



ABH Substances Secretion Status, ABO & Rhesus Blood Groups Typing and P24 Antigen Screening in HIV 1 and 2 Screened Antibody-negative Apparently Healthy Prospective Blood Donors in Calabar, Nigeria

Forwah Jacques Ndeh^{1*}, Enosakhare A. Asemota², Dorathy Chioma Okpokam² and Immaculate I. Ekeagba³

¹Chemical Pathology Unit, Department of Medical Lab Science, Faculty of Allied Medical Sciences, University of Calabar, Nigeria.

²Haematology and Blood Transfusion Unit, Department of Medical Lab Science, Faculty of Allied Medical Sciences, University of Calabar, Nigeria.

³WORCACCE Group Integrated Healthcare Services, Training and Research Foundation (WGIHSTARF), Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author FJN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAA, DCO and FJN managed the analyses of the study. Authors FJN and IIE managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):

(1) Dr. Dharmesh Chandra Sharma, G. R. Medical College & J. A. Hospital, India.

Reviewers:

(1) J. Lissa, JSS Academy of Higher Education and Research, India.

(2) Marin Abraham, Rajiv Gandhi University of Health Sciences, India.

(3) El Amrani Souad, Université Hassan II, Morocco.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/60481>

Original Research Article

**Received 14 July 2020
Accepted 18 September 2020
Published 02 November 2020**

ABSTRACT

Aim: To investigate ABH Secretion status, ABO/ Rhesus blood groups and P24 antigen in 400 HIV screened antibody-negative apparently healthy prospective blood donors aged above 20 years recruited within Calabar, Nigeria.

*Corresponding author: E-mail: ndehforwah@gmail.com;

Study Design: experimental, work carried out in the Haematology & Blood Transfusion Unit, Department of Medical Lab Science, University of Calabar Nigeria between December 2009 and December 2010.

Methods and Materials: About 5ml of blood and 3ml of saliva samples were collected by standard procedures from 400 consented selected subjects prior to selection and sera were serologically screened for HIV 1 & 2 antibodies using Stat Pak, Determine and Unigold test Kits, while HIV 1 & 2 antigens were screened using HIV1 and 2 P24 antigens Combi test Kit. Saliva samples analysed for ABH secretor status using BIOTECH Anti-H reagent. ABO/Rhesus blood groups typing were done using blood grouping Anti-sera (A, B, AB, & D) reagents and known red blood cells.

Results: Only 10 samples (02.5 %) reacted positively to the HIV antibody screening panel with two samples (0.2%) out of the 10 samples reacted positive to HIV P24 antigens screening kit. About 11 samples (2.82%) from the tested HIV antibody-negative samples reacted positively to HIV P24 antigens screening kit with 13 positive samples (3.25%). 317 samples (79.25%) were ABH Substance secretors and 83 samples (20.75%) were non-secretors. 4 samples (1%) that were HIV P24 positive were also positive for non-secretor while 9 samples (2.25%) that were positive to P24 antigen screening test were also secretor.

The order positive results of P24 antigens screening amongst ABO/Rhesus blood groups positive samples were 2(.5%) for A+, 4(1%) for B+, 7(1.75%) for O+. A significant statistical difference exists between HIV Antibody and HIV P24 antigen tests ($P < 0.5$). Chi-Square X^2 test shows positive relationship between HIV P24 antigen screening, secretor status, ABO and Rhesus typing results ($P < 0.5$).

Conclusion: ABH Substance secretors were less susceptible to HIV 1 and 2 P24 antigens screened positive test than ABH Substance non-secretors. Blood group O+ subjects are predisposed to HIV 1 and 2 P24 antigens screened positive test than A+, B+, AB+, and Rhesus D negative subjects. The risk of infected HIV antibody-negative blood donors has been implicated with a prevalence rate of 3.3%.

Keywords: ABH, antibody; ABO Rhesus blood groups; blood transfusion; blood grouping; HIV; hematology; P24 Antigen; reagent; saliva.

ABBREVIATION

Anti-A.	Antibody A		
Anti-B	Antibody B	AIDS	Acquired Immune Deficiency Syndrome
Anti-H	Antigen H Substances	ARVs	Anti-Retroviral Drugs
Yrs	Age	BYSACA	Bayelsa State AIDS Control Agency
A ⁻	Blood group A Rh D Negative	CDC	Centre of Disease Control
A ⁺	Blood group A Rh D Positive	CD4	Cluster of Differentiation 4
AB ⁻	Blood group AB Rh D Negative	CHOs	Community Health Officers
AB ⁺	Blood group AB Rh D Positive	WHO	World Health Organization
ABO	Group A, B, & O Blood Group System	eMTCT	Elimination of Mother-To-Child Transmission
ANOVA	Analysis of Variance	FCT	Federal Capital Territory
		FMOH	Federal Ministry of Health
FP	Family Planning		
FHI	Family Health International	P < 0.05	P-values indicating the Level of significance
		P > 0.05	P-values indicating the Level of no significance
		P24	HIV 1 and 2 core protein
GF	Global Fund	PV	Prevalance rate
GH	General Hospital	Rh	Rhesus Blood Groups System

GHAIN	Global HIV/AIDS Initiative in Nigeria	SACA	State Agency for the Control of AIDS
HTC	HIV Testing and Counseling	Se,Se	Pair of dominant alleles for the secreting genes controlling secretors
(HREC)	Health Research Ethical Committee	Se,se	Pairs of recessive alleles for the secreting genes controlling non secretors
HIV	Human Immuno-deficiency Virus	SD	Standard Deviation of the Mean
LGA	Local Government Area	SPSS	Statistical Package for Social Science
MTCT	Mother-to-Child Transmission	SASCP	State AIDS and STD Control Programme
NACA	National Agency for Control of HIV/AIDS	SIDHAS	Strengthening Integrated Delivery of HIV/AIDS Services
NASCP	National AIDS and STD Control Programme	t	Stendent independent test
NGOs	Non-Governmental Organizations	UCTH	University Of Calabar Teaching Hospital
NPHCDA	National Primary Health Care Development Agency	UN	United Nations
NAT	Nucleic Acid-based Tests	UNAIDS	United Nations Joint Programme on HIV/AIDS
RT-PCR	Real time Polymerase Chain Reaction tests	UNICEF	United Nations Children Emergency Fund
PCR	Polymerase Chain Reaction	USAID	United States Agency for International Development
PEPFAR	President's Emergency Fund For AIDS Relief	WHO	World Health Organization
PHC	Primary health care	X²	Spearson Chi Square Statistics
PMTCT	Prevention of Mother-to-Child Transmission		

1. INTRODUCTION

1.1 Background of the Study

About 38.0 million people were living with the Human immunodeficiency virus (HIV) infection in 2019 worldwide, with adult prevalence rate of 0.8 percent and 75.7 million people have been infected with HIV since the beginning of the global epidemic [1,2]

Human immunodeficiency virus infection, first described in the 1980s in the USA has continued to spread rapidly [3]. HIV is a lentivirus (belonging to the retrovirus family) known to cause Acquired Immunodeficiency Syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections and some malignancies [4,5].

The adult prevalence rate of HIV in Nigeria was 1.4 percent for 2019 amongst adults aged

between 15-49 years [6], While Cross River State current prevalence rate is 7.1 % [7,8].

The routes of HIV transmission are unsaved sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (perinatal transmission) and transfusion of infected blood or blood products. Around 5 percent of the AIDS cases in the world are through blood or blood products [9,10,11,12].

Five to ten percent of all new HIV cases in Africa are caused by contaminated blood used in blood transfusion therapy. This translates to between 250 and 500 patients every single day [13] HIV transmission through unsaved blood is the second largest source of HIV infection in Nigeria [14].

The first attempt to correlate the human blood group systems to some types of pathology was by [15] while [16] established the first relationship between the human blood group systems and diseases. More new findings are assumed to be

showing that non-secretors are more susceptible to some diseases than secretors [17,18,19,20].

It was the work of [21] that first showed an inconsistent relationship between the incidence of blood group types and the natural defense mechanism against HIV infection.

Recently [22] showed that Pk blood group has a direct association with resistance to HIV infection. However [23] concluded this report by saying that Pk blood group variation may be another new genetic mutation and risk factor for HIV infection.

The ideology behind the existence of infected HIV antibody-negative individuals was first hypothesized by [24] and later by other researchers [24abc].

Today in most resource poor countries of the world, the risk of human immunodeficiency virus (HIV) transmission through blood and blood products from infected but HIV antibody -negative blood donors is still an issue of concern. Blood donated by these individuals remains a major avenue of transmission of HIV in that much of the blood supply is simply not pre- tested with HIV 1 AND 2 antigens screening panels [25].

This is in sharp contradiction to most developed countries of the world where the use of fourth generation HIV 1 and 2 kits such as P24 antigen screening, Nucleic acid-based tests (NAT) ,RT-PCR tests have largely reduced the risk of HIV transmission through blood transfusion especially from infected but HIV antibody-negative blood donors [26].

Infected but HIV antibody-negative blood donors are those donors that test negative to antibody tests but positive to the antigen test .There may be some percentage risk of HIV transmission from screened units donated by these donors [24,25,26,27,28,29,30].

1.2 Research Questions

- Is there any difference between the results of HIV1 and 2 Antibody tests and that of HIV 1 and 2 Antigens screening tests in these donors?
- Is there any relationship between the results of secretor status, ABO & Rh blood groups and that of P24 antigen screening test?

- Is there any reason for adapting the two methods simultaneously for routine screening of blood donors?
- Is there any considerable risk and significant contribution to onward transmission of transfusable HIV where only the HIV antibody assays algorithms are employed?
- Is there any increase in false negative results in infected HIV antibody -negative blood donors where HIV antigen assays algorithms are unaffordable?

1.3 Research Hypothesis

- There is no difference between the results of HIV Antibody tests and that of HIV Antigen tests in screening apparently healthy prospective blood donors.
- There is no relationship between the results ABH Secretor Status, ABO & Rh blood groups and the results of P24 antigen screening test.
- There is no reason for adapting the two methods simultaneously for routine screening of these donors
- There is no considerable risk and significant contribution to onward transmission of transfusable HIV.
- There is no increase in false negative results in HIV (antibody -negative) blood donors.

1.4 Justification and Rationale for the Study

- There is an urgent and great need to reduce the unusual long incubation period of the HIV infection thereby enhancing early detection of HIV infection especially in infected HIV blood donors, where the antibody screening test is always negative instead of positive.
- There is a great quest to know the antigenic interaction of HIV 1 & 2 and secretor status, ABO, & Rh blood groups in early HIV infection.
- There is an increasing rate of false negative results amongst infected HIV antibody -negative blood donors when done only with the HIV antibody screening test and undetermined or indeterminate results when done with the ELISA or Western Blot confirmatory tests due to the long HIV window period or HIV incubation period.

1.5 Aim

The aim of this research work was set to find out the prevalence rate of HIV 1 and 2 infection in apparently healthy prospective blood donors screened using HIV antibody screening kits in Calabar and the prevalence rate of HIV 1 and 2 infection in HIV antibody-negative prospective blood donors screened using P24 antigen screening kit.

1.6 Specific Objectives

- 1 .To find out the prevalence rate of HIV 1 and 2 infection in apparently healthy prospective blood donors and HIV Antibody-negative blood donors that were both positive for the two test methods.
- 2. To find out the distribution of infected HIV Antibody-negative blood donors screened using HIV P24 antigen test method and their corresponding secretor status, ABO & Rh blood groups.
- 3.To make a comparative study of the results of HIV Antibody screening test and the HIV P24 Antigen screening test techniques for routine screening of these blood donors.

1.7 Scope of Study

The scope of this study was limited to the target population in the area under study which was Calabar .The number of samples used was limited to the calculated sample size and the selected method for subjects recruitment was based on the inclusive and exclusive criteria.

2. SUBJECTS

2.1 Study Area

Calabar, where this study was carried out, is the capital of Cross River State in the south eastern part of Nigeria. Geographically Calabar has a total Surface of land area of 142 km² while the total local government area population is estimated to be 320,826 of which 166,203 are males and 154,659 females [31]. The inhabitants are mainly the Efiks, Quas, Ejagham, Efut, Ibibio, Annang and others – the migrant workers. They are mainly civil servants, subsistence farmers, traders and fishermen.

2.2 Subjects Recruitment Recruitment, Selection, Screening and Samples Collection Sites

These were Haematology/blood transfusion department of University of Calabar Teaching

Hospital Temporary Site (UCTH) and General Hospital Calabar (GHC), in Calabar, Cross River State, Nigeria.

2.3 Study Design

Experimental.

2.4 Duration of Study

The work was carried out in the Haematology & Blood Transfusion Unit, Department of Medical Lab Science, University of Calabar Nigeria between December 2009 and December 2010.

2.4.1 Calculation of sample size

The Formula of Cochran, 1977, for calculating the sample size (S) was adopted in this study and is given by [32]:

$$S = t^2 p (1-p) / e^2$$

Where t= t value (The alpha level used in determining sample size in most educational research studies is either .05 [33]. In Cochran's formula, t-value for alpha level of .05 is 1.96 for 95% confidence level for sample sizes above 120.

p= prevalence rate in percentage (%) of infected HIV antibody-negative prospective blood donors population in Calabar and in this case it is taken to be 0.5 or 50% since nobody had ever worked on this population [34,35]

While e = tolerance error or confidence interval expressed as decimal and it is taken to be 0.05. Therefore

$$S = (1.96)^2 (.5(1-0.5) / (0.05)^2$$

$$S = (1.96)^2 (0.5)^2 / (0.05)^2 = 384.16$$

S = ~ 400 subjects were used in cases of any loss data or specimen during the study.

2.4.2 Inclusive and exclusive criteria for subject selection

A total of 400 apparently healthy prospective voluntary blood donors of both genders, aged between 20 to 50 years were randomly recruited from Haematology / blood transfusion department of University of Calabar Teaching Hospital Temporary Site (UCTH) and General Hospital Calabar (GHC) in Calabar, Cross River State, Nigeria. The subjects were divided into six study groups according to their ages and genders and a questionnaire form

was used for both inclusive and exclusive criteria.

2.4.3 Administration of questionnaire

The harmless nature and advantage of the research was also explained to each donor in the form of pre-donation counselling in which a questionnaire was administered on each of the subjects to obtain more medical information about the clinical history donors. After the Pre-blood donation counselling, informed consent forms were filled and signed by these donors for screening to start. They were screened in accordance with the blood transfusion national algorithms and standard parameters set forth in these blood transfusion centres.

2.5 Treatment of Collected Blood Samples

2.5.1 Blood samples for ABO/ Rh typing

Venous blood from the selected and screened blood donors were collected from Monday to Friday for both subjects between the hours of 7.00 am and 1.00 pm continually for four months. About 5ml of blood samples were withdrawn from the ante-cubital vein of the arms of pre-counselled prospective blood donors of both genders by a means of disposable plastic 5mls syringe fitted with 21 SWG needles. The area of the venipuncture was first of all cleansed with 70 percent Alcohol and allowed to dry. A tourniquet was tied just for a short time. About 3mls out of the 5mls of blood withdrawn were dispensed into dried and labeled samples bottles to be used for serum and cell ABO & Rh blood groupings. Samples which were not analyzed immediately were stored in the refrigerator at 4- 6 OC.

2.5.2 Blood samples for HIV Antibody and antigen screening assays

The remaining 2mls of blood samples were dispensed into dried, labelled plain tubes to be centrifuged for 10 minutes at 4000 revolutions per minutes after being allowed to retract for two hours. Finally, the clear supernatants were removed from the retracted, centrifuged samples and dispensed into another cleaned, labelled dried tubes for HIV antibody and antigen screening assays.

2.5.3 Collection and treatment of saliva for determining Secretor Status (ABH substances.)

About 2mls saliva were collected from the mouth of these subjects into sterilized universal

containers before transferring into clean, dried and labelled 16 x 100mm centrifuge Pyrex tubes. Commercially bottled sterilized clean water was used to stimulate the secretions. The collected saliva samples were placed in a boiling water bath for 10minutes to inactivate enzymes that might otherwise destroy blood group substances. The saliva samples in the boiled test tubes were cooled. After cooling the saliva samples were centrifuged for about 5minutes at 4000 revolution per minute and the supernatant was collected.

3. MATERIALS AND METHODOLOGY

3.1 Materials and Methods for the Determination of ABH Secretor Status - Saliva Test

(The Method of Haemagglutination Inhibition as outlined in the Manufacturer Technical Manual was adopted).

3.1.1 Principle

Approximately 80 % of the population has the secretor (Se) gene. These people secrete water-soluble blood group substances in their saliva and other body fluids. Group A secretes A substance and a small amount of H, group B secretes B (and H) substance, group O secretes H substance only, and group AB secretes A, B, and a small amount of H. To determine if a person is a secretor, the principle of Agglutination Inhibition is utilized, where the presence of agglutination means a negative test, and no agglutination is interpreted as a positive result.

3.1.2 Part I - antibody neutralization

Saliva is mixed with commercial antiserum (Anti-A, Anti-B or Anti-H) and allowed to incubate briefly. If the patient is a secretor, the soluble blood group antigens in the saliva will react with and neutralize the antibodies in the commercial antiserum. It is necessary, however, to dilute the commercial antiserum so that its antibody titer more closely matches the antigen level in the saliva.

3.1.3 Part II - agglutination inhibition

When commercial RBC of the appropriate blood group are then added to the test mixture, there should be no free antibody to agglutinate them if the patient is a secretor, because the antibodies have already reacted with the blood group

antigens in the saliva. The reaction will be negative for agglutination, but is interpreted as positive for secretor status. If the patient is a non-secretor, there will be no blood group antigens in the saliva; the antibodies in the antiserum will not be neutralized and will be free to react when the test cells are added. Therefore, agglutination is a negative test for secretor status.

3.1.4 Interpretation

Agglutination in all of the patient TEST tubes indicates a negative result for secretor status. If any one of the patient TEST tubes is not agglutinated, this indicates a positive test for secretor status, and the tube showing the non-agglutination should indicate the ABO type

3.2 Materials & Methods for HIV 1 AND 2 Antibody Assay

3.2.1 Method for HIV 1 & 2 Antibody assay

Three different types of HIV & Antibody rapid test kit methods were used as approved by (UNSAID, 2011).

3.2.2 Determine HIV1& 2 Antibody rapid test kit

Produced by Inverness Medical Japan Co, Ltd.

3.2.3 Principles of the test

Determine HIV-1 & 2 Abs Combo is an immunochromatographic test for the qualitative detection of antibodies to HIV 1 & 2. The manufacturer's instructions were strictly followed as follows:- Specimen was added to the sample pad. The specimen mixes with a biotinylated antibody and selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized avidin, recombinant antibodies and synthetic peptides at the patient window sites. If antibodies to HIV-1 and / or HIV-2 are present in the specimen, the antibodies bind to the antigen selenium colloid and to the immobilized recombinant antigens and synthetic peptides, forming one red bar at the patient HIV Antibody window site. If antibodies to HIV-1 and / or HIV-2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the patient HIV Antibody window site.

3.2.4 Procedure of the test

All reagents and the test samples were removed from the refrigerator and allowed to assume

room temperature. One strip from the right side of the package was torn and the cover removed. Exactly 50µl of serum was added to the sample pad and followed by the addition of the buffer and was timed for 20 minutes. After 20 minutes the results were read for HIV-1 & 2 antibodies (Ab).

3.2.5 Built-in control feature

The control line appeared as a visible pink/red band in the control region of the device to indicate that the test device was functioning correctly. A positive result was visualized by a pink/red band in the test region of the device. A negative reaction occurred in the absence of detectable levels of human immunoglobulin antibodies to HIV-1 and / or HIV-2 in the specimen; consequently no visually detectable band develops in the test region of the device.

3.3 Stat-Pak HIV 1 & 2 Antibody Rapid Screening test kit

Produced by Chembio Diagnostic System Inc.

3.3.1 Principle

The Chembio HIV 1& 2 Stat-Pak assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles and HIV 1&2 antigens, which are bound to the membrane solid phase. The sample is applied to the Sample (S) well followed by the addition of a running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the Test (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the Test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the Control (C) area containing immunoglobulin G antigens. This procedural control served to demonstrate that specimens and reagents have been properly applied and have migrated through the device.

3.3.2 Procedure of the test

Specimens to be tested if refrigerated, were removed from the refrigerator and allowed to

come to room temperature of (approximately 18 to 30°C or 64 to 86°F) prior to testing. The Chembio HIV 1 & 2 Stat-Pak test device was removed from its pouch and placed on a flat surface. The test device was then labeled with the test identification number. Exactly 5 µL of the test specimen was added to the sample pad in the centre of the Sample (S) well of the device. Exactly 3 drops of buffer was added slowly, drop wise, into the Sample (S) well. The mixture was timed after the addition of the running buffer. The test results were read after 15 minutes.

3.3.3 Built-in control feature

When the test was completed a pink/purple line appeared in the Control (C) area of the test device, on non-reactive as well as reactive samples. This control line served as an internal control and gave confirmation of sample addition and proper test performance. A pink/purple line appeared in the Control (C) area. This showed that the test has been performed correctly and the device was working properly.

3.4 HIV Uni-gold Rapid Test Kit

Produced and supplied by Trinity Biotech USA.

3.4.1 Principles

Uni-Gold Recombigen HIV was designed as a rapid immunoassay based on the immunochromatographic sandwich principle and is intended to detect antibodies to HIV in human serum. Uni-Gold Recombigen HIV test employs genetically engineered recombinant proteins representing the immunodominant regions of the envelope proteins of HIV. The recombinant proteins are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. If antibodies to HIV are present in the sample, they combine with an HIV antigen/colloidal gold reagent and this complex binds to the immobilized antigens in the test region of the device forming a visible pink/red band.

3.4.2 Procedure of the test

Specimens to be tested if refrigerated, were removed from the refrigerator and allowed to come to a temperature of (approximately 18 to 30°C or 64 to 86°F) prior to testing. The Chembio

HIV 1 & 2 test device was removed from its pouch and placed on a flat surface, the desiccant from the pouch as recommended by its manufacturer. The test device was labeled with the test identification number. Exactly 5 µL of sample were dispensed into the sample pad in the center of the Sample (S) well of the device. About 3 drops (~ 105 LL) of buffer was slowly, added drop wise, into the Sample (S) well. 4) Timing was started after the addition of the Running Buffer. The test results were read exactly after 15 minutes.

3.4.3 Built-in control feature

The control line was always appearing as a visible pink/red band in the control region of the device to indicate that the test device was functioning correctly. A positive result was visualized by a pink/red band in the test region of the device. A negative reaction occurs in the absence of detectable levels of human immunoglobulin antibodies to HIV-1 in the specimen; consequently no visually detectable band develops in the test region of the device.

3.5 Method for HIV 1 & 2 P24 Antigens Assay

3.5.1 Determine HIV-1 & 2 P24 Ag/ Ab Combo test kit method

Produced by Inverness medical Japan Co, Ltd.

3.5.2 Principles of the test

Determine HIV-1 & 2 Ag/ Ab Combo is an immunochromatographic test for the qualitative detection of p24 antigen and antibodies to HIV-1 and HIV-2. Specimens are added to the sample pad. The specimen mixes with a biotinylated anti-P24 antibody and selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized avidin, recombinant antigens and synthetic peptides at the patient window sites. If antibodies to HIV-1 and / or HIV-2 are present in the specimen, the antibodies bind to the antigen selenium colloid and to the immobilized recombinant antigens and synthetic peptides, forming one red bar at the patient HIV Antibody window site. If antibodies to HIV-1 and / or HIV-2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the patient HIV Antibody window site. If p24 antigen is present in the specimen, the antigen binds to the biotinylated anti-p24 from

the sample pad and the selenium colloid anti-p24 antibody and it binds to an immobilized avidin forming a red bar at the patient HIV Antigen window site. If p24 antigen is not present both the biotinylated anti-p24 and selenium colloid anti-p24 antibody flow past the patient window, and no red bar is formed at the patient HIV Antigen window site. To ensure assay validity, a procedural control bar is incorporated in the assay device.

3.5.3 Procedure of the test

All reagents and the test samples were removed from the refrigerator and allowed to assume room temperature. One strip from the right side of the package was torn and the cover removed. Exactly 50µl of serum was added to the sample pad and followed by the addition of the buffer and was timed for 20 minutes. After 20minutes the results were read for both the HIV-1 p24 antigen (Ag) and HIV-1/2 antibodies (Ab) respectively.

3.5.4 Built-in control feature

The control line appeared as a red bar for all results. If it does not appear, the results were considered invalid. A positive result was visualized by a pink/red band in the test region of the device. A negative reaction occurred in the absence of detectable levels of human immunoglobulin antibodies to HIV-1 and / or HIV-2 in the specimen; consequently no visually detectable band develops in the test region of the device.

Three different types of rapid HIV1 and 2 Antibody test kits recommended by WHO were used:

- The HIV 1 and 2 Antibody assay was done with HIV 1 and 2 STAT-PAK Assay produced by CHEMBIO Diagnostic System Inc.

3.5.5 Principle of the Test

The Chembio HIV 1 and 2 STAT-PAK™ assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1 and 2 antigens, which are bound to the membrane solid phase. The sample is applied to the SAMPLE (S) well followed by the addition of a running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the

antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimens and reagents have been properly applied and have migrated through the device.

Determine HIV1 and 2 Antibody test kit produced by Inverness Medical Japan Co, Ltd.

3.6 Materials & Method for HIV 1 AND 2 P24 Antigens Assay

(Determine HIV- 4 and 2 P24 Ag/AbCombi Method) produced by Inverness Medical Japan Co, Ltd.

3.6.1 Principle

Determine® HIV-1 and 2 Ag/Ab Combo is an immunochromatographic test for the qualitative detection of p24 antigen and antibodies to HIV-1 and HIV-2. Specimens are added to the sample pad. The specimen mixes with a biotinylated anti-p24 antibody and selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized avidin, recombinant antigens and synthetic peptides at the patient window sites. If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to the antigen selenium colloid and to the immobilized recombinant antigens and synthetic peptides, forming one red bar at the patient HIV Antibody window site. If antibodies to HIV-1 and/or HIV-2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the patient HIV Antibody window site. If p24 antigen is present in the specimen, the antigen binds to the biotinylated anti-p24 from the sample pad and the selenium colloid anti-p24 antibody and it binds to an immobilized avidin forming a red bar at the patient HIV Antigen window site. If p24 antigen is not present both the biotinylated anti-p24 and selenium colloid anti-p24 antibody flow past the patient window, and no red bar is formed at the patient HIV Antigen window site. To ensure assay validity, a procedural control bar is incorporated in the assay device.

3.7 Statistical Analysis

The raw data base of the results for both sexes was subjected to statistical analysis data were represented with frequency and percentages while continuous data were expressed as mean and standard deviations. One sample Kolmogorov-Smirnov test was used to assess the normality of the data. All data were normally distributed; hence, parametric procedure was used. SPSS Statistics software version 20 (SPSS Inc., Chicago, USA) was used for the statistical analysis of the data. The Pearson chi square χ^2 test was used to calculate the hypothesis one-to five. The prevalence rate formulae were used to calculate the prevalence rate of HIV 1 & 2 infections. A two tailed p-value of <0.05 was considered indicative of a statistically significant difference. T-test and fisher exact test was used. Comparison of the parameters and variables between the samples were performed using independent t-test while comparison among various age groups were analyzed using ANOVA. Association between variables was analyzed using Chi Square and Fisher exact test. Risk estimates were analyzed using an odd ratio. Alpha value of 0.5 was used.

4. RESULTS

The results have been presented in the following Tables 1,2,3,4,5,6,7,8,9,10,11,12,13, 14 and 15 below.

Table 1 shows total numbers of 400 samples randomly collected by standard method from apparently healthy subjects recruited, selected, screened in the two sites which were Haematology/blood transfusion department of University of Calabar Teaching Hospital Temporary Site (UCTH) and General Hospital Calabar (GHC), in Calabar, Cross River State, Nigeria. The Total number of registered male and female apparently healthy prospective blood donors were 224 (56%) and 176 (44%) respectively from both sites with UCTH Calabar had the highest number of samples.

Table 2 shows the distribution of the 400 apparently healthy prospective blood donors based on gender and age range. It was observed that more female subjects fall between the age ranges of 20-25 years while male subjects fall between the age ranges of 26-30. The percent distribution indicates a lower age range frequency and percent distribution in female subjects.

Table 3: Results of ABO/Rh blood typing done on 400 voluntary apparently healthy prospective blood donors samples according gender. Blood group O Rhesus D positive had the highest percentage of 58.25% of samples with frequency of 233 samples followed by blood group A Rhesus D positive (19.25%,77) and blood group B Rhesus D positive (17.25%, 70), and the least percentage frequency is that of blood group AB Rhesus D positive (3.75%,15), blood group O Rhesus D positive (0.5%,2).

Table 4 Frequency distribution of HIV 1 & 2 Antibody screening test results amongst ABO & Rh blood groups There is no significant difference between the female and male positivity ($P>0.05$) It was observed that there was no statistical significance difference between the % positivity in female and male samples ($p>0.05$).

Table 5 Frequency distribution of P24 Antigen screening test results amongst ABO/Rh blood groups All the positive result of antigen test come from positive blood group (A+, B+, O+) .The result of the chi-square test between P24 and ABO blood group shows that there is no significant different amongst antigen test and blood group despite the fact that the distribution shows that all the positive cases of P24 are in positive blood group.

Table 6 Frequency distribution of HIV1&2 P24 antigen screening test Results amongst Rh blood groups.

Table 7 Frequency distribution of HIV 1& 2 Antibody Screening test results amongst Rhesus blood group. These observations might mean that Rh D negative subjects are more resistant to certain pathological conditions despite hazards encountered in infancy.

Table 8 shows the results of the three HIV I &2 antibody screening test kits based on gender. Out of 400 samples 12 (3%), 10(2.5 %) and 9(2.25 %) subjects tested positive to HIV 1& 2 Determine, Stat-Pak, and Unigold antibody screening test kits respectively. There was no statistical significance different between the three HIV 1 & 2 antibody screening test kits ($p>0.05$). There were 2 discordant samples between HIV 1 & 2 Determine and Stat-Pak, 3discordant samples between HIV 1 & 2 Determine and Unigold antibody test screening kits, 1 discordant sample between Stat-Pak, and Unigold antibody test screening kits.

Table 1. Demographic frequency distribution of voluntary apparently healthy prospective blood donors samples according to recruitment and screening sites and gender

Parameters	Number of Samples From** Collected UCTH(Temp. site)		Number of Samples Collected From GHC		Total number of Samples collected from both centres		p-values
	Frequency <i>f</i>	Percentage (%)	Frequency <i>f</i>	Percentage (%)	Frequency <i>f</i>	Percentage (%)	
Female	160	(39.6%)	16	(4.4%)	176	(44%)	P<0.05*
Male	200	(50%)	24	(6%)	224	(56%)	P<0.05 *
Total (N)	360	(89.6%)	40	(10.4%)	400	(100%)	

*Significance difference, N=total of sample collected P<0.05 **UCTH Calabar had the highest number of samples

Table 2. Demographic distributions of 400 apparently healthy prospective blood donors according to Age group

Study Groups	Age Range(Years)	Female		Male		Total		p- values
		Frequency <i>f</i>	Percentage (%)	Frequency <i>f</i>	Percentage (%)	Frequency <i>f</i>	Percentage (%)	
1	20-25	79	(44.98%)	036	(13.59%)	115	(28.75%)	*P<0.05
2	26-30	51	(29.97%)	100	(44.69%)	151	(37.75%)	*P<0.05
3	31-35	36	(20.45%)	060	(26.78%)	096	(24.00%)	*P<0.05
4	36-40	10	(05.68%)	013	(05.80%)	023	(05.75%)	*P<0.05
5	41-45	00	(00.00%)	010	(04.64%)	010	(02.50%)	*P<0.05
6	46-50	00	(00.00%)	005	(04.03%)	005	(01.25%)	*<0.05
Total	06	176	(44%)	224	(56.00%)	400	(100%)	

*Significance difference, N=total of sample collected more female subjects fall between the age ranges of 20-25 years with (45%) of the 79 samples more male subjects fall between the age ranges of 26-30years with (44.7%) of the 100 samples lowest between the age range of 41-50years with 04.3-4.63%.of the samples

Table 3. Results of ABO/Rh blood typing done on 400 voluntary apparently healthy prospective blood donors samples according gender

Parameters ABO/Rh	Gender						P-value
	Female		Male		Total		
	Frequency <i>f</i>	Percentage %	Frequency <i>f</i>	Percentage %	Frequency <i>f</i>	Percentage %	
A-	00	(0%)	1	(0.25%)	1	(0.25%)	P>0.05*
A+	34	(8.5%)	43	(10.75%)	77	(19.2%)	P>0.05**

Parameters ABO/Rh	Gender						P-value P=0.05
	Female		Male		Total		
	Frequency <i>f</i>	Percentage %	Frequency <i>f</i>	Percentage %	Frequency <i>f</i>	Percentage %	
AB-	00	(0%)	1	(0.25%)	1	(0.25%)	P>0.05*
AB+	6	(1.5%)	9	(2.25%)	15	(3.75%)	
B-	00	(0%)	1	(0.25%)	1	(0.25%)	P>0.05*
B+	29	(7.25%)	41	(10.25%)	70	(17.5%)	P>0.05**
O-	1	(0.25%)	1	(0.25%)	2	(0.5%)	P>0.05*
O+	106	(26.5%)	127	(31.75%)	233	(58.2%)	P<0.05**
Total	176	(44%)	224	(56%)	400	(100,%)	

****Significance difference, *not Significance difference, N=total of sample collected, Blood group O Rhesus D positive had the highest percentage of 58.25% with frequency of 233 samples, blood group A Rhesus D positive (19.25%,77) and blood group B Rhesus D positive (17.25%, 70), and the least percentage frequency is that of blood group AB Rhesus D positive (3.75%,15), blood group ORhesus D positive (0.5%,2)**

Table 4. Frequency distribution of HIV 1 & 2 Antibody screening test results amongst ABO & Rh blood groups

Parameters ABO/Rh Blood groups	HIV 1 & 2 antibody screening test results						P-value P=0.05
	Number of samples that tested Negative		Number of samples that tested Positive		Total number of samples collected		
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)	
A-	1	(0.25%)	0	(0%)	1	(0.25%)	p>0.05.
A+	77	(19.25%)	0	(0%)	77	(19.25%)	P<0.05
AB-	1	(0.25%)	0	(0%)	1	(.25%)	p>0.05.
AB+	15	(3.75%)	0	(0%)	15	(3.75%)	p>0.05.
B-	1	(0.25%)	0	(0%)	1	(0.25%)	p>0.05.
B+	67	(16.75%)	3	(0.75%)	70	(17.5%)	P<0.05
O-	2	(0.5%)	0	(0%)	2	(0.5%)	p>0.05.
O+	226	(56.5%)	7	(1.75%)	233	(58.25%)	P<0.05
TOTAL	390	(97.5%)	10	(2.5%)	400	(100%)	

Pearson chi square test χ^2 (at $df=1, n=400$) was =0.130 and level of $\alpha=0.05$, and critical value = 0.719. The χ^2 calculated value < χ^2 table calculated value. The obtained chi test χ^2 value (0.130) was less than the critical value (0.719). Fisher's exact test at $df=1, n=400$, was =0 $p>0.05$. There is no significant difference between the female and male positivity ($P>0.05$) It was observed that there was no statistical significance difference between the % positivity in female and male samples ($p>0.05$)

Table 5. Frequency distribution of P24 Antigen screening test results amongst ABO/Rh blood groups

Parameters ABO/Rh Blood groups	HIV1 & 2 p24 antigen screening test results						P-value P=0.05
	Number of sample that tested Negative		Number of sample that tested Positive		Total number of sample tested		
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage%	
A-	1	(0.25%)	0		1	(0.25%)	<i>P</i> >0.05*
A+	75	(18.75%)	2	(0.5%)	77	(19.25%)	<i>P</i> >0.05*
AB-	1	(0.25%)	0		1	(0.25%)	<i>P</i> >0.05*
AB+	15	(3.75%)	0		15	(3.75%)	<i>P</i> >0.05*
B-	1	(0.25%)	0		1	(0.25%)	<i>P</i> >0.05*
B+	66	(16.5%)	4	(1%)	70	(1.5%)	<i>P</i> <0.05**
O-	2	(0.5%)	0		2	(0.5%)	<i>P</i> >0.05*
O+	226	(56.5%)	7	(1.75%)	233	(58.25%)	<i>P</i> <0.05**
Total	387	(96.75%)	13	(3.25%)	400	(100%)	

The Pearson chi square test χ^2 (df=1, n=400) = 0.170, *p*<0.05, the CV=3.84. The obtained Pearson chi square test χ^2 value (0.170) is less than the critical value (CV) which is =3.84). **Significance difference, *not Significance difference, N=total of sample collected. All the positive result of antigen test come from positive blood group (A⁺, B⁺, O⁺)

Table 6. Frequency distribution of HIV1&2 P24 antigen screening test Results amongst Rh blood groups

Parameters Rh blood group type	HIV 1 & 2 P24 antigen screening test result						P-value P=0.05
	Negative		Positive		Total		
	Frequency f	Percentage %	Frequency f	Percentage %	Frequency f	Percentage %	
Rh D Negative	0	(1.25%)	0	(0%)	5	(1.25%)	<i>P</i> <0.05*
Rh D Positive	382	(95.5%)	13	(3.25%)	395	(98.75%)	<i>P</i> <0.05*
Total	387	(96.75%)	13	(3.25%)	400	(100%)	

The Pearson chi square test χ^2 (df=1, n=400) =0.130, *p*<0.05, the CV=3.84. The obtained Pearson chi square test χ^2 value (=0.130) is less than the critical value (CV) =3.84.

*Significance difference, N=total of sample collected. The Fisher's test gives a *p*-value of 1.00 which is above 0.05. This means that the nil hypothesis of independence between the two variables is not rejected. So, despite the fact that all the positive results of antibody test come from positive blood group, the data don't provide a significant difference

Table 7. Frequency distribution of HIV 1& 2 Antibody Screening test results amongst Rhesus blood group

Parameters	HIV 1& 2 antibody screening test result						P-value
	Negative		Positive		Total		
Rh blood group type	Frequency	Percentage %	Frequency	Percentage %	Frequency	Percentage%	P=0.05
Rh D Negative	5	(1.25%)	0	(0%)	5	(1.25%)	P<0.05*
Rh D Positive	385	(96.25%)	10	(2.5%)	395	(98.75%)	P<0.05*
Total	390	(97.5%)	10	(2.5%)	400	(100%)	

The Pearson chi square test X^2 (df=1, n=400)=0.130, p<0.05, the CV=3.84. The obtained Pearson chi square test X^2 value (=0.130) is less than the critical value (CV) =3.84. *Significance difference, N=total of sample collected. The Fisher's test give a p-value of 1.00 which is above of 0.05, this means that the nil hypothesis of independence between the two variables is not rejected. So, despite the fact that all the positive results of antibody test come from positive blood group, the data don't provide a significant difference

Table 8. Summary of the results of the three HIV I &2 antibody screening test Kits according to gender of study subjects

Gender	Determine		Stat-Pak		Uni-gold		P-values
	Positive (n=12 (3%))	Negative (n=388 (97%))	Positive (n=10 (2.5 %))	Negative (n=390 (97.5%))	Positive (n=9 (2.25 %))	Negative (n=391 (97.75%))	
Female	6(1.5)	170(42.5)	7(1.75)	169(42.25)	4(1%)	172(43)	p>0.05*
Male	6(1.5)	218(54.5)	3(.75)	221(55.25)	5(1.25)	219(54.75)	p>0.05*

Chi square test (x^2) was =0.1807, at df=1, n=400, level of alpha=0.05, and CV or tab cal =3.84. The x^2 cal. < x^2 tab cal. *Significance difference, n= No. of subjects involved, %= percentages. P>0.05

Table 9. P24 Antigen test results of HIV antibody- negative apparently healthy peospective blood donor samples according to Gender

Gender	Determine		Stat-Pak		Unigold		P-values
	Positive (n=15 (3.87%))	Negative (n=373(96.13%))	Positive (n=12(3.1 %))	Negative (n=378 (96.9%))	Positive (n=10(2.55 %))	Negative (n=381(97.44%))	
Female	14(3.60)	162(43.43)	7(1.8)	169(44.70)	6(1.5%)	170(44.61)	P<0.05*
Male	1(0.26)	211(56.56)	5(1.3)	209(55.29)	4(1)	205(51.38)	P<0.05*

The Chi square test (x^2) was =15.825, at df=1, n=388, level of alpha=0.05, and CV or tab cal =3.84. The x^2 cal > x^2 tab cal. The obtained Chi square test x^2 value (15.825) was greater than the critical value CV (3.84). P<0.05. *Significance difference, n= No. of subjects involved, %= percentages There is significant difference between male and female positivity (P<0.05). It was observed that there was statistical significance difference between the % positivity in female and male subjects (p<0.05)

Table 10. P24 Antigen test results of HIV antibody-positive apparently healthy prospective blood donors samples according to Gender

Gender	Determine		Stat-Pak		Unigold		P-values
	Positive (n=2(16.66%))	Negative (n=10 (83.33%))	Positive (n=2 (.2 %))	Negative (n=8 (.88%))	Positive (n=1(.11 %))	Negative (n=8(.88%))	
Female	2 (16.66)	4(33.33)	2(.2)	5(.5)	0 (0.00)	4(.44)	p>0.05*
Male	00(0.00)	6(50.00)	0(0.00)	3(.3)	1(.11)	4(.44)	p>0.05*

No significance difference, n= No. of subjects involved, %= percentages

Table 11. Prevalence rates (pv) and HIV 1 & 2 positive results for HIV p24 antigen (ag) & HIV antibody (ab) tests based on gender of study subjects

Gender	Determine Ab	Stat-PakAb	UnigoldAb	P24 Ag	Total	p-values
	Positive &PV	Positive & PV	Positive & PV	Positive & PV	Positive &PV	
Female	4(1.0%)	6	4	12(3%)	24(4%)	P<0.05*
Ag+Ab test	2(0.5%)	1	0	2(0.5%)	5(1%)	
Male	6(1.5%)	2	4	1(.25%)	13(1.75%)	P<0.05*
Ag+Ab test	0(00.0%)	1	1	0(00.0%)	2(00.0%)	
Total	12(3.0%)	10(2.5%)	9 (2.25%)	15(3.75%)	46 (11.5%)	

Antigen= (Ag) ,Antibody =(Ab),Prevalence Rates = (PV) Chi square test (x^2) was =6.512, at df=1, n=27, level of alpha=0.05, and cv or tab cal =3.84. the $x^2 cal > x^2 tab cal$. P<0.05. The obtained Chi square test x^2 value (6.512) was greater than the critical value CV (3.84). There is *significant difference between the two tests method (P<0.05). Hence the research hypotheses one to four were rejected. Significance statistical difference was observed when comparing the two methods.

Table 12. Secretor status typing results done on the 400 saliva samples collected from apparently healthy prospective voluntary blood donors per gender

Secretor Status	Female		Male		Total		P-value
	Frequency F	Percentage (%)	Frequency f	Percentage (%)	Frequency f	Percentage (%)	
ABH substance Non-secretor	39	(9.75%)	41	(10.25%)	80	(20%)	P<0.05*
ABH substance Secretor	137	(34.25%)	183	(45.75%)	320	(80%)	P<0.05*
Total	176	(44%)	224	(56%)	400	(100%)	

The Pearson chi square test x^2 (1,n=400)=1.209,p<0.05, the CV=3.84 . The obtained Pearson chi square test x^2 value (1.209) is less than the critical value CV (3.84). P<0.05, There is no significant difference between the results of P24 according to Secretor Status(P>0.05). Secretor Status doesn't influence the result of P24 test as shown by the data

Table 13. Distribution of p24 antigen screening test results amongst secretors & non secretors of ABH substances

Secretor status	HIV 1& 2 P24 antigen screening test result						P-Value
	Negative		Positive		Total	P=0.05	
	Frequency	% of P24 in SS	Frequenc y	% of P24 in SS	Frequenc y		
Non-Secretors of Abh substances	72	94,7%	4	5,3%	76	100,0%	<i>P</i> <0.05*
Secretor of Abh Substances	315	97,2%	9	2,8%	324	100,0%	<i>P</i> <0.05*
Total	387	96,75%	13	3,25%	400	100,0%	

The Pearson chi square test χ^2 (1, n=400) =0.007, *p*<0.05, the CV=3.84. The obtained Pearson chi square test χ^2 value (0.007) is less than the critical value CV (3.84). *significant difference between the two tests method (*P*<0.05). The result of chi-square test between Antibody screening test and Secretor status shows that there was no significant different between the antibody screening test and secretor status (*P*>0.05)

Table 14. Frequency distribution of between HIV 1 & 2 Antibody screening test results amongst Secretor & Non-Secretors

Secretor Status(SS)		HIV 1& 2 antibody screening test result				p-vau e
		Negative	Positive	Total		
Non- secretors of Abh substances	Frequency	74	2	76	<i>p</i> >0.05*	
	% of antibody test in SS	97,4%	2,6%	100,0%		
Secretors of Abh substances	Frequency	316	8	324	<i>p</i> >0.05*	
	% of antibody test in SS	97,5%	2,5%	100,0%		
Total	Frequency	390	10	400		
	% of antibody test in SS	97,5%	2,5%	100,0%		

The Pearson chi square test χ^2 (1, n=400) =0.007, *p*<0.05, the CV=3.84. The obtained Pearson chi square test χ^2 value (0.007) is less than the critical value CV (3.84). No significant different between the antibody screening test and secretor status (*P*>0.05)

Table 15. Frequency Distribution of secretor status test results amongst ABO/RH blood groups according to gender

Parameters ABO/Rh	Male (n =224 , 56%)				Female (n=176, 44%)				Total (n=400,100%)	p-value	
	Male Secretors		Male Non-secretors		Female secretors		Female Non-secretors				
	F	%	F	%	F	%	F	%	F	%	P=0.05
A-	1	(0.25%)	00	00	00	00	00	00	01	(0.25%)	p>0.05*
A+	41	(10.25%)	2	(.5%)	28	(7%)	6	(1.5%)	77	(19.25%)	P<0.05**
B-	1	(0.25%)	00	00	00	00	00	00	01	(0.25%)	p>0.05*
B+	34	(08.5%)	7	(1.75%)	24	(6%)	5	(1.25%)	70	(17.5%)	P<0.05**
AB-	1	(0.25%)	00	00	00	00	00	00	01	(.25%)	p>0.05*
AB+	8	(02%)	1	(0.25%)	4	(1%)	2	(0.5%)	15	(3.75%)	P<0.05**
O-	1	(.25%)	00	00	1	(0.25%)	00	00	02	(0.5%)	p>0.05*
O+	96	(24%)	31	(7.75%)	80	(20%)	26	(6.5%)	233	(58.25%)	P<0.05**
Total	183	(45.75%)	41	(10.25%)	137	(34.25%)	39	(09.75%)	400	(100%)	

The Pearson chi square test $\chi^2 (1, n=400) = 0.007, p < 0.05, \text{ the } CV = 3.84. \text{ The obtained Pearson chi square test } \chi^2 \text{ value } (0.007) \text{ is less than the critical value } CV (3.84). \text{ *No Significance difference, **Significance difference. Both blood O, and A Rh D positive are 100\% male and female secretors, and poor are poor male and females non-secretors. While Both blood B and AB Rh D positive are } < 100\% \text{ male and female secretors and are poor male and females non-secretors. All the Rh D negative ABO blood groups are very poor male and female secretors and non-secretors}$

Table 9 shows the result of the HIV 1 & 2 P24 antigens screening test done on the 388, 390 and 391 subjects screened as HIV 1 & 2 antibody-negative using Determine, Stat -Pak and Unigold rapid test kits respectively. HIV 1 & 2 P24 antigens was detected in 15(3.75%), 12(3.07%) and 10(2.55%) out of 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively. It is observed that out of the 388 HIV 1&2 Antibody–negative screened for p24 antigens, 15 were positive while 373 were negative. 14 positive samples were from the female group and only 1 positive sample was from the male group. A total of 162 negative samples were from the female sex group while 211 negative samples were from the male sex group. For Stat -Pak there were a total of 12(3.1%) that tested positive to HIV 1& 2, 7(1.8%) were female and 5 (1.3%) were male. There were 169 (44.70%) negative for females, 209(55.29%) for males which gave a total of 378 (96.9%). For Unigold 6 (1.5%) for female and 4 (1%) for male and total positive was (10(2.55 %) and negative were 170 (44.61) for female, male 205(51.38) and total negative of (381 (97.44%).

Table 10 shows the result of p24 antigen screening test done on 12, 10 and 9 HIV antibody -positive samples screened using Determine, Stat-Pak and Unigold respectively. It is observed that 2(16.66%) female, 2 (0.2%) female, and 1(0.11 %) male samples were positive to Determine, Stat-Pak and Unigold HIV 1&2 test kits respectively.

Table 11 shows the prevalence rates & HIV 1 & 2 positive results for the three antibody test kits and p24 antigens tests according to gender. It is observed that the prevalence rate was 12(3%) with HIV 1 & 2 Determine test kit, 10(2.5%) with Stat Pak, and 9(2.25%) with Unigold rapid test kits. The disparity in these results were not statistically significant ($P>0.05$). HIV 1 & 2 P24 antigens was detected in 15(3.75%), 12(3.07%) and 10(2.55%) out of the 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively. while that of p24 antigen test method was 15(3.86%).

Table 12 Secretor status typing results done on the 400 saliva samples collected from apparently healthy prospective voluntary blood donors per gender. There is no significant difference between the results of P24 according to Secretor Status($P>0.05$). In this case, Secretor Status

doesn't influence the result of the P24 test as shown by the data.

Table 13 Frequency distribution of p24 antigen screening test results amongst secretors & non secretors of ABH substances The result of chi-square test between Antibody screening test and Secretor status shows that there was no significant difference between the antibody screening test and secretor status ($P>0.05$). Despite the fact that the distribution shows that almost all the positive cases of the Antibody screening test are secretors, the data collected don't enable us to conclude a significant difference between the two variables.

Table 14 Frequency distribution of between HIV 1 & 2 Antibody screening test results amongst Secretor & Non-Secretors.

Table 15 Frequency Distribution of secretor status test results amongst ABO/RH blood groups according to gender.

5. DISCUSSION

Table 1 shows that total numbers of 400 samples had been randomly collected by standard method from apparently healthy residents of Calabar metropolis in the two blood recruitment, selection, screening and donation sites which were Haematology/blood transfusion department of University of Calabar Teaching Hospital Temporary Site (UCTH) and General Hospital Calabar (GHC), in Calabar, Cross River State, Nigeria. The Total number of registered male and female apparently healthy prospective blood donors were 224 (56%) and 176 (44%) respectively from both sites with UCTH Calabar had the highest number of samples.

Table 2 shows that out of the six study groups, a total of 400 samples collected from both sites 224 (56 percent) were male and 176 (44 percent) were female apparently healthy potential blood donors respectively. The Male study group 2 with age between 26-30 years had the highest percentage (44.7%) 100 of samples and highest frequency of number of potential blood donors while this was closely followed by female study group one with age between 20-21 years with highest percentage (45%) 79 of sample and highest frequency of number of potential blood donors. The lowest were between the age range of 41-50years with percentage range of 04.3-4.63%. This means that more male subjects were willing to participate in the study than females in

the various age groups. It also means that more awareness about HIV infection would have to be targeted towards this female age group.

Table 3 Blood group O Rhesus D positive had the highest percentage of 58.25% of samples with frequency of 233 samples followed by blood group A Rhesus D positive (19.25%,77) and blood group B Rhesus D positive (17.25%, 70), and the least percentage frequency is that of blood group AB Rhesus D positive (3.75%,15), blood group O Rhesus D positive (0.5%,2). These results reflect those observed in previous studies by [41,42,43] and [44]. Many other studies have -also shown that blood group ORhesus D positive was the most common blood group and blood group AB Rhesus D positive was the least common blood group in different ethnic groups [41a]. Also among Western Europeans populations blood group type O Rhesus D positive records 46%, blood group type A Rhesus D positive records 42%, blood group type B records 9%, and blood group type AB Rhesus D positive records 3% [45,46].

Table 4 shows the frequency distribution of HIV 1 & 2 Antibody screening test results amongst ABO & Rh blood groups systems. The Pearson chi square test χ^2 (at $df = 1, n = 400$) was $= 0.130$ and level of $\alpha = 0.05$, and critical value $= 0.719$. The χ^2 calculated value $< \chi^2$ table calculated value. The obtained chi test χ^2 value (0.130) was less than the critical value (0.719). There was no significant difference between the female and male positivity ($P > 0.05$). It was observed that there was no statistical significance difference between the % positivity in female and male samples ($p > 0.05$). Fisher's exact test at $df = 1, n = 400$, was $= 0$, $p > 0.05$ showing there were significant differences in the results.

Table 5 shows the frequency distribution of P24 Antigen screening test results amongst ABO/Rh blood groups. The Pearson chi square test χ^2 ($df = 1, n = 400$) $= 0.170$, $p < 0.05$, the $CV = 3.84$. The obtained Pearson chi square test χ^2 value (0.170) is less than the critical value (CV) which is $= 3.84$). The result of the chi-square test show that between P24 and ABO/Rhesus blood groups there is no significant different amongst antigen test and blood group despite the fact that the distribution shows that all the positive cases of P24 are in positive blood group which are blood group (A+, B+, O+) confirming the report of [36a,37a,38a] observed other places.

Table 6 shows Frequency distribution of HIV1&2 P24 antigen screening test Results amongst Rh negative and positive blood groups. The Pearson chi square test χ^2 ($df = 1, n = 400$) $= 0.130$, $p < 0.05$, the $CV = 3.84$. The obtained Pearson chi square test χ^2 value ($= 0.130$) is less than the critical value (CV) $= 3.84$.

Fisher's test gives a p-value of 1.00 which is above 0.05, this means that the nil hypothesis of independence between the two variables is not rejected. So, despite the fact that all the positive results of antibody tests come from Rhesus positive blood groups, the data don't provide a significant difference. This finding was in consonance with the results of [39] which reported that Rhesus D positive subjects were more susceptible to certain infections.

Table 7 shows the Frequency distribution of HIV 1& 2 Antibody Screening test results amongst Rhesus negative and positive blood groups The Pearson chi square test χ^2 ($df = 1, n = 400$) $= 0.130$, $p < 0.05$, the $CV = 3.84$. The obtained Pearson chi square test χ^2 value ($= 0.130$) is less than the critical value (CV) $= 3.84$. Fisher's test gives a p-value of 1.00 which is above 0.05, this means that the nil hypothesis of independence between the two variables is not rejected. So, despite the fact that all the positive results of antibody tests come from positive blood groups, the data don't provide a significant difference. These observations might mean that Rh D negative subjects are more resistant to certain pathological conditions despite the hazards encountered in infancy, Sayalet al.,[21a].

Table 8 shows the results of the three HIV I & 2 antibody screening test kits based on gender. Out of 400 samples 12(3%), 10(2.5 %) and 9(2.25 %) subjects tested positive to HIV 1& 2 Determine, Stat-Pak, and Unigold antibody screening test kits respectively. There was no statistical significance different between the three HIV 1 & 2 antibody screening test kits ($p > 0.05$). There were 2 discordant samples between HIV 1 & 2 Determine and Stat-Pak, 3 discordant samples between HIV 1 & 2 Determine and Unigold antibody test screening kits, 1 discordant sample between Stat-Pak, and Unigold antibody test screening kits. Chi square test (χ^2) was $= 0.1807$, at $df = 1, n = 400$, level of $\alpha = 0.05$, and CV or $\text{tab cal} = 3.84$. The χ^2 cal. $< \chi^2$ tab cal. The obtained Chi square test χ^2 value (0.1807) was less than the critical value CV (3.84). There is no significant difference between the female and male positivity ($P > 0.05$) It was observed that

there was no statistical significance difference between the % positivity in female and male subjects ($p>0.05$).

A total of 27 (6.86 percent) were declared positive for at least one of the two tests and among these 27 positive cases, there was a convergent result in only 2 of them. So, using only the HIV 1 & 2 antibody screening test for example, take along 12 positive cases over the 27. This will mean that more than 50 percent of the positive cases will not be taken into consideration thus going undetected. This will mean that there was still a considerable risk and significant contribution to onward transmission of transfusion HIV where only the HIV antibody assays algorithms are employed and implemented for laboratory diagnosis of early HIV infection or infected HIV (antibody -negative) blood donors.

This will also mean that there are still increased false positive or negative results in early HIV infection or infected HIV (antibody -negative) blood donors especially where HIV antigen assays algorithms are unaffordable.

The prevalence rate of HIV 1 & 2 infection amongst apparently healthy voluntary potential blood donors done in Calabar using HIV 1 & 2 antibody screening test Kits method were 1.5 percent for female, 1.5 percent for male and a mean value of 3% at 95 percent confidence interval. The 3% prevalence rate of the HIV 1 & 2 infection amongst in apparently healthy voluntary potential blood donors in Calabar are lower than the current 10% in the general population from which these blood donors were drawn as reported by [47,48,49] However this results falls within the range of 1- to 9 percent reported by others researchers in different region of Nigerian and different part of the world.

Table 9 shows the result of the HIV 1 & 2 P24 antigens screening test done on the 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively. HIV 1 & 2 P24 antigens was detected in 15(3.75%), 12(3.07%) and 10(2.55%) out of 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively. It is observed that out of the 388 HIV 1&2 Antibody-negative screened for p24 antigens, 15 were positive while 373 were negative. 14 positive samples were from the female group and only 1 positive sample was

from the male group. A total of 162 negative samples were from the female sex group while 211 negative samples were from the male sex group. For Stat -Pak there were a total of 12(3.1%) that tested positive to HIV 1& 2, 7(1.8%) were female and 5 (1.3%) were male. There were 169 (44.70%) negative for females, 209(55.29%) for male which gave a total of 378 (96.9%). For Unigold 6(1.5%) for female and 4 (1%) for male and total positive was (10(2.55 %) and negative were 170 (44.61) for female, male 205(51.38) and total negative of (381 (97.44%).

The Chi square test (χ^2) was =15.825, at $df=1$, $n=388$, level of $\alpha=0.05$, and CV or tab cal =3.84. The χ^2 cal > χ^2 tab cal. The obtained Chi square test χ^2 value (15.825) was greater than the critical value CV (3.84). There is a significant difference between male and female positivity ($P<0.05$). It was observed that there was statistical significance difference between the % positivity in female and male subjects ($p<0.05$).

Table 10 shows P24 Antigen test results of HIV antibody- positive apparently healthy prospective blood donor samples according to Gender.

There were 14 female samples (3.60 percent and 1 male samples (1.26 percent) which reacted positive to the HIV 1 & 2 antigens using P24 antigens screening test with a total number of 15 positive samples (3.86 percent) out of the screened 388 HIV 1 & 2 antibody negative potential blood donors.

Table 11: shows the prevalence rates & HIV 1 & 2 positive results for the three antibody test kits and p24 antigens tests according to gender. It is observed that the prevalence rate was 12(3%) with HIV 1 & 2 Determine test kit, 10(2.5%) with Stat Pak, and 9(2.25%) with Unigold rapid test kits. The disparity in these results were not statistically significant ($P>0.05$). HIV 1 & 2 P24 antigens was detected in 15(3.75%), 12(3.07%) and 10(2.55%) out of the 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively. while that of p24 antigen test method was 15(3.86%). Chi square test (χ^2) was =6.512, at $df=1$, $n=27$, level of $\alpha=0.05$, and CV or tab cal =3.84. The χ^2 cal > χ^2 tab cal. The obtained Chi square test χ^2 value (6.512) was greater than the critical value CV (3.84). There is a significant difference between the two test methods ($P<0.05$). Hence the Research hypotheses one to four were rejected. Significance statistical difference was observed when comparing the two methods.

The mean prevalence rate was 3.9% at 95% confidence interval. This prevalence rate of 3.9% amongst the 388 samples according to the HIV 1 & 2 antibody screening method are qualified to be acceptable for blood transfusion. However the use of the p24 proved that some of them are actually infected with the virus.

From these results it means that approximately 3.9 percent of the subjects who tested as HIV 1 and 2 Antibody negative have detectable HIV 1 and 2 P24 antigens in their serum and thus are actually infected with the HIV 1 and 2 and could be described as infected HIV 1 and 2 antibody negative potential blood donors. If the blood of these HIV 1 and 2 antibody negative potential blood donors were to be transfused directly without HIV 1 and 2 P24 antigens screening about 15 (3.86) recipients unavoidably would stand the risk of receiving transmissible transfusion HIV 1 and 2 infections.

These results are within normal range when compared with the prevalence rate of 2.7 percent of HIV amongst infected HIV Antibody Negative Pregnant Women [50] in Calabar but lower than 14.5 percent reported by [51] amongst infected HIV Antibody Negative Pregnant Women in district hospitals in Calabar and in Asokoro Hospital, Abuja. This is the first time the Prevalence rate of HIV 1 & 2 infection amongst female and male apparently healthy voluntary potential blood donors was done in Calabar using HIV 1 & 2 P24 antigen screening test Kit. These results were slightly higher than that of [52] but are totally in line with that of [53,54].

The Chi-square Test χ^2 was used to find out whether there was any difference between the positive results of both tests per sex group. Pearson chi square test χ^2 was =6.512, at $df=1$, $n=27$, level of $\alpha=0.05$, and CV or $tab\ cal =3.84$. $\chi^2\ cal < \chi^2\ tab\ cal$. The obtained Pearson chi square test χ^2 value (6.512) was less than the critical value CV (3.84). There is a significant difference between the two tests methods ($P<0.05$). Hence the Research hypothesis one was rejected.

Table 12 shows secretor status typing results done on the 400 saliva samples collected from apparently healthy prospective voluntary blood donors according to their gender. About 41(10.25%) subjects were male non secretor and 39 (9.75%) were female non secretors .183(45.75%) subjects were male secretors while 137(34.25%) were female secretors .This gives

an overall total of 80(20%) subjects that were Non-secretors, while about 320(80%) were Secretors. This result agrees with that [55,56].

Table 13 shows the frequency distribution of p24 antigen screening test results amongst secretors & non secretors of ABH substances .Out of 15 samples reacted positively to the p24 antigen test, 6 are non- secretors and 9 are Secretors. About 1.5 % of non -secretors are positive while 2.25 % of Secretors are positive. The prevalence rate according to P24 is higher in non-secretor patients than in Secretors.

The Chi-square Test χ^2 of was used to find out whether there is any Relationship between HIV 1 & 2 P24 Antigen Screening test Results and Secretor Status typing result .The Pearson chi square test χ^2 ($1, n=400$)=1.209, $p<0.05$, the $CV=3.84$. The obtained Pearson chi square test χ^2 value (1.209) is less than the critical value CV (3.84). There is no significant difference between the results of P24 according to Secretor Status ($P>0.05$). In this case, Secretor Status doesn't influence the result of the P24 test as shown by the data.

Table 14 shows frequency distribution of between HIV 1 & 2 Antibody screening test results amongst Secretor & Non-Secretors. Among the 12 positive antibody tests, 4 are Non -Secretors and 8 are Secretors. The prevalence rate of antibody tests is almost the same in both Secretors (2 percent) and Non- Secretors (1percent) and consequently the general prevalence rate (3 percent). So, the table shows a similarity of antibody test whatever the secretor Status.

The Chi-square Test χ^2 was used to find out whether there is any relationship between HIV 1& 2 antibody screening test results and Secretor Status result. The Pearson chi square test χ^2 ($1, n=400$) =0.007, $p<0.05$, the $CV=3.84$. The obtained Pearson chi square test χ^2 value (0.007) is less than the critical value CV (3.84). The result of chi-square test between Antibody screening test and Secretor status shows that there was no significant difference between the antibody screening test and secretor status ($P>0.05$). Despite the fact that the distribution shows that almost all the positive cases of the Antibody screening test are secretors, the data collected don't enable us to conclude a significant difference between the two variables (this is in line with the observation of [40a].

In Table 15 (24%) of 96 male subjects of Blood group O are secretors and (20%) 80 female subjects are secretors (10.25%) of 41 male subjects of Blood group A are secretors, (7%) of 28 female subjects of blood group B are secretors while (08.5%) of 34 male subjects of blood group B are secretors, (02%) of 8 male subject of blood group AB are secretors. Both blood O, and A Rh D positive are 100% male and female secretors and poor male and females non-secretors. While both blood B and AB Rh D positive are <100% male and female secretors and are poor male and females non-secretors. The entire Rh D negative, ABO blood groups are very poor male and female secretors and non-secretors. This is in with [57].

6. CONCLUSION

Based on the finding of this work it was concluded that the HIV1&2 antibody screening test method alone does not provide accurate results. This is a big risk in the case of screening of blood donors since it will not provide safe and HIV 1 & 2 free blood for transfusion because some of the samples that tested HIV antibody negative were positive with the P24 antigen screening test method.

Hence, P24 antigen screening test method is observed to give a higher safety because of its high degree of sensitivity.

7. RECOMMENDATION

Although the results of this study strongly support and agree with those hypothesized and cited in most current literature and journals, the reality and risk of 3.3 percent infected HIV 1 and 2 Antibody Negative Blood Donors in Calabar have been implicated. Adding HIV 1 and 2 P24 Antigen, or other HIV fourth generation test kit to the current HIV antibody screening Panels cannot be regarded as a high cost-effective health-care intervention since the life of blood many recipients will be saved from infected HIV 1 and 2 Antibody Negative Blood Donors. This work is therefore the first attempt to establish epidemiological prevalence rate of infected HIV antibody negative blood donors in Calabar.

AVAILABILITY OF DATA AND MATERIALS

Datasets generated and analysed in this study are available from the corresponding author on request.

CONSENT AND ETHICAL APPROVAL

This study was approved by Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital, Calabar, Cross Rivers State Nigeria and the Research Ethical Committee, Centre for Clinical Governance, Research & Training Ministry of Health Calabar, and Cross Rivers State, Nigeria. Oral informed consent was obtained from the donors before inclusion in the study.

REFERENCES

1. WHO GHO. World Health Organization Global Health Observatory data on HIV/AIDS; 2020.
2. UNAIDS. United Nation Joint Programme on AIDS, Global HIV/AIDS 2020 fact sheet; 2020.
3. Fauci A, Masur H, Gelmann EP, Markham PO, Halin BH, Lane HC. The AIDS, an annual update of internal medicine. 1985;102:800-813
4. Weiss RA. "How does HIV cause AIDS? Science. 1993;260(5112):1273-9.
5. Douek DC, Roederer M, Koup RA. "Emerging concepts in the immunopathogenesis of AIDS". Annu. Rev. Med. 2009;60:471-84.
6. United Nation Joint Programme on AIDS ABUJA/GENEVA Declaration Report; 2020.
7. Cross River State eMTCT Operation Plan for the Elimination of Mother -to- Child Transmission of HIV, Bayelsa State Ministry of Health ,Family Health International- FHI 360 and United Nation Joint Programme on AIDS Report; 2013-2015
8. Osuchukwu NC, Onovo AA, Atobatcle AO, Nta IE. Disproportionate Burden of HIV Prevalence among Men and Women assessing HIV Testing and Counseling Services in Cross River State Nigeria, IOSR Journal of Pharmacy and Biological Science (IOSR-JPBS). 2013;7(6):19-24.
9. Wahdan MH. Epidemiology of acquired immunodeficiency syndrome. Alexandria, World Health Organization, Regional Office for the Eastern Mediterranean, (WHO-EM/GPA/014/E/L/95); 1995.
10. Barré-Sinoussi F, Chermann JC, Rey F. Isolation of a T-lymphotropic retrovirus from a patient at risk For Acquired Immuno - Deficiency syndrome (AIDS). Science 220. 1983;868-871.

11. WHO. Guidelines for antimicrobial resistance surveillance. WHO Regional Publications. Eastern Mediterranean Series No. 151929021213, Border No. 1440015; 2007.
12. UNAIDS. The latest statistics of the global HIV and AIDS epidemic: published by UNAIDS in November. Refer to the end of; 2009.
13. Alison Walkley. HIV transmission via transfusions in Africa remains high, MEDIAGLOBAL published by the United Nations Secretariat, Room L-221 K, Dag Hammarskjold Library, New York, NY 10017; 2009.
Available:<http://mediaglobal.org/article/2009-08-08/cote-divoire-approved-for-fi>.
14. FMOH. Federal Ministry of Health, Nigeria :'National Blood Transfusion Service-About Us; 2009.
15. Alexander W. An inquiry into the distribution of the blood group patient suffering from malignant disease. British Journal of Experimental Pathology. 1921;2:66-69.
16. Aird I, Bentall HH, Mehigan JA, Roberts JA. The blood groups in relation to peptic ulceration and carcinoma of colon, rectum, breast, and bronchus; an association between the ABO groups and peptic ulceration. British Medical Journal. 1954;7;488(3):315-21.
17. Mourant AE. Identification of the Antibody Anti-e, International Journal of Transfusion Medicine VoxSanguinis. Wiley Online Library. 1983;44(4):260-26.
Available:<https://doi.org/10.1111/j.1423-0410.1983.tb01892.x>
18. Dickey W, Wylie JD, Collins JS, Porter KG, Watson RG, McLoughlin JC. Lewis phenotype, secretor status, and coeliac disease. Gut. 1994;35(6):769-770.
19. Mollicone R, Cailleau A, Oriol R. Molecular genetics of H, Se, Lewis and other fucosyltransferase genes. Transfusion in Clinical Biology. 1995;2(4):235-42.
20. Nakano T, Shimanuki T, Matsushita M, Koyama I, Inoue I, Katayama S, Alpers DH, Komoda T. Involvement of intestinal alkaline phosphatase in serum apolipoprotein B-48 level and its association with ABO and secretor blood group types. Biochemistry, Biophysical Research Communication. 2006;341(1):338.
21. (a) Sayal SK, Das AL, Nema SK. Study of blood groups in HIV seropositive patients. Indian Journal of Dermatology Venereology Leprology. 1996;62:295-7.
22. Martin L. Olsson, Nicole Lund, Stephanie Ramkumar, Darinka Sakac, Vered Yahalom, Cyril Levene, Asa Hellberg, Xue-Zhong Ma, Beth Binnington, and Daniel Jung. Blood group antigen provides protection against HIV-1 infection. Blood. 2009;03:143.
23. Lund N. "The human Pk histo-blood group antigen provides protection against HIV-1 infection." Blood. E.pub ahead of print; 2009.
24. Imagawa DT, Lee MH, Wolinsky SM. Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods. New England Journal of Medicine. 1989;320:1458-1462.
- 24^a) Larry Reimer Susan Mottice, Charles Schable, Patrick Sullivan, Allyn Nakashima, Mark Rayfield, Randy Den, Charles Brokopp, Absence of Detectable Antibody in a Patient Infected with Human Immunodeficiency Virus in Clinical Infectious Diseases 1997;25:98–100.
- 24^b) Centers for Disease Control and Prevention (CDC), Persistent Lack of Detectable HIV-1 Antibody in a Person with HIV Infection -- Utah, Morbidity and Mortality Weekly Report (MMWR), 1996;45(09):181-8.
Available:mmwrq@cdc.gov.
- 24c) Joseph Sonnabend, Natural Immunity and HIV journal of infection disease, 2010;19/10/10 POZ.
25. Egesie OJ, Joseph DE, Egesie UG, Odeh CI. Trends in the incidence of hepatitis B, C and Human Immunodeficiency Virus among blood donors in a tertiary hospital in Nigeria. Journal of Medicine in the Tropics. 2011;13(1).
26. Coovadia H. Antiretroviral agents how best to protect infants from HIV and save their mothers from AIDS". New England Journal of Medicine. 2004;351(3):289–292.
27. Scott, Katherine C Philip, Susan, Ahrens Katherine, Kent, Charlotte K, Klausner, Jeffrey. High Prevalence of Gonococcal and Chlamydial Infection in Men Who Have Sex With Men With Newly Diagnosed HIV Infection An Opportunity for Same-Day Presumptive Treatment JAIDS Journal of Acquired Immune Deficiency Syndromes: May 1st. 2008;48(1):109-112.
DOI:10.1097/QAI.0b013e318165dc0b

28. Jayasri Basu, Seymour L, Romney, Ruth H. Angeletti, Sten H. Vermund, Edward Nieves, Anna S. Kadish, Magdy S. Mikhail, and George A. Orr. Human Immunodeficiency Virus (HIV) Antigens and RNA in HIV-Seronegative Women with Cervical Intraepithelial Neoplasia, AIDS research and human retroviruses. 2009;25(3):249-59.
29. Zhu T, Corey L, Akridge R, Change Y, Feng F, Kim J, Alef C, Mcelroy J, Mullins J, Mcelrath J. Evidence for HIV-1 latent infection in exposed seronegative individuals. 6th Conference on Retroviruses and Opportunistic Infections. Chicago. Abstract No.8; 1999.
30. Zhu-Tuofu, Corey-Lawrence, Hwangbo-Yon, Lee-Jean-M, Learn-Gerald-H, Mullins-James-I, McElrath-M-Juliana. Persistence of extraordinarily low levels of genetically homogeneous human immunodeficiency virus type 1 in exposed seronegative individuals. Journal of virology. 2003;77(11):6108-16.
31. National Population Commission (NPC) [Nigeria] and ICF Macro. Nigeria Demographic and Health Survey. Abuja, Nigeria: National Population Commission and ICF Macro; 2009.
32. Cochran WG. Sampling techniques (3rd ed.), 605 third Avenue, New York, New York, 10016 by John Wiley & Sons inc, ISBN0-471-02939-4; 1977.
33. Ary D, Jacobs LC, Razavieh A. Introduction to research in education. Fort Worth, TX: Harcourt Brace College Publishers; 1996.
34. Bartlett JE, II, Kotrlik JW, Higgins C: "Organizational research Determining appropriate sample size for survey research", Information Technology, Learning, and Performance Journal. 2001;19(1):43-50.
35. Rao JNK. Sample size calculation, by Rao soft, Inc; 2004.
36. (a)Neil SJ, McKnight A, Gustafsson K, Weiss RA. HIV-1 incorporates ABO histo-blood group antigens that sensitise virions to complement-mediated inactivation. Blood. 2005;105(12):4693-9.
37. (a)Saarloos MNTF Lint, GT Spear. Efficacy of HIV-specific and 'antibody-independent' mechanisms for complement activation by HIV-infected cells Clin Exp Immunol. 1995;99(2):189-95. DOI:10.1111/j.1365-2249.1995.tb05531.x
38. (a)Friedli Fabienne Robert Rieben Edgar Wegmüller Martine Moerenhout Urs E. Nydegger 1996: Normal Levels of Allo- but Increased Levels of Potentially Autoreactive Antibodies against ABO panel Clinical Immunology and Immunopathology. 1996;80(1):96-100. Available:https://doi.org/10.1006/clin.1996.0099
39. Omoregie Richard Bankole Henry Oladeinde, Eguagie Osareniro Osakue, and Tola Ohiengbomwan Onaiwu. Asymptomatic Malaria among Blood Donors in Benin City Nigeria Iran J Parasitol. PMID: PMC4316574 PMID: 25678927. 2014;9(3):415-422.
40. (a)D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status, Alternative Medicine Review. 2001;6(4):390-405.
41. (a)Ejele OA, Erhabor O, Nwauche CA. The risk of transfusion-transmissible viral infections in the Niger-Delta area of Nigeria.Sahel Medical Journal. 2005;8(1):1619.
42. Kulkarni AG, Peter B, Ibazebo R, Dash B, Fleming AF. The ABO and Rhesus groups in the north of Nigeria Journal annal of Tropical Medicine & Parasitology. 1983;79(1):83-88. Available:https://doi.org/10.1080/00034983.1985.11811891
43. Falusi AG, Ademowo OG, Latunji CA, Okeke AC, Olatunji PO, Onyekwere TO, Jimmy EO, Raji Y, Chedo C, Otukonyong EE, Itata EO. Distribution of ABO and RH genes in Nigeria Afr J Med Med Sci. 2000;29(1):23-6.
44. Encyclopædia Britannica. ABO /Rhesus written by Eugene M. Berkman, Editor of Fundamentals of a Pheresis Program; 2010.
45. Pramanik T, Pramanik S. Distribution of ABO and Rh blood groups in Nepalese students: A report. East. Mediterr. Health J. 2000;6(1):156-158.
46. Adeyemo OA, Soboyejo OB. Frequency distribution of ABO, RH blood groups and blood genotypes among the cell biology and genetics students of University of Lagos, Nigeria. Afr. J. Biotechnol. 2006;5(22):2062-2065
47. FMOH. 'National Blood Transfusion Service- About Us'. Federal Ministry of Health, Nigeria; 2009.

48. WHO. "WHO and UNAIDS announce recommendations from expert consultation on male circumcision for HIV prevention". WHO International; 2007.
49. Inyang-Etoh PC, Udofia GC, Alaribe AAA, Udonwa NE. Asymptomatic bacteriuria in patients on antiretroviral drug therapy in Calabar, Journal of Medical Science. 2009;9:270-275.
50. Ekure EN, Etuk SJ. Factors influencing HIV seroprevalence rate among pregnant women in Calabar, Nigeria Sexual Health Matters, Published Quarterly by Express Print Works, Middlesbrough, U.K; 2001.
51. Oriaku Doris, Uko EK. Evaluation of HIV I P24 Antigenemia in HIV seronegative antenatal attendees at Asokoro District Hospital Abuja; 2004.
52. A Kuliya-Gwarzo. Screening For Blood Transfusion Transmissible Viruses In Resource Limited Settings. The Internet Journal of Infectious Diseases. 2009;(9):1.
53. Kwaru AH, GA Kuliya, FE Jegede, T Ofula, MO Imoru, AG Abubakar, AA Samaila, Al Dutse: Prevalence of Human Immunodeficiency Virus (HIV-1) P24 Antigen Among HIV Antibody - Negative Blood Donors in Kano, Nigeria. Journal of Medical Laboratory Science. 2006;15(1).
54. Kleinman S. "Patient information: Blood donation and transfusion". Uptodate; 2004.
55. Dacie JV, Lewis SM. Practical Haematology 7th edition Churchill Livingstone, Edingburg, London, Melbourne and New York. 1991;1(2):5.
56. Emeribe AO, Igweagu CA, Osim EE. ABH secretor status in residents of Calabar Municipality, East African Medical Journal. 1992;69(1):27-30.
57. Rashmi Metgud, NidiKhajauria Gayathri Ramesh, Journal Clinical Diagnostic Research: ZC01-ZC03. 2016:10(2).

© 2020 Ndeh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/60481>