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Clinical Utility of Fourth Generation Alere™ HIV P24 Core Antigen Rapid Combo Test Panels for Improved Detection of Human Immunodeficiency Virus Infection among Pre-Screened HIV 1 and 2 Antibody Sero-Negative Long-Distant Truck Drivers in Calabar Municipality, Cross River State, Nigeria

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Abstract:- Recent studies have shown that transmission of human immunodeficiency virus (HIV) infection from infected but screened HIV antibody sero-negative individuals have continued to be a public challenge. This unprecedented finding may be due to the long pre-seroconvertion / window period or other predisposing factors like resource poor settings especially where there are no availability of ideal HIV p24 antigen /antibody fourth generation panels or biomarkers during early phase of this infection. The current study investigated HIV p24 core antigen status of healthy Long Distant Truck Drivers (LDTDs) of commercial and public vehicles, who were previously screened and documented as HIV antibody- sero-negative males with ages above 20 years in Calabar Municipality, Cross River State, Nigeria. About five milliliters of blood samples were collected from precounseled and consented 400 apparently healthy married and unmarried male participants who were initially screened for HIV 1 and 2 antibodies using Determine™ HIV-1 and 2 (Inverness Medical Japan company limited), Stat-Pak HIV-1 and 2 (Chembio Diagnostic System International Inco-operation ,United State of America) and HIV Uni-gold rapid test kit (Trinity Biotech, United State of American) respectively and repeated for HIV p24 antigens screening using Alere TM HIV-1 and 2 p24 Ag/ Ab Combo test kit-tool (Inverness Medical Japan company limited, United State America).After data analysis using IBM-SPSS version 26, about 12 (3%), 10 (2.5%) and 9 (2.25%) of participants were reactive to HIV 1 and 2 Determine, Stat-Pak and Unigold test panels respectively, with statistical significant difference between the results according to marital status (p=0.7065) and mean age range in years .While some of the 15 (3.75%), 12 (3.0%) and 10 (2.5%) participants who reacted to HIV 1 and 2 antibodies panels including the 388, 390 and 391 participants respectively who initially tested non-reactive for the three HIV antibody kits become reactive to HIV p24 core antigens with no statistically significant difference between the results of the three HIV 1& 2 antibody test kits and HIV p24 antigen/antibody tests (P=0.901).In the current study, the use of fourth generation Alere™ HIV P24 Core Antigen rapid test kit tool had not only improved detection of HIV infection in the index population but had also demonstrated the discrepancies, limitations and short-coming associated with the routine antibody screening testing panels when done alone in this population at early stage of HIV infection and long HIV window period.

Keywords:- *HIVI &II P24 Antigens Test Tool Kits , HIV 1 & II Antibody Screening Test Tool Kits , HIV 1 & II Antibody-Sero-Negative Screened Long-Distant Truck Drivers, Commercialized Private And Public Vehicle's Terminals , Calabar Municipality.*

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I. INTRODUCTION

Long-distance truck drivers (LDTDs) have been defined as those individual drivers who take a consignment of goods and materials from one place which is usually point of departure to another destination, using heavy lorries or trucks through various roads located along major national highways covering a distance of more than 800 kilometers, one-way before returning to the place of origin place of departure [1]. Long-distance truck drivers (LDTDs) could be considered also as a group of individual drivers who are vulnerable to human immunodeficiency virus (HIV 1and 2) infection [2-3] and other sexually transmitted infections (STI) or urinary tract infection (UTI) due to the nature of their work which usually involved long hauling of transportation of goods and materials and frequent mobility in different routes from one city to another [4]. Studies have shown that the spread of HIV along major truck roads and the crucial role played by Long-distance truck drivers (LDTDs) and their helpers traveling about 800 kilometers or more in a single direction with high risk of carrying HIV infections and other sexually transmissible infections from one place to another thus helping in transmission of these infections from a very high-risk populations (high-risk population includes female sex workers, men who have sex with men, and injecting drug users) to low-risk populations (low-risk population primarily includes spouses of migrants /mobile populations) [5-7].

A lot of researchers believed and supposed that there are always some chances, proportion or probability, although said to be very low percent risk of HIV transmission from screened but infected HIV antibody-sero-negative long distant truck drivers (LDTDs) of commercial and public vehicles especially in some developing countries [8]. The ideological concept behind this phenomenal and existence of infected HIV antibody-negative individuals were first hypothesized by [9] and later by other researchers who also observed and confirmed this phenomenal [10-12].In those days these researchers believed and thought that the only explanation offered for this phenomenal may be possibly due to the host factors and clinical conditions namely : a) Seroconversion of person(s) being in unusual long window (incubation) period of HIV infection; b) A false-negative HIV antibody test; c) Human operational and technical errors and d) Lack of trained and technical personnel and unaffordable advanced blood-screening techniques or equipment and reagents, e) Host genetic and immune characteristics, size of the inoculums, and the amount of infecting virus, f) Route of infection, the particular virus strain and the presence of associated systemic infections, capable of providing signals for activating HIV proviral DNA [13-18].Unfortunately despite decades of aggressive study of human immunodeficiency virus (HIV) infection, prevention, transmission, and significant advances in its laboratory diagnosis, treatment and management in the developed countries, there are still many challenges to overcome in some developing countries [19-21].It is undeniable and undisputable facts that the human immunodeficiency virus / acquired immune deficiency syndrome (HIV/AIDS) epidemic had created serious devastating, substantive negative impacts on health, social status, and economic growth and development of individuals, households, local, national and international communities and life of the human race [22-25]. This is due to the fact that there was no vaccine for its prevention, no definite eradicative therapy and the high risk of continuous transmissible infection [26]. According to the World Health Organization Global Health Observatory (WHO-GHO) data on HIV/AIDS, 2020, about 38.0 million people were living with the 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 start of the epidemic [27, 28].

A. Statement of the Problem

According to World Health Organization (WHO), it is on record that the Human immunodeficiency virus infection was first described in the 1980s in the United Stated American (USA) and has continued to spread rapidly and globally [29]. The HIV is a lentivirus (belonging to the retrovirus family) reputed to cause the Acquired Immuno-Deficiency Syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections and some malignancies [30-31]. 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[35].The adult prevalence rate of HIV in Nigeria was 1.4 percent for 2019 amongst adults aged between 15-49 years [32], while Cross River State current prevalence rate is 7.1 percent [33-34]. HIV transmission through unsaved blood is the second largest source of HIV infection in Nigeria [36-38].

B. Rationale And Justification of the Current Study

Despite all the advanced research and innovated study in HIV infection there are still some on- going challenges and unbridged gaps of knowledge in HIV transmission and any potential risk of HIV transmission, no matter how small it may be, it must be avoided as fast as possible to prevent loss of human life [30].Therefore, this unfilled gap of knowledge need to be bridged and this means that identifying primary HIV infection in infected HIV antibody sero-negative individuals is clearly a matter of urgency and is of global public health importance , essential and crucial in preventing further transmission of HIV through these infected HIV antibody sero- individual individuals and identified infected HIV antibody negative individuals so that the affected group may benefit from early therapeutic interventions [40-41].

C. Research Questions

- What is the prevalence rate of HIV I and II infection in the married male long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigen screening test?
- What is the prevalence rate of HIV I and II infection in unmarried male long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigens screening test?

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- What is the prevalence rate of HIV I and II infection in both the male married and unmarried long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigen screening test?

D. Research Hypothesis (Null and Alternate Hypothesis)

- HO = There was no statistically significant difference between the prevalence rate of HIV I and II infection in the male married and unmarried long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using the HIV p24 core antigen screening test?
- Ha = There was statistically significant difference between the prevalence rate of HIV I and II infection in the male married and unmarried long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using the HIV p24 core antigen screening test.

E. General Objective of the Current Study.

The current study is aimed at the determining the prevalence rate of HIV p24 core antigens status of apparently healthy married and unmarried male long-distant truck drivers of commercialized private and public vehicle's terminals in Calabar municipality (who had been previously screened as HIV 1 and 2 antibody- sero-negative using three different types of HIV 1 & 2 antibody testing kit tool) and are now being screened using HIV p24 core antigen point of care testing (POCT).

F. Specific Objectives

- To determine the prevalence rate of HIV I and II infection of unmarried male long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigens point of care testing kit tools.
- To determine the prevalence rate of HIV I and II infection of married male long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigens point of care testing kit tools.
- To determine the prevalence rate of HIV p24 core antigen positive screening test among unmarried and married male long distant truck drivers of commercialized private and public vehicle's terminals in Calabar Municipality, Cross River State, Nigeria using HIV p24 core antigens point of care testing kit tools.

G. Significance and Justification of the Study

- The results of the current study are hoped to contribute information and knowledge to ongoing activities of HIV I and II testing and prevention amongst the study population and to bridge the gap of knowledge in this domain.
- It is hoped that the results obtained from this study will help provide the status of HIV P24 core antigen screening test for the studied population, thus equipping and informing the various stakeholders and actors in the field.
- More so, the current prevalence rate of HIV p24 core antigen and the transmissible rate of HIV infection among HIV antibody -seronegative long-distant truck drivers' population, who may be in the early stage of the HIV infection will be established for time in the study area.
- In addition, it is hope that the outcome will contribute knowledge to humanity, scientific and research community.
- Furthermore, the current study is expected to help us better understand how certain health conditions, behavior, and work environment combine to affect long-haulers' safety and health.
- Finally, it is hoped that the information gathered from the current study will help guide truck driver's health, safety policy and address all the health and safety concerns that may be facing these individuals.

II. STUDY SETTING

The current study was carried out in Calabar municipality which it is one of Local Government Area that makes up the city of Calabar. It is also the capital of Cross River State located in the south eastern part of Nigeria. Geographically, Calabar Municipality has a total Surface land area of 142 kilometer squared (km^2) while the total local government area population is estimated to be 320,826 of which 166,203 are males and 154,659 females [42]. The inhabitants are mainly the Efiks, Quas, Ejagham, Efut, Ibibio, Annang and others – the migrant workers. The inhabitants of the study area are mainly civil servants, subsistence farmers, traders and fishermen (See figure 1).

A. Recruitments of Participants, Sample Collection and Analysis:

The participants of the current study were made up of long-distant truck drivers (LDTDs). LDTD was defined as a trucker who takes a consignment from one place to another destination located along the national highways and who travels more than 800 km one-way before returning back to the place of origin. There were six main different terminal bus stop station in Calabar city, Cross River State, Nigeria, that were selected randomly for recruitment and HIV pre-counselling for sample collection in this study. Experimental and analytic designed were adopted in this study and the analysis of the collected samples were carried out in the Hematology and Blood Transfusion and Chemical Pathology Departments of Medical Lab Sciences, University of Calabar, Nigeria between January ,2017 and December 2018.

B. Calculation of Sample Size:

The Formula of Cochran, 1977, for calculating the sample size (S) was adopted in this study and is given by [43]: S= t2 p (1-p)/ ٍe2 , Where t= t value (The alpha level used in determining sample size in most educational research studies is either .05 % [44]. 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 long distant truck drivers population in Calabar and in this case it is taken to be 0.5 or 50% since

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nobody had ever worked on this population [45,46] While e = tolerance error or confidence interval expressed as decimal and it is taken to be 0.05.Therefore S = (1.962)2 (.5(1-0.5)/ (0.05)2, S = (1.962)2 (0.5)2/ (0.05)2 = 384.16 ,hence S = ~ 400 subjects were used in cases of any loss data or specimen during the study or in cases of non-respondent individuals.

C. Inclusive and Exclusive Criteria for Subject Selection:

A total of 400 apparently healthy voluntary subjects of male genders, aged between 20 to 50 years and who were randomly recruited from the various terminal bus stop of the long -distant truck drivers of commercialized private and public vehicle's terminals in Calabar, Cross River State, Nigeria. The participants were divided into six study groups according to their ages and marital status and a questionnaire form was employed for both inclusive and exclusive criteria.

D. Ethical consideration and institutional approvals:

These were sought and obtained from the Research Ethical Committee, Centre for Clinical Governance, Research & Training Ministry of Health Calabar, and Cross Rivers State, Nigeria and institutions concerned. Informed and written consents were sought and obtained from these participate before inclusion in the study. 2.6) The nature of questionnaire forms and mode of administration: The harmless nature and advantage of the research was explained to each participant in the form of HIV pre- counselling in which a questionnaire was administered on each of the subjects to obtain more medical information about the clinical history. After the HIV Pre- counselling, informed consent forms were filled and signed by these participants for screening to start. They were screened in accordance with the current HIV national algorithms and standard parameters set forth in these HIV screening centers.

E. Collection and Treatment of Collected Blood Samples:

Venous blood from the selected and screened participant were collected from Monday to Friday between the hours of 7.00 am and 1.00 pm continually for four months. About five milliliters of blood samples were withdraw from the ante-cubital vein of the arms of pre- HIV counseled individual by a mean of disposable plastic five milliliters syringe fitted with 21 SWG needle. 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. The five milliliters of blood withdrawn were dispensed into dried and labeled samples bottles were dispensed into dried, labelled plain tubes to be centrifuged for ten 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. Samples which were not analyzed immediately were stored in the refrigerator at 4- 6 OC.

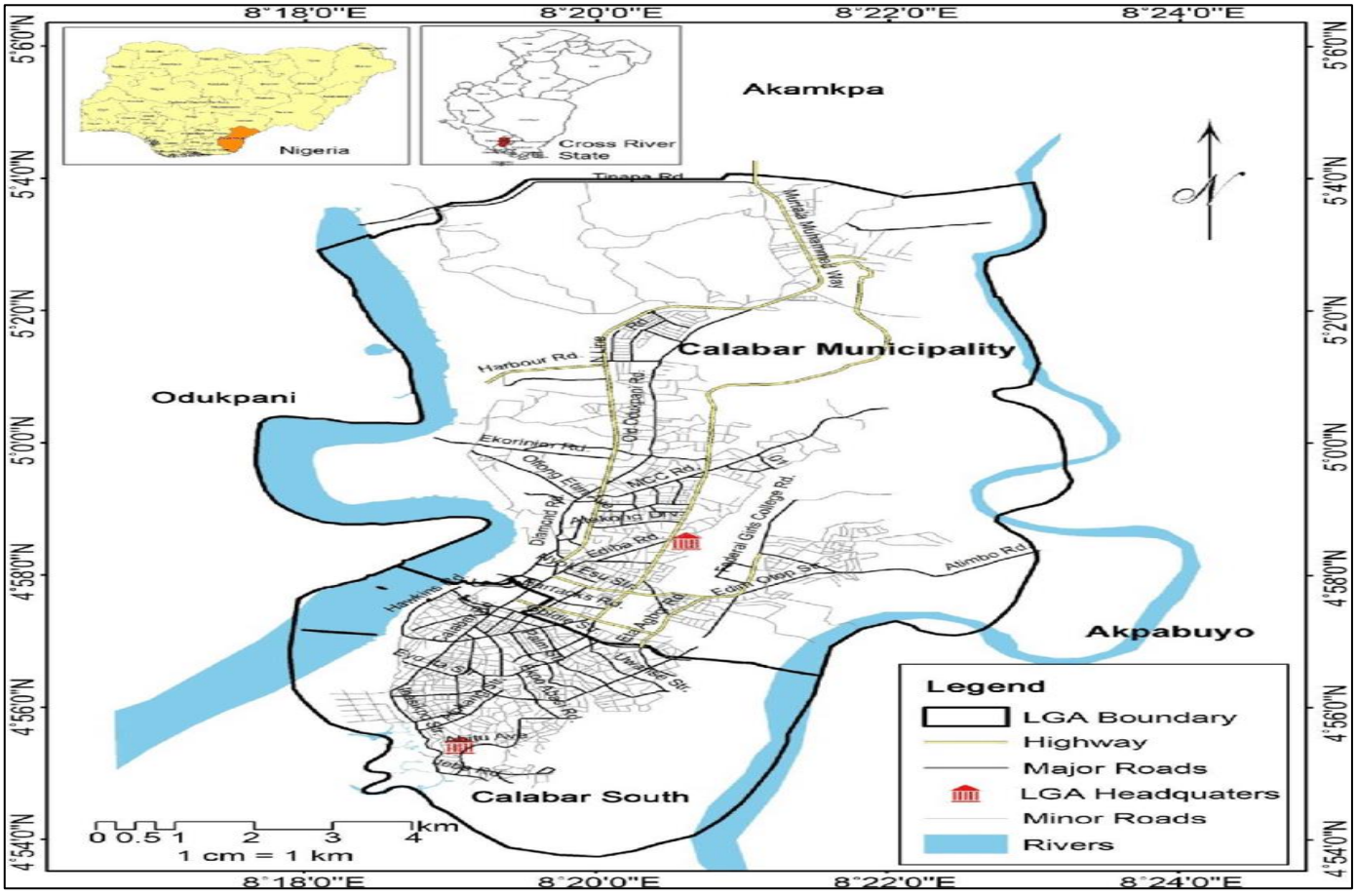

Fig 1: Map of Calabar Metropolis Showing Roads in and Out of Calabar South and Calabar Municipality Local Government Area Source: Office of the Surveyor General, Cross River (OSG-CR), 2015)

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III. MATERIALS AND METHODOLOGY FOR LABORATORY TESTS

➢ *Materials & Methods for HIV 1 AND 2 Antibody Assay*

A. Method for HIV 1 & 2 Antibody Assay

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

➢ *Determine HIV1& 2 Antibody Rapid Test Kit*
(Produced by Inverness Medical Japan Co, Ltd).

- *Principles of the Test:*

Determine HIV-1 and 2 Abs Combo is an immunochromatographic test for the qualitative detection of antibodies to HIV 1 and 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.

- *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 and 2 antibodies (Ab).

- *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.

➢ *Stat-Pak HIV 1& 2 Antibody Rapid Screening Test Kit*
(Produced by Chembio Diagnostic System Incorporation.)

- *Principle:*

The Chembio HIV 1and 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 served to demonstrate that specimens and reagents have been properly applied and have migrated through the device.

- *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 and 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.

- *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 shown that the test has been performed correctly and the device was working properly.

➢ *HIV Uni-Gold Rapid Test Kit*
(Produced and supplied by Trinity Biotech USA).

- *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.

- *Procedure of the Test:*

Specimen 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.

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The Chembio HIV 1and 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.

- *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.

B. Method for HIV I and 2 P24 Antigens Assay.

Determine HIV-1 and 2 P24 Ag/ Ab Combo test kit method (Produced by Inverness medical Japan Company, Limited) a) 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. Specimen is 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.

- *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.

- *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.

C. Data Collection and Statistical Analysis

The raw data of the results was collated, codified and subjected to statistical analysis using Statistical Package for Social Students software version 26 (SPSS Incorporation, Chicago, United State America). Data were represented with frequency and percentages while continuous data were expressed as mean plus or minus two standard deviations (X±SD). One sample Kolmogorov-Smirnov test was used to assess the normality of the data. All data were normally distributed; hence, parametric procedure was used for the statistical analysis of the data. The prevalence rate formulae were used to calculate the prevalence rate of HIV 1 and 2 infections. A two tailed p-value of <0.05 was considered indicative of a statistically significant difference. 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 Fischer exact test. Alpha value of 0.5 was used.

IV. RESULTS

A total of 400 samples were randomly collected from apparently healthy male long-distant truck drivers (LDTDs) of commercialized private and public vehicle s selected and recruited from six terminal bus stop in Calabar Metropolis, Cross Rivers State, Nigeria as shown in the following Tables 1-5 below.

Table1shows the distribution of 400 voluntary participants according to number of bus terminals, Age range, sex, turn out rate and marital status. There were 224 (56%) from male unmarried participants and 176 (44%) from male married participants. It is observed that more unmarried participants fall between the age ranges of 20-25 years while unmarried subjects fall between the age ranges of 26-30 years. The prevalence percent distribution indicates a lower age range percent distribution in married participants.

Table2 shows the distribution of blood samples randomly collected by standard method from 400 voluntary apparently healthy participants in each recruitment and HIV pre-counselling sites which were in each bus terminal or final bus stations of these long-distant truck drivers of commercialized private and public vehicle's terminals.

Table 3 shows the results of the three HIV I &2 antibody screening test kits based on marital status. Out of 400 samples 12(3%), 10(2.5 %) and 9(2.25 %) participants tested positive to HIV 1& 2 Determine, Stat-Pak, and Unigold

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antibody screening test kit tools respectively. There was no statistically significant different between the three HIV 1 & 2 antibody screening test kits tools (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.

Table 1: Distribution of voluntary participants according to number of bus terminals, Age range, sex, turn out rate and marital status of long-distant truck drivers in vehicle's terminals in Calabar municipality Cross River State, Nigeria

Demographic parameters of Participants			**Number of unmarried males that turned out**		**Number of married males that turned out**		**Total number of males that turned out**			
No of bus Terminals S	**Age Range (Years)**	**Sex**	**(f)**	**(%)**	**(f)**	**(%)**	**(f)**	**(%)**	**F-ratio**	**p-value Remark**
1	20-25	M	79	44.98	36	13.59	115	28.75	5.125	0.008 S* (P<0.05)
2	26-30	M	51	29.97	100	44.69	151	37.75		
3	31-35	M	36	20.45	60	26.78	96	24.00		
4	36-40	M	10	5.68	13	5.80	23	5.75		
5	41-45	M	00	00	10	4.64	10	2.50		
6	46-50	M	00	00	5	4.03	5	1.25		
Total (N)			**176**	**44**	**224**	**56**	**400**	**100**		

N =total number of samples, frequency =f and percentage =%, long distant truck drivers= LDTDs, Number of bus terminals selected and studied=NOBTSS

*Using t-test there was statistically significant difference between unmarried and married subjects (Calculated t- test =3.0, Degree of freedom (df) =6, alpha value =0.05, t-test critical value =1.943, Right-tail p-value is 0.012, P<0.05) and

**Using analysis of variance (ANOVA) there was statistically significant difference between age range and number of groups F-ratio=5.125, Degree of freedom (df) =6, 12, alpha value =0.05, F-test critical value =3.00, Right-tail p-value was 0.008, P<0.05)

Table 2: Distribution of voluntary participants' blood samples collected from commercial public and private LDTDs according to their marital status in vehicle's terminals in Calabar Municipality, Cross River State, Nigeria

Parameters	**Number of Samples Collected From Unmarried male**		**Number of Samples Collected From Married male**		**Total number of Samples collected from both participants**		**Calculated Chi Squared**		
Type of vehicle terminal	F	(%)	F	(%)	F	(%)	X2	p-values	Remarks
Private	76	(43.18)	100	(44.64)	176	(44)	3.10	0.013	S**
Public	100	(56.82)	124	(55.35)	224	(56)			
Total (N)	176	(100)	224	(100)	400	(10)			

N =total number of samples, frequency =f and percentage =%, long distant truck drivers= LDTDs

*Using Chi Square X2 test there was statistically significant difference between the numbers of samples collected from the two centers. Calculated Chi Square test (X2) was =5.825, at degree of freedom (df) =1, Total of sample collected (N) =400, alpha value =0.05, and Chi Square test (X2) critical value or Table value =3.84. The obtained Chi Square test x2 value (5.825) was greater than the critical value (3.84) or (X2 calculated value > X2 table value) and right-tail p-value is 0.0158 (P<0.05).

**Using Chi Square X2 test there was statistically significant difference between the numbers of samples collected from unmarried and married male subjects. Calculated Chi Square test (X2) was =, at degree of freedom (df) =1, Total of sample collected (N) =400, alpha value =0.05, and Chi Square test (X2) critical value or Table value =3.84. The obtained Chi Square test x2 value (5.825) was greater than the critical value (3.84) or (X2 calculated value > X2 table value) and right-tail p-value is 0.0158 (P<0.05).

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Table 3: Negative and Positive Results of the Three HIV I & 2 Antibody Screening Test Kits According to Married Status of Participants in Vehicle's Terminals Calabar Municipality, Cross River State, Nigeria

	HIV DETERMINE ANTIBODY TEST KIT				**HIV STAT-PAK ANTIBODY TEST KIT**				**HIV UNI-GOLD ANTIBODY TEST KIT**				**T0TAL**	
Marital status	**Number of participants tested Positive**		**Number of participants tested Negative**		**Number of participants tested Positive**		**Number of participants tested Negative**		**Number of participants tested Positive**		**Number of participants tested Negative**		**participants who reacted to both test kits**	
	f	%	f	%	f	%	F	%	f	%	f	%	f	%
Unmarried	6	(.75)	174	(44.4)	7	(1.75)	169	(42.25)	4	(1)	172	(43)	17	62.96
Married	2	(.25)	218	(55.6)	3	(.75)	221	(55.25)	5	(1.25)	219	(54.75)	10	37.04
Total (N)	8	(1)	392	(99.0)	10	(2.5)	390	(97.5)	9	(2.25)	391	(97.75)	27	100.0

N =Total Number of Samples, Frequency =f and Percentage =%

*There was no statistically significant different between the positive results of the three HIV 1 & 2 antibody screening test kits despite the disparity in the percentage positivity. (F -ratio = 1.7997, df1 = 2, df2 = 3, F-critical value = 9.55, at alpha value of 0.05, Right-tail p-value is 0.307) (p>0.05).

**Using t-test there was no statistically significant different between the mean positive results of the three HIV 1 & 2 antibody screening test kits in unmarried and married male subjects (Calculated t- test =2.5, Degree of freedom (df) =2, alpha value =0.05, t-test critical value =1.943, Right-tail p-value is s 0.06481, P>0.05).

The results in Table 4 shows the outcome of the HIV 1 & 2 P24 core antigens screening test done on the various 388, 390 and 391 subjects screened as HIV 1 & 2 antibody -negative using Determine, Stat -Pak and Unigold rapid test kits respectively .According to these results of HIV I & 2 P24 core antigens screening test kit ,it was observed that the kit was able to detects HIV I and 2 in 15(3.75%), 12(3.07%) and 10(2.55%) samples 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 also 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 unmarried group and only 1 positive sample was from the married group. A total of 162 negative samples were from the unmarried group while 211 negative samples were from the married group. For Stat -Pak there were a total of 12(3.1%) that tested positive to HIV 1& 2, 7(1.8%) were unmarried and 5 (1.3%) were married men. There were 169 (44.70%) negative for unmarried, 209(55.29%) for married group which give a total of 378 (96.9%). For Unigold there were 6 (1.5%) samples for unmarried and 4 (1%) for married groups and Total positive was 10 (2.55 %) and negative were 172 (43%) for both unmarried and married 219(54.75) and total negative of (391 (97.75%). The Total Number of subjects tested positive for both kits were 37 (7.75%) which comprises of 27 (6.75%) samples from unmarried group and 10 (2.5%) from married group and respectively.

Table 4: Frequency distribution of the results of P24 core antigen screening test kit done on the screened HIV 1and 2 antibody-seronegative sera according to marital status in Calabar Municipality, Cross River State , Nigeria

	HIV DETERMINE ANTIBODY TEST KIT				**HIV STAT-PAK ANTIBODY TEST KIT**				**HIV UNI-GOLD ANTIBODY TEST KIT**				TOTAL	
MARITAL STATUS	**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Total Number of subjects tested positive for both kits**	
	F	**%**	**F**	**%**	**F**	**%**	**F**	**%**	**F**	**%**	**F**	**%**	F	%
Unmarried	14	(1.5)	162	(42.5)	7	(1.75)	169	(42.25)	6	(1.5)	172	(43)	**27**	**(6.75)**
Married	1	(1.5)	211	(54.5)	5	(1.3)	221	(55.25)	4	(1.25)	219	(54.75)	**10**	**(2.5)**
Total (N)	**15**	**(3)**	**373**	**(97)**	**12**	**(3.1)**	**378**	**(97.5)**	**10**	**(2.25)**	**391**	**(97.75)**	**37**	**(7.75)**

N =total number of samples, frequency =f and percentage =%

*Using ANOVA there was statistically significant difference between the % positivity in unmarried and married male subjects (F - ratio = 0.10734, df1 = 2, df2 = 3 , Alpha value =0.05, F-critical value =9.55, Right-tail p-value is 0.9015)

Table 5: shows the prevalence rates of HIV 1 & 2 positive results for the three antibody test kits and p24 antigens tests according to age and marital status. 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 statistical significant (P>0.05). HIV I & /2 P24 antigens was detected in 15(3.75%), 12(3.07%) and

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10(2.55%) out of the 388, 390 and 391subjects 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 5: Frequency distribution of the results of HIV 1 and 2 p24 antigen screening test done on the HIV antibody- positive subjects according to gender in Calabar, Nigeria

	HIV DETERMINE ANTIBODY TEST KIT				**HIV STAT-PAK ANTIBODY TEST KIT**				**HIV UNI-GOLD ANTIBODY TEST KIT**				**TOTAL**			
Gender	**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Number of subjects tested Positive**		**Number of subjects tested Negative**		**Overall number of subjects that tested reactive to P24 test kit**		**Overall number of subjects that tested non - reactive to P24 test kit**	
	F	**%**	**F**	**%**	**F**	**%**	**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**		
Unmarried	2	(20)	2	(20)	2	(25)	3	(37.5)	00	(00)	4	(50)	**4**	**1**	**9**	**2.25**
Married	00	(00)	6	(80)	00	(00)	3	(37.5)	1	(12.5)	3	(37.5)	**1**	0.25	**12**	3.0
total N	**2**	**(20)**	8	(80)	**2**	**(25)**	**6**	**(75)**	**1**	**(12.5)**	**7**	**87.5**	**5**	1.25	**21**	5.25

N =total Number of Samples, Frequency =f and Percentage =%

*Using ANOVA statistical stool there was no statistical significance difference between the % positivity in female and male subjects (the calculated F-ratio =0.4096, at degree of freedom (df) =2, 3, alpha value =0.05, the F-critical value =9.55. The obtained F-ratio value (0.4096) is less than the F- critical value (9.55) and P=0.3038 at alpha value =0.05).

Table 6 shows the result of calculated sensitivity, specificity of the three types of HIV test kits. The observed sensitivity of the test kits was 85.7%, 82.4% and 73.68% for Determine, Stat-Pak and Unigold respectively while their respective specificity was 70%, 70% and 55.56%. These differences were not statistically significant (p>0.05).

Table 6: The result of calculated sensitivity, specificity of the three types of HIV test kits

Parameter	**Determine**	**Stat-Pak**	**Unigold**	**p- Value**	**Remarks**
Specificity	70%	70%	55.56%	p>0.05	NS*
Sensitivity	85.7%	82.4%	73.68%		NS**

V. DISCUSSION

The current study was designed to determine the prevalence rate of HIV p24 status of apparently healthy long-distant truck drivers (LDTDs) of commercialized private and public vehicle's terminals in Calabar municipality (that had been previously screened as HIV 1 and 2 antibody- sero-negative using three different types of HIV 1 & 2 antibody) and now are being screened using HIV p24 core antigen point of care testing (POCT) screening method. Long-haul truck drivers are individuals who operate heavy trucks and tractor-trailers (with a capacity of at least 26,000 pounds Gross Vehicle Weight) [47].These individuals are essential to the transportation of varieties of goods and materials in both the public and commercial sectors of the economy and in many industrialized countries of the world [48]. Recent studies have shown that these type of jobs are very highly demanding especially when low self-control , tight delivery schedules, no delays, and sometimes over-stressing can cause stress related sicknesses which finally cumulate or lead to poor health[49]. By occupational law, drivers of commercial private and public vehicles or trucks are permitted 14 hours of duty per day worked. They are required to take a mandatory 10-hours break before they can drive again for work. As a result, long freight delivery roads often require them to sleep away from home. Life on the road makes it more difficult to live healthy because of irregular schedules, long hours, little physical activity, limited access to healthy foods on the interstates stress [50].The results in Table 1 shows the frequency distribution of demographic parameters of 400 voluntary apparently healthy subjects according to turn out rate, level of awareness of HIV infection, age and marital status in Calabar, Nigeria. There was a high turnout rate for voluntary counselling and testing (VCT) among married group of long distant truck drivers (LDTDs) than the unmarried group long distant truck drivers (LDTDs).The reasons for this result is because of high level of awareness of voluntary counselling and testing (VCT) and good knowledge ,good perception ,good attitude ,good preventive majors and good practices of HIV I and 2 by the married groups before their respective marriages .Another reason for differences in this result is that married group were mindful or afraid of the wives and children and the damaging impacts and effects HIV I and 2 that they might have already seen on the life of other infected victims .On the other hand unmarried group have had no premarital experience ,no wives and children to worried about and hence they never bothered to show up. These results were in line with of that of [51] who had a similar observation in Indian [52]. However, these results were not in line with that of [53]. This might be so because of evolution in the epidemiological update and aggressive fight against the HIV I and II infection. Similarly, the results in Table 2 shows the frequency distribution of voluntary apparently healthy participants and their blood samples collected from the commercialized public and private LDTDs vehicle's terminals according to their marital status in Calabar, Cross River State, Nigeria. There were more participants in then married group of public long-distant truck divers than private groups. Our results were in line with the studies of the following researchers who had advanced

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three theories for the explanation of these results. The first is that there is the general conception that the public companies are more lucrative than the private companies in eyes of Nigeria population and the theory that more unmarried groups are usually in the private sector than in public sector which is in line with [54]. Secondly, it is on records that public companies usually pay more salary than private companies [55] and since more married men need increase wages or higher salary because of their family's financial burdens, they will usually prefer to join the public companies and hence more married men are involved. In sharp contrast to this, the unmarried groups of men are not bothered whether it is private or public companies .The third theory is that the private companies preferred employing unmarried men than married men .however ,these results were not in line with the study of [56] .The results in Table 3 are based on the national HIV tests algorithm in Nigeria that have been recommended by WHO,2021 [57], which employed three different HIV toolkits, the first is considered as the baseline usually DetermineTM and Sat-Pak and Unigold which are tiebreakers .The results obtained were not in line with previous studies [58 ,59,60] and reasons for this result was due to low level of knowledge existing between HIV married men and unmarried individuals [61].In spite of the disparities of the results of three individual test kits there was an overall no different in the two groups of participants and this was in line with study of [62] and the discordant rate was also not low but insignificant which is also in line with the study of [63]. The results obtained in Table 4 were based on the recommendations of WHO, 2021 and these results were perfectly in line with previous studies published by [64,65].The reason for this results were due to exceptionally long window period by some of individuals though having HIV antibody sero-negative status were still in the process of sero-convertion .This may have been attributed to the some individuals having the status of non-progressors and having certain blood group types [66].The results of the prevalence of HIV I and II antibody test kits shown in Table 5 were higher than the current prevalence of HIV antibody test kits results in the Cross Rivers State, Nigeria which have been reduced drastically from 2.1% to 1.7% [67].The reasons for these observed differences were expected and have been attributed to the aggressive measures, strategic effort needed to step up effort to reduce the prevalence, furthermore, if possible eradicate it through aggressive publicity campaign and follow-up of existing cases, situation room monitoring that have put in by Cross River state Ministry of Health for an outstanding performance which has led to drastic and significant reduction in the prevalence and spread of HIV/AIDS in the state. In addition to this organizations like USAID, FHI360 and SIDHAS project and NACA have made a great contributions and support from partners like USAID working through FHI360, on SIDHAS Project. Also other partners like UNICEF, AHF, Hartland alliance Jepigo etc, have been actively involved in this process [68-70].The HIV I & II P24 antigen test result were higher than expected and the reasons were due to exceptionally long window period by some of individuals though having HIV antibody sero-negative status were still in the process of sero-convertion .This may have been attributed to the some individuals having the status of non-progressors and this agrees with observations made by [66].The results in Table 6 shows the various calculated values of the sensitivity and specificity of the various antibody screening test kits and p24 in the current study as already defined and reported by [71] and [72] .The differences in this results were due to improve of decades of aggressive study of human immunodeficiency virus (HIV) infection, prevention, transmission, and significant advances in HIV/AIDS laboratory diagnosis, treatment and management in the developed and developing countries The results are line with that of [73-77].

VI. CONCLUSIONS

In the current study, the use of P24 antigens/antibody Point of Care testing kits had not only improved detection of the HIV infection but had also demonstrated and implicated the discrepancies associated with the routine antibody screening testing panel kits alone in subjects at early stage of HIV infection and hence serving as eye-opener where there is still a lukewarm and indifferent attitude of implementing both panels in routine utilization for the index populations of these individuals under study and as well as other populations with long HIV window period.

RECOMMENDATIONS

From the findings of this study it is recommended that all focal persons in all centers where all HIV 1 and 2 tests are done, HIV1 and 2 p24 antigen screening or any other fourth generation test kit should be used alongside with HIV antibody screening test which is currently used for routine work, to reduce any possibility of transfusion of HIV 1 and 2 infected blood or false negative results.

We also strongly recommend that government should make it as a policy that HIV1 and 2 p24 antigen screening or any other fourth generation test kit should be used alongside with HIV antibody screening test which is currently used for routine work in all public and private institutions at all levels of health care and service centres.

AUTHORS' CONTRIBUTIONS

- Conception of study: FJN, (2) Design of study: FJN IIE & OCJ, (3)Sample analysis: FJN, EWO, ASE (4) Data analysis: FJN, EWO & IIE; (5) Statistical analysis: FJN, EWO ,JOOO and AIS ; (6) Initial manuscript draft: FJN, EWO ,EPC and OJM, (7) All authors read and approved the final manuscript.
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ACRONYMS (ABBREVIATION)	FULL MEANING	ACRONYMS (ABBREVIATION)	FULL MEANING
AIDS	Acquired Immune Deficiency Syndrome	PCR	Polymerase Chain Reaction
ARVs	Anti-Retroviral Drugs	PEPFAR	President's Emergency Fund For AIDS Relief
BYSACA	Bayelsa State AIDS Control Agency	PHC	Primary health care
CDC	Centre of Disease Control	$P < 0.05$	P-values indicating the Level of significance
CD4	Cluster of Differentiation 4	PMTCT	Prevention of Mother-to-Child Transmission
CHOs	Community Health Officers	$P > 0.05$	P-values indicating the Level of no significance
WHO	World Health Organization	P24	HIV 1 and 2 core protein
Emtct	Elimination of Mother-To-Child Transmission	PV	Prevalence rate
FCT	Federal Capital Territory	Rh	Rhesus Blood Groups System
FMOH	Federal Ministry of Health	SACA	State Agency for the Control of AIDS
FHI	Family Health International	Se,Se	Pair of dominant alleles for the secreting genes controlling secretors
GF	Global Fund	Se,se	Pairs of recessive alleles for the secreting genes controlling non secretors
GH	General Hospital	SD	Standard Deviation of the Mean
GHAIN	Global HIV/AIDS Initiative in Nigeria	SPSS	Statistical Package for Social Science
HTC	HIV Testing and Counseling	SASCP	State AIDS and STD Control Programme
(HREC)	Health Research Ethical Committee	SIDHAS	Strengthening Integrated Delivery of HIV/AIDS Services
HIV	Human Immuno-deficiency Virus	T	Student independent test
LGA	Local Government Area	UCTH	University Of Calabar Teaching Hospital
MTCT	Mother-to-Child Transmission	UN	United Nations
NACA	National Agency for Control of HIV/AIDS	UNAIDS	United Nations Joint Programme on HIV/AIDS
NASCP	National AIDS and STD Control Programme	UNICEF	United Nations Children Emergency Fund
NGOs	Non-Governmental Organizations	USAID	United States Agency for International Development
NPHCDA	National Primary Health Care Development Agency	WHO	World Health Organization
NAT	Nucleic Acid-based Tests	X^2	Pearson Chi Square Statistics
RT-PCR	Real time Polymerase Chain Reaction tests		

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