Kebbi

2- Department of Microbiology, Faculty of Life Sciences, Kebbi State University of Science and Technology Aliero

3- Department of Biological Sciences, Faculty of Science, Federal University Birnin Kebbi

4- Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University Sokoto

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## ABSTRACT

**Background:** Blood transfusion is a known risk factor for transmitting infectious diseases, including the human immunodeficiency virus, due to false negatives and false positives in HIV rapid diagnostic test results. This study determines the percentage of false negatives and positives among prospective blood donors in two major referral hospitals in Kebbi State, Northwest Nigeria. **Method:** A total of 900 sera were collected from potential blood donors from January to July 2020 in two general hospitals in Kebbi State. All donors were screened for HIV infection using Determine HIV-1/2, Uni-Gold, and STAT-PAK. The presence of viral antigen in the donor serum was tested using the p24 diagnostic technique. Data were analyzed using SPSS version 16. Descriptive statistics were used to determine frequencies and percentages. The chi-square test compared categorical variables. **Result:** Out of 900 blood donors, 27 (3%) were reactive to determine HIV-1 and HIV-2. One-third (9) of the 27 reactives on Determine HIV 1/2 were non-reactives on Uni-Gold. The nine (1%) non-reactive on Uni-Gold were also non-reactive on STAT-PAK and p24 viral antigens. The 873 (97%) samples that were non-reactive on Determine HIV 1/2, Uni-Gold, and STAT-PAK were non-reactive on HIV p24 antigens. The result showed that one-third of the blood donors who were not positive on determination were false positives, as confirmed on Uni-Gold, STAT-PAK, and the Ultra HIV Ag–Ab p24 antigen ELISA. **Conclusion:** The results suggest that using a lone rapid technique for HIV diagnosis is not recommended. Hence, a double-blind strategy should reduce HIV endemicity and optimize blood safety.

## Introduction

The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS), a blood-borne viral infection. The virus is

spread by direct contact with infected blood or other bodily fluids contaminated with the infected virus [1]. Human HIV infection induces gradual immune system damage [2,3]. In untreated individuals, the

illness has a near-100 percent death rate [3]. In the absence of effective treatment intervention, infection prevention is the principal method for HIV/AIDS control [3,4].

Blood transfusion is a known risk factor for infectious disease transmission, including HIV [5]. The prevalence of seroconversion following transfusion of HIV-infected blood is greater than 90% in HIV infection [6,7]. The danger of getting HIV via a blood transfusion was practically removed in industrialized nations with the advent of the HIV antibody diagnostic test [5]. The danger was further decreased by developing susceptible point-of-care diagnostic tests [3,8].

HIV/AIDS control depends on an accurate laboratory HIV infection diagnosis [9,10]. Rapid HIV-1/2 antibody-based point-of-care (POC) tests are evolving and making HIV testing feasible in the developing world [10]. Despite this, screening techniques can produce false-negative results for acute HIV infection and false-positive results for HIV vaccine recipients who are not sick and newborns who have passive maternal antibodies produced by mothers who are HIV-positive [11, 12]. To control the infection, it is crucial to apply the molecular approach in addition to the rapid HIV-1/2 antibody-based tests among potential blood donors [13, 14].

In Nigeria, the demand for blood and blood products is high due to road traffic accidents, surgical blood loss, and anaemia [1]. Despite the demands, blood transfusion programmes use a single antibody-based test to screen donor blood for HIV infection. To prevent the transfusion of contaminated blood and to limit the spread of HIV/AIDS in the community, screening techniques for blood donors must be continuously reviewed and evaluated. The objectives of this study were to assess and determine the percentage of false negatives and positives among prospective blood donors in two major referral hospitals in Kebbi State, northwest Nigeria.

## Materials and methods

### Study design

The present study included 900 prospective blood donors, males between the ages of 21 and 60 from January to July 2020 at General Hospital Yauri and Sir Yahaya Memorial Hospital Birnin Kebbi, Kebbi State. The study did not include children, pregnant women, and subjects with chronic diseases.

### Sample collection

The prospective blood donors were screened for HIV using conventional HIV rapid antibody-based test kits. The volunteers were invited into the laboratory, and five millimetres (5 ml) of the blood was aseptically collected using a sterile syringe and transferred into a clean EDTA container. The blood samples were screened for HIV infection using HIV rapid test kits Determine® HIV 1/2 strips (Alere Medical Co. Ltd., Chiba, Japan). STAT-PAK (Chembio Diagnostic Systems, NY, USA) and Uni-Gold kit (Trinity Biotech, Wicklow, Ireland) were used as a tiebreaker and for confirmation. All presumed unreactive samples from conventional HIV rapid antibody-based test kits were tested for the presence of p24 viral antigen using ULTRA HIV Ag–Ab p24 ELISA. Questionnaires were also administered to the prospective blood donors to obtain their demographic characteristics, blood donation history, HIV knowledge, awareness/risk, and practices. Ethical approval was obtained from the ethics and research committees of the Kebbi State Ministry of Health and the two general hospitals used in the study. Informed consent was obtained from the prospective blood donors.

### Rapid HIV screening using Determine® HIV 1/2 strips

The conventional HIV screening method was conducted using the Determine® HIV 1/2 strips (Alere Medical Co. Ltd., Chiba, Japan) to detect HIV type 1 (HIV-1) and (HIV-2) antibodies within 15 minutes by using 50 µl of serum or plasma, according to the manufacturers. The strip has two horizontal lines labelled "control and "patient" bars. A single red line on the strip at position C (control) indicated a reasonable control. A red line in the patient bar indicated a positive result for HIV-1 or HIV-2, whereas its absence signified a negative result.

### Confirmation test with Uni-Gold and STAT-PAK

The positive samples were subjected to a confirmatory test using the Uni-Gold kit (Trinity Biotech, Wicklow, Ireland). According to the manufacturers, a sample with a negative result after utilizing Uni-Gold was verified using a STAT-PAK (Chembio Diagnostic Systems, NY, USA). Briefly, blood is collected from the punctured finger using a pipette, and then two drops are placed into the sample port of the Uni-gold device. Subsequently, two drops of running buffer were added to the

sample port. The result was red after ten minutes. A "reactive" result indicates that the person who supplied the blood is HIV-positive, whereas a "non-reactive" result indicates that they are HIV-negative. The STAT-PAK was employed as a tiebreaker. It is a rapid point-of-care assay for detecting HIV-1 and HIV-2 antibodies in fingerstick whole blood, whole venous blood, serum or plasma. The interpretation of STAT-PAK is similar to that of Uni-Gold.

#### **ULTRA HIV Ag–Ab p24 antigen ELISA**

The unreactive samples from conventional antibody-based HIV rapid test kits (Determine® HIV 1/2, Uni-Gold, and STAT-PAK) were tested for p24 antigens. The samples were screened for the presence of p24 viral antigen using an ELISA kit (Genscreen Ultra HIV Ag–Ab), and the procedure was followed according to the test kit. Greenscreen Ultra HIV Ag–Ab is a qualitative enzyme immunoassay kit for detecting HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2. Briefly, three wells were marked: negative control, positive control, and blank. 20 µl of biotin conjugate was added into each well except the blank one, and 100 µl of each positive and negative control was added into the marked wells. 100 µl of the sample was added to the well-marked specimen wells, leaving the blank empty. The plates were washed five times with a diluted wash buffer after being incubated at 37 °C for 1 hour. Following that, each well except the blank received 100 µl of horse reddish peroxidase (HRP) conjugate. After another round of washing, the plate was covered and incubated for 30 minutes at 37°C. Each well received a 50 µl volume of chromogen solutions A and B. The plates were read with a plate reader. The results were calibrated with the blank at 450 nm absorbance for 10 minutes after stopping the reaction with 50 µl of stop solution.

#### **Statistical analysis**

Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to determine frequencies and

percentages. The study used the chi-square test to compare categorical variables. Spearman's rank correlation coefficient investigated the significance of the correlation between variables. All tests were at the 5% level of statistical significance.

#### **Result**

Overall, 900 out of 1,239 individuals were tested for HIV between January 2020 and July 2020 (**Figure 1**). The highest frequency of volunteers is within the age group of 31–35 years (24.4%), followed by individuals within the age group of 26–30 years (23%), while the least was aged 46 years and above (8%) (**Table 1**). Of the 900 blood donors, 27 (3%) were reactive to Determine HIV-1 and HIV-2. Of the 27 that tested positive for Determine HIV 1/2, two-thirds were reactive on Uni-Gold, while nine were non-reactive and reported negative (**Table 2**). The nine (1%) non-reactive on Uni-Gold were also non-reactive on STAT-PAK. All nine non-reactive samples on Uni-Gold and STAT-PAK were also not reactive using the p24 viral antigen. In addition, 873 (97%) samples that were non-reactive on Determine HIV 1/2, Uni-Gold, and STAT-PAK were non-reactive on HIV p24 antigens.

From the knowledge of respondents concerning HIV/AIDS, most of the respondents (72%) have enough awareness about HIV/AIDS and its transmission routes and believe that HIV/AIDS is not curable. Most of the respondents (73%) were not at risk of HIV infection. In addition, only 27 percent of the respondents are at high risk of HIV infection. The result showed that most of the study respondents (61%) observed good practices regarding HIV infection (**Table 3**). The bivariate correlations between respondents' knowledge and educational level and their practices and risk of HIV/AIDS show that respondents with moderate educational levels and knowledge were likely to have positive practices regarding HIV/AIDS and a lower risk of infection (**Table 4**).

**Table 1.** Demographic characteristics of the respondent's blood donors ( $n = 900$ ).

Variables	Category	Frequency ( <i>f</i> )	Percent (%)
Age (years)	21-25	129	14.3
	26-30	207	23.0
	31-35	219	24.4
	36-40	153	17.0
	41-45	120	13.3
	46-60	72	8.0
Education level	SSCE	159	17.7
	OND/COE	351	39.3
	HND/BSc	273	30.3
	MSc	42	4.7
	PHD	0	0
	Others	75	8.3

**Table 2.** HIV knowledge, awareness, and practices of the prospective blood donors ( $n = 900$ ).

Variables	Yes		No		I do not know	
	<i>Freq.</i>	%	<i>Freq.</i>	%	<i>Freq.</i>	%
Knowledge of HIV/AIDS	576	64	315	24	108	12
Risk of HIV/AIDS infection	243	27	558	62	117	13
Good practice regarding HIV/AIDS	549	61	333	37	18	2

**Table 3.** HIV-1/2 antibody screening based on conventional techniques and the p24 viral antigen test ( $n = 900$ ).

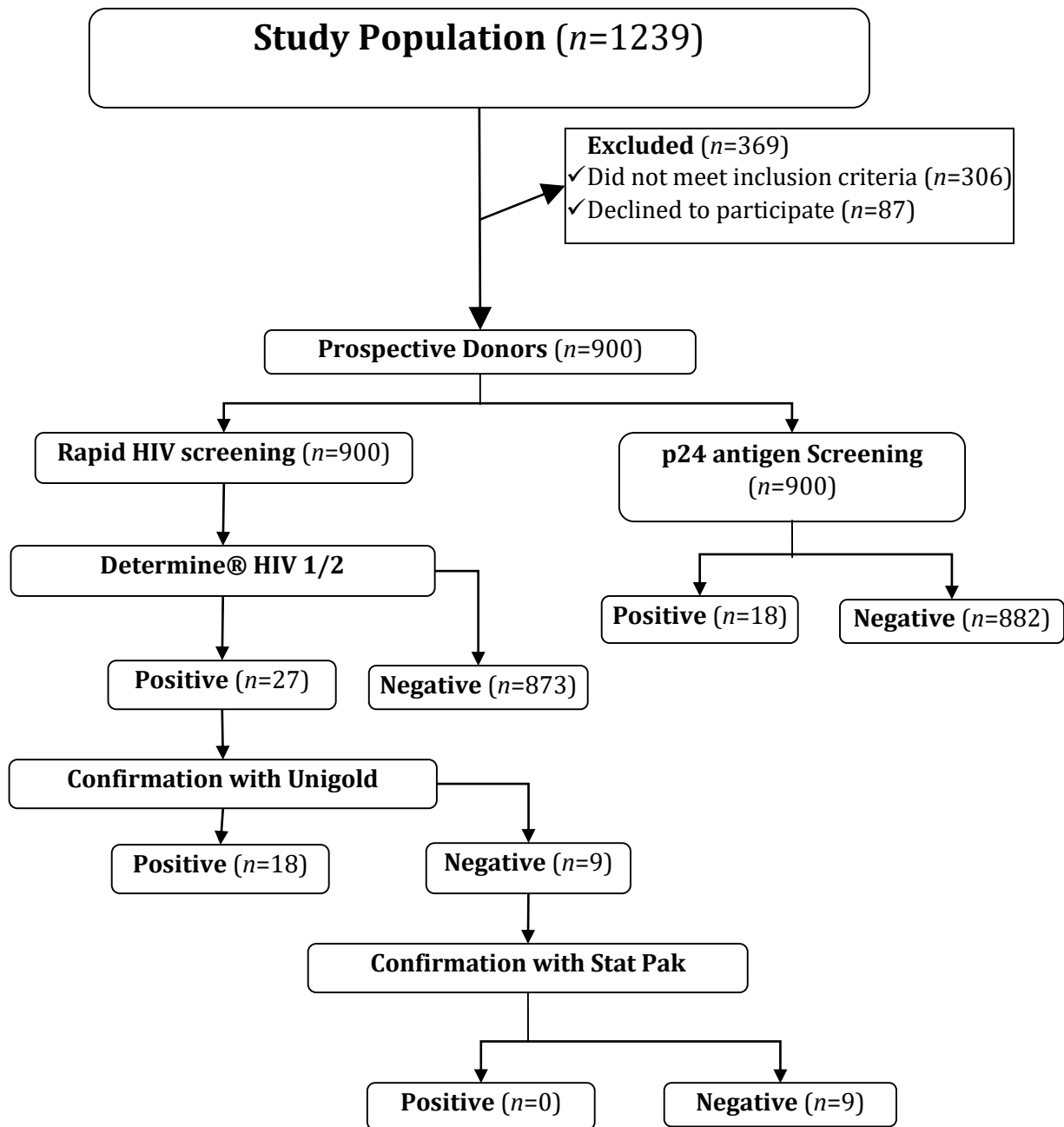
HIV status	HIV 1/2 (Determine®)		Uni-Gold™		STAT-PAK™		p24 antigen	
	<i>f</i>	(%)	<i>F</i>	(%)	<i>f</i>	(%)	<i>f</i>	(%)
Reactive	27	(3%)	18	(2%)	18	(2%)	18	(2%)
Non-Reactive	873	(97%)	873	(98%)	882	(98%)	882	(98%)

*f* = frequency; % = percent. Reactive = positive; non-reactive = negative.

**Table 4.** Associations between the respondent's knowledge, educational level, practices, and risk of HIV/AIDS.

Variable	Educational Level		Knowledge	
	<i>r</i>	<i>p</i> -value	<i>R</i>	<i>p</i> -value
Practice	0.848**	< 0.001	0.938**	< 0.001
Risk	0.737**	< 0.001	0.587**	< 0.001

\*\* The correlation is significant at the 0.01 level (2-tailed).

**Figure 1.** A flow diagram of the study design.**Discussion**

The result from the study showed that 3% of the prospective blood donors were reactive on conventional rapid HIV diagnostic tests. In contrast, only two-thirds of the donors who were reactive to the conventional antigen were reactive to the p24 viral antigen [11, 15]. The study indicates one-third of false positive results when Determine® is used alone, as confirmed by Uni-Gold and STAT-PAK. This result is significantly higher than Osun's (0.43%) result but similar to the 2.92% found among

blood donors using point-of-care diagnostics in resource-limited settings [16]. However, it was lower than the result recorded in prospective blood donors in Ile-Ife, Nigeria (5.9%) [17], and nearly a 5-fold increase among voluntary blood donors in Namibia [7]. The frequency of reactive antibodies among prospective blood donors in this study has nearly corresponded to the results reported by the WHO in HIV-endemic populations [18]. The findings from this study show the potential for false positive HIV test results in the subset of individuals who tested positive on Determine but negative on

Uni-Gold and STAT-PAK [11, 12, 19]. The false positive on Determine® indicates the possibility of cross-reacting antibodies such as HIV [19, 20]. These results showed the weakness of conventional rapid HIV test results compared with p24 antigen ELISA diagnostic test results in blood screening and surveillance testing [21, 22].

Previously, a cohort study on known HIV-positive patients tested with Determine® showed 100.0% sensitivity, following the manufacturer's claim. However, the current one was a study that focused on the screening for HIV among prospective blood donors. Hence, the Determine® technique's sensitivity was obtained based on a few positive cases confirmed by the p24 antigen test [15, 22]. The conventional screening technique with Uni-Gold confirmed only eighteen seropositive donors (i.e., 2% HIV seroprevalence). The inclusion of Stat-Pak as a tiebreaker kit resolved the discrepancy, as it confirmed 2% HIV seroprevalence [5, 15, 23]. The results suggest that a single HIV rapid diagnostic technique is not recommended in a high seroprevalence population because of the possibility of false positive and negative results [5, 6, 14, 15, 23]. The single HIV antibody test has the advantages of simplicity and cost-effectiveness for confirming infection. However, it falls short due to the risk of transfusing an antibody-negative donor unit during the window period of HIV infection [1, 16]. The absence of HIV infection among the prospective blood donors using the WHO-recommended two-test diagnostic technique (the conventional rapid test and the p24 antigen ELISA) raised confidence in the specificity of the blood before transfusion. Misdiagnosis of HIV can cause mental illness and public health repercussions and should thus be avoided to the extent possible [9, 10, 24].

Knowledge, practices, and risk studies are valuable tools deployed to assess the extent to which individuals adopt risk-free behaviours. The study found that the prospective blood donors were young, ranging in age from 26 to 35 years old [16, 26]. These findings are in line with previous results, where the most voluntary blood donors are youth in the age group of 18–32 years. This group has been discovered to be low-risk and more willing to donate blood in Nigeria and Africa [26, 27]. Overall, the knowledge, risk, and practice levels of respondents regarding HIV/AIDS were found to be positive. The findings agreed with a recent report by **Dzah et al.** among senior high school pupils [25], who observed

that >60% of their study respondents had good knowledge of HIV/AIDS [25, 26]. Overall, the findings in the study confirm the level of exposure of blood donors to campaigns of awareness on HIV/AIDS. Knowledge and awareness have contributed to the preference of donors and patients to receive HIV-tested banked blood from their relatives for transfusion [3, 8, 9]. Moreover, the literacy level of the respondents could explain the findings of this current study, thereby suggesting a direct relationship between literacy level and the risk associated with blood donation in the high HIV seroprevalence population [27, 28]. That established reasons for a more rigorous approach to the system's effectiveness in educating and selecting prospective blood donors [22, 26].

In conclusion, conventional (serological) HIV rapid screening was conducted using Determine®, and confirmation was carried out using Unigold and Stat-Pak. The findings revealed that one-third of the blood donors who were not positive for Determine® were false negatives, as confirmed on Uni-Gold, STAT-PAK, and further evaluation using the Ultra HIV Ag–Ab p24 antigen ELISA. The study shows no statistically significant difference among the blood donors screened using the conventional and molecular ULTRA HIV Ag–Ab p24 antigen ELISA techniques. However, using a single rapid technique to screen for HIV among blood donors is not recommended due to the possibility of false negative results. Hence, a double-blind strategy should be adopted to reduce HIV endemicity and optimize blood safety.

#### **Competing interests**

The authors declare that they have no competing interests.

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#### **References**

- 1-**Olusola BA, Olaleye DO, Odaibo GN.** New infections and HIV-1 subtypes among febrile persons and blood donors in Oyo State, Nigeria. *J Med Virol* 2021;93(8):4891-900.
- 2-**Boasso A, Shearer GM, Chougnnet C.** Immune dysregulation in human immunodeficiency

- virus infection: know it, fix it, prevent it? *J Intern Med* 2009;265(1):78-96.
- 3-**Custer B, Quiner C, Haaland R, Martin A, Stone M, Reik R, et al.** HIV antiretroviral therapy and prevention use in US blood donors: a new blood safety concern. *Blood* 2020;136(11):1351-8.
- 4-**Adimora DE, Ogba FN, Omeje MO, Amaeze FE, Adene FM.** Social support as a correlate of depression among people living with HIV and AIDS in Nigeria. *Afr Health Sci* 2021;21(3):1016-26.
- 5-**Tagny CT, Bissim M, Djeumen R, Ngo Sack F, Angandji P, Ndoumba A, et al.** The use of the Geenius(TM) HIV-1/2 Rapid confirmatory test for the enrolment of patients and blood donors in the WHO Universal Test and Treat Strategy in Cameroon, Africa. *Vox Sang* 2020;115(8):686-94.
- 6-**Guerrero-Garcia JJ, Zuniga-Magana AG, Barrera-De Leon JC, Magana-Duarte R, Ortuno-Sahagun D.** Retrospective Study of the Seroprevalence of HIV, HCV, and HBV in Blood Donors at a Blood Bank of Western Mexico. *Pathogens* 2021;10(7):878.
- 7-**Mavenyengwa RT, Mukesi M, Chipare I, Shoombe E.** Prevalence of human immunodeficiency virus, syphilis, hepatitis B and C in blood donations in Namibia. *BMC Public Health* 2014;14:424.
- 8-**Slev P.** The status of laboratory testing for the diagnosis of HIV infection. *MLO Med Lab Obs* 2016;48(9):34, 6.
- 9-**Martin D, Sim J.** The laboratory diagnosis of HIV infection. *S Afr Med J* 2000;90(2):105-9.
- 10-**Vermeulen M, van Schalkwyk C, Jacobs G, van den Berg K, Stone M, Bakkour S, et al.** The Impact of Early Antiretroviral Treatment (ART) for HIV on the Sensitivity of the Latest Generation of Blood Screening and Point of Care Assays. *Viruses* 2022;14(7):1426.
- 11-**Schupbach J, Gebhardt MD, Tomasik Z, Niederhauser C, Yerly S, Burgisser P, et al.** Assessment of recent HIV-1 infection by a line immunoassay for HIV-1/2 confirmation. *PLoS Med* 2007;4(12):e343.
- 12-**Klarkowski D, O'Brien DP, Shanks L, Singh KP.** Causes of false-positive HIV rapid diagnostic test results. *Expert Rev Anti Infect Ther* 2014;12(1):49-62.
- 13-**Menitove JE, Richards WA, Bauer P.** False-positive anti-HIV tests and blood donation. *Lancet* 1987;2(8569):1213.
- 14-**Levi JE, Wendel S, Takaoka DT, Silva IC, Castro JP, Torezan-Filho MA, et al.** Replacement of HIV p24 Ag test by a multiplex RT-PCR method for primary screening of blood donors. *Rev Inst Med Trop Sao Paulo* 2007;49(3):171-6.
- 15-**Gendler SA, Pascuccio MS.** Routine HIV screening among blood donors in Buenos Aires (Argentina): results from six years' experience and report of a single window-period donation. *Enferm Infecc Microbiol Clin* 2007;25(2):82-90.
- 16-**Glynn SA, Smith JW, Schreiber GB, Kleinman SH, Nass CC, Bethel J, et al.** Repeat whole-blood and plateletpheresis donors:unreported deferrable risks, reactive screening tests, and response to incentive programs. *Transfusion* 2001;41(6):736-43.
- 17-**Heidt B, Siqueira WF, Eersels K, Dilien H, van Grinsven B, Fujiwara RT, et al.** Point of Care Diagnostics in Resource-Limited Settings: A Review of the Present and Future of PoC in Its Most Needed Environment. *Biosensors (Basel)* 2020;10(10):133.
- 18-**Buseri FI, Muhibi MA, Jeremiah ZA.** Sero-epidemiology of transfusion-transmissible

- infectious diseases among blood donors in Osogbo, south-west Nigeria. *Blood Transfus.* 2009;7(4):293-9.
- 19-Faryar KA, Ancona RM, Luketic KR, Braun RS, Lyons MS. Missed HIV diagnoses when screening only emergency department patients who have blood samples obtained for other clinical purposes. *Am J Emerg Med* 2021;50:102-5.
- 20-Salgado CD, Flanagan HL, Haverstick DM, Farr BM. Low rate of false-positive results with use of a rapid HIV test. *Infect Control Hosp Epidemiol* 2002;23(6):335-7.
- 21-Langedijk JP, Vos WF, van Doornum GJ, Rotman HA, Meloen RH, Huisman JG. Identification of cross-reactive epitopes recognized by HIV-1 false-positive sera. *AIDS* 1992;6(12):1547-8.
- 22-Orkuma JA, Egesie JO, Banwat EB, Ejele AO, Orkuma JH, Bako IA. HIV screening in blood donors: rapid diagnostic test versus enhanced ELISA. *Niger J Med* 2014;23(3):192-200.
- 23-Acar A, Kemahli S, Altunay H, Kosan E, Oncul O, Gorenek L, et al. The significance of repeat testing in Turkish blood donors screened with HBV, HCV and HIV immunoassays and the importance of S/CO ratios in the interpretation of HCV/HIV screening test results and as a determinant for further confirmatory testing. *Transfus Med* 2010;20(3):152-9.
- 24-Sacks R, Omodele-Lucien A, Whitbread N, Muir D, Smith A. Rapid HIV testing using Determine HIV 1/2 antibody tests: is there a difference between the visual appearance of true- and false-positive tests? *Int J STD AIDS* 2012;23(9):644-6.
- 25-Stoyanov E, Gozlan Y, Wax M, Levin E, Shvartz L, Shinar E, et al. HIV-1/2 screening in blood centers: implementing a two-step serological screening assay approach to reduce donor deferral. *Transfusion* 2019;59(6):2054-60.
- 26-Sharma UK, Schreiber GB, Glynn SA, Nass CC, Higgins MJ, Tu Y, et al. Knowledge of HIV/AIDS transmission and screening in United States blood donors. *Transfusion.* 2001;41(11):1341-50.
- 27-Dzah SM, Tarkang EE, Lutala PM. Knowledge, attitudes and practices regarding HIV/AIDS among senior high school students in Sekondi-Takoradi metropolis, Ghana. *Afr J Prim Health Care Fam Med* 2019;11(1):e1-e11.
- 28-Adejumo OA, Adebayo BI, Adesola S, Bowale A, Adejumo EN, Atewe S, et al. Factors associated with risky sexual behaviour among clients undertaking HIV testing and counselling services at a secondary referral hospital Lagos, Nigeria. *Afr Health Sci* 2022;22(1):51-61.
- 29-Umeobieri AK, Gyang HJ, Aniwada EC. Prevalence and Factors Associated with HIV Sero-Discordance among In-Union HIV Patients Receiving Care in a Private Health Facility in Jos, North Central, Nigeria. *West Afr J Med* 2022;39(4):415-24.
- 30-Danlami MB, Aliyu B, Samuel G. Incidence of Rifampicin-Resistance Presumptive M. Tuberculosis Cases among Outpatients in Kebbi State, Nigeria. *Afr J Infect Dis* 2021;15(1):47-52.