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RESEARCH ARTICLE

RDT BASED DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) AMONG BLOOD DONORS AT SPECIALIST HOSPITAL, SOKOTO

Isiaka Bala Barga[1], Aliyu Kane[1], Shu'aibu Muhammad Gobir[2], Yusha'u Shu'aibu Baraya[2], Ahmad Rufai Umar[2], Alhassan Musa Mohammed[2], Bashar Umar[2] and Muhammad Habibu Yahya[2]

1. Sultan AbdulRahman School of Health Technology Gwadabawa. Sokoto State.
2. Usmanu Danfodiyo University Sokoto, Department of Biochemistry.

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Abstract

Hepatitis B infection is a major global health problem and a potentially life-threatening infection that attacks liver cells caused by Hepatitis B virus and it is characterised by liver cirrhosis, liver scarring, liver failure or cancer of the liver as the disease progresses. This study was carried out to determine theRDT based detection of hepatitis B surface antigen (HbsAg) among blood donors at specialist hospital, Sokoto, Sokoto State Nigeria. Two hundred (200) centrifuged sera from blood donors who have given prior verbal consent to participate in the study were screened for HBsAg using rapid diagnostic test (RDT). The result obtained shows that, 51 out of the 200 blood donors which represented (25.5%) were reactive while 149 were non-reactive. With Chi-square analysis only blood donors that are none previous blood donors showed a significant association with HBV infection, (p-value 0.05). This study however, indicates that, hepatitis B infection occurs in one out of every four blood donors. It also observed that the virus antigen is highly endemic among blood donor and these blood donors can serve as potential reservoir for the transmission of the virus to susceptible host individuals in the study area.

Introduction:-

Hepatitis B infection is a major global health problem and a potentially life-threatening infection that attacks liver cells caused by Hepatitis B virus and it is characterised by liver cirrhosis, liver scarring, liver failure or cancer of the liver as the disease progresses (Isah*et al.*, 2015). The disease is highly contagious and has been found to be fifty to one hundred (50-100) times more contagious and infectious than humans immunodeficiency virus and it is ten (10) times more infectious than hepatitis C virus, because it replicates profusely and produces high titter in the blood (10^8-10^{10}virions/ml). The virus can resist antibody product by the defence system, only people who have been vaccinated successfully or those who have developed HBs antibodies after hepatitis B infection are immune to hepatitis infection (Kaoje*et al*., 2018). Hepatitis B virus enters the liver through the bloodstream and its replication occurs only in the liver tissues (cytoplasm of the hepatocytes) thereby interfering with the liver function (Samuel, 2010). Hepatitis B virus has been detected in peripheral mononuclear cells, tissues such as pancreas, spleen, kidney and body fluids like blood, saliva, semen, sweat, breast milk, faeces, urine and vaginal secretion (Isah*et al.*, 2015).

Corresponding Author:- Isiaka Bala Barga
Address:- Sultan Abdul Rahman School of Health Technology Gwadabawa. Sokoto State.

There are high risk groups for hepatitis B virus infection; these include parenteral drug users, institutionalized parsons, health care personnel, organ transplant patients, blood transfusion and haemodialysis patients and staff that are highly promiscuous and infants born to infected mothers (Isah*et al.,* 2015). Hepatitis B virus infection is mostly asymptomatic but in few cases the infection is symptomatic often with high morbidity and mortality (WHO, 2012). The virus (HBV) may establish a chronic infection especially in those infected as infants. Signs and Symptoms of an infection include: itchy skin, body aches, joint pains, fatigue, loss of appetite, nausea, vomiting, dark urine and jaundice (yellow colouration of skin and eyes). The highest concentrations of infectious Hepatitis B virus are found in blood and serum. However, other body fluids, such as semen and saliva have also been reported to be infectious (Colins *et al.,* 2016).

There is a high level of occurrence of blood demanding health conditions in many parts of sub-Saharan Africa. In Nigeria, the increase in road accidents, pregnancy-related haemorrhages, armed robbery attacks, and violent events, increase the possibility of the transmission of HBV (and other blood-borne pathogens) through contaminated blood as reported by United Nations System in Nigeria (UNSN 2001) (Uneke*et al.,* 2005). Blood transfusion service (BTS) is an integral and indispensable part of the healthcare system. The priority objective of blood transfusion service (BTS) is to ensure safety, adequacy, accessibility, and efficiency of blood supply at all levels (Islam, 2009). Transfusion of blood and blood components, as a specialized modality of patient management saves millions of lives worldwide each year and reduces morbidity. It is well known that blood transfusion is associated with a large number of complications, some are only trivial while others are potentially life threatening, demanding for meticulous pre-transfusion testing and screening. The use of unscreened blood and ineffective blood screening transfusion keeps the patient at risk of acquiring many transfusion transmitted infections (TTI) like hepatitis viruses (HBV, HCV), human immune-deficiency viruses (HIV), syphilis, malaria (Khan *et al.,* 2007). Hepatitis B virus infection is a potentially life threatening liver infection caused by the hepatitis B virus, in some people, the hepatitis B virus can cause chronic liver infection and puts people at high risk of death from cirrhosis and liver cancer. However, some people have acute illness with symptoms that last several weeks, including yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain (Idioha *et al.,* 2010).

Materials and Methods:-

The study was carried out for a period of four month (February to April) in 2018. The study was conducted in Specialist Hospital Sokoto. The hospital is located in Sokoto South Local Government, Sokoto state. According to Sokoto State Diary SSGOD(2015) the state is located between latitudes 11^0 30`and $13^0$ 50`N and longitudes 4^0 and 6^0 E. Sokoto state shares border with Niger Republic to the north eastern part of the state. It shares common boundaries with Kebbi state to the west and Zamfara state to the south. The total land area is about 32,000 square kilometers, with total population, according to provisional figures of 2006 census, of 3,696,999 people (SSGOD, 2015). The state is characterized by alternating wet and dry seasons with a short spell of cold period (Harmattan) which usually lasts from December to February, with 3 to 4 months rainfall (from June to September or October). The duration and intensity of rainfall increase from north to south and environmental temperature ranges from 21 to 42^0C and highest of (47 ^{O}C) recorded in April and the lowest 13 ^{0}C recorded in January (Newtrack india.com, 2018). Culturally, the state is homogenous. The people of the state are Muslims and Islamic religion provides them with code of conduct and behaviours, their mode of dressing is also in line with Islamic tenets (Garba, 2015). The state has an estimated population of more than 4.5 million as according to census, (NPC, 2006)

Study population

The study included male and female blood donors which comprises two hundred (200) participants during the study time. Individuals with no history of hepatitis B infection or prior treatment against hepatitis B virus infection attending Sokoto State Specialist Hospital for blood donation were recruited and screened for the study.

Ethical consideration

Ethical approval was obtained from Institutional Ethical Review Committee of the Specialist Hospital and the Ministry of Health, Sokoto State.

Inclusion criteria and exclusion criteria

The blood donors of both sexes aged (18 and above years) who have given prior verbal consent to participate in the study and attendees off specialist hospital sokoto were included.

Theexclusion criteria were the individuals who are less than 18 and are non-attenders of specialist hospital, sokoto were excluded.

Sample collection
Blood samples were collected aseptically using venepuncture method, a tourniquet were applied about two inches above the elbow or above the forearm and the site of puncture was disinfected using 70 % ethyl alcohol vigorously and allowed to dry (Jeon *et al*., 2011).Five millilitre of blood was collected using a 5 ml syringe into a properly labelled vacutainer plane bottles and transported to the central research laboratory, Usmanu Danfodiyo University for analysis.

Sample processing
Blood specimens were allowed on the bench for 30 minutes to allow coagulation after which the serum was separated by centrifuged at 1000 rpm for 5 minutes. Pasteur pipettes were used to carefully transfer the supernatant (sera) to plane cryovials. All serum samples were analysed for HBsAg using rapid strip test based on the principle of one-step test (Aspen, one strip Hepatitis B-surface antigen Test strip, USA),

Hepatitis B virus rapid strip test
Hepatitis B virus (HBsAg) strips test were used to detect the reactive and non- reactive hepatitis B virus surface antigen (HBsAg) among blood donors, the samples were analysed following standard procedures as described by the manufacturer. Each serum was screened for the presence of hepatitis B virus antigen (HBsAg) using one step test strip, which is a rapid chromatographic immunoassay for qualitative determination of HBV antigen in the serum

Principles of the test
This test device is a chromatographic lateral-flow immunoassay, including membrane pre -coated with anti-HBsAg antibodies and a pink coloured pad with colloidal gold reagents labeled with anti-HBsAg antibodies. The membrane has two coated lines: a test line (T line) and a control line (C line). At the T line, a pink coloured band develops if HBsAg is present in the specimen tested. If antigen is not present, no T line will develop. At the C line, a pink colored band should appear regardless of the presence of HBsAg. The C line serves as an internal qualitative control indicating that an adequate volume of solution added to the test system (according to manufacturer specification Instant-View HBsAg One-step serum test. ALFA Scientific Design USA).

Assay Procedure
The test strips were removed from the sealed pouch it was vertically immersed in the serum for 10 seconds. Then, removed and placed on a clean, flat, non-absorbent surface were allowed to stand for 15 minutes. The result was read between control and patient line (according to manufacturer specification).

Statistical analysis
Data obtained were recorded on excelspread sheet which was subsequently analysed using statistical Graph Pad software. Descriptive statistics of socio-demographic variable were computed. The prevalence was determined at 95% confidence interval (CI). A *p* value less than 0.05 was calculated to be statistically significant. The statistical difference was also evaluated by applying the Chi-square test. All statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software package version 10 (SPSS Inc. Chicago, USA).

Results:-

Interpretation of result
Observation for band information as done and results recorded. At the T line, a pink coloured band developed in the present of HBsAg in the specimen tested. But in the absence of antigen, no T line was developed. At the C line, a pink coloured band should appear regardless of the presence of HBsAg.

A total of two hundred (200) of milliter blood sample of blood donors were analysed for seroprevalence of hepatitis b surface antigen which indicate the presence of HBV in blood. The result of this study revealed the overall prevalence of hepatitis B Sero-antigen among the blood donors to be 25.5%.were 149 tested negative for hepatitis B surface antigen.

Result obtained in relation to gender shows that, 51 were positive cases, 48 among them were male while 3 were female with 27.9% and 10.7 respectively (Table 4.1).

Distribution of hepatitis b sero-antigen among blood donors in relation to marital status shows that, out of two hundred (200) samples subjected to general screening using rapid strips test, one hundred and forty-one 141 were married while 59 were unmarried. Out of the 141 married study subjects, 35 were positive for HBV (24.8%) of the 59 unmarried donors 16 were positive for HBV (27.1%), (Table 4.2).

Hepatitis B sero-antigen detected in serum sample of intravenous drugs users, were 6/22 (27.27%) were intravenous drug user blood donors while one hundred and seventy-eight 178 blood donors were non intravenous drugs user. Out of which 45 were positive cases of hepatitis B sero antigen with 25.2% prevalence (Table 4.3).

Out of the two hundred study subject, 123 had no history of previous blood donation, and 31 were positive for 27.2% prevalence while 77 had history of blood donation, out of which 20 were positive cases with an incidence of 25.9% respectively **(**Table 4.4**).**

The age distribution of Hepatitis B virus sero-antigen among blood, among (200) subjects, subjected to general screening using rapid strips test base on the principle. It was observed that, majority belong to the age group of 15-30 years with proportion percentage of 32.0%, flowed by age group 31-45 years and 45 above with percentage of 21.5% and 21.6% respectively (Table 4.5**)**.

Hepatitis B virus sero-antigen among blood donors in relation to occupational status (Table 4.6**)** shown that, hepatitis b sero-antigen positive cases were detected in 24 business persons (24/93) with 25.8% flowed by civil servants (16/66) 24.2% and others (11/41) 26.8% respectively.

Table 4.1:- Gender distribution of Hepatitis B sero-antigen among the study population.

Gender distribution	**Number tested**	**Reactive**	**Non-reactive**	**Proportions (%)**
Male	172	48	124	27.9
Female	28	03	25	10.71
Total	200	51	149	25.5

The result shows that Male were 48/172 (27.9**%**). Female were 03/28 (10.7**%**) positive. x^2 = 3.747, DF = 1, p-value 0.053, odd ratio 0.310 and 95% C I = 0.087- 1.075. Statistically not significant.

Table 4.2:- Marital status-based distribution of HBV infection among study population.

Marital Status	**Number tested**	**Reactive**	**Proportions (%)**
Married	141	35	24.8
Single	59	16	27.1
Total	200	51	25.5

The result shows that Married were 36/141 (24.87**%**) positive and single were 16/59 (27.11**%**) positive respectively.

Statistically using chi-square shows that x^2 = 0.115, DF = 1, p-value 0.734, old ration 01.127 and at 95% C I = 0.5 – 2.246 not significance

Table 4.3:- Hepatitis B sero antigen distribution among indiscriminate intravenous drugs users.

Intravenous-drugs users	**Number tested**	**Reactive**	**Proportion (%)**
No	178	45	25.28
Yes	22	06	27.27
Total	200	51	25.5

The result shows that indiscriminate intravenous drugs uses were 6/22 (27.27**%**) positive and non-intravenous drug uses were 45/178 (25.28**%**) positive respectively. Chi-square shows that x^2 = 0.041, DF = 1 and p-value 0.840 at 95% C I = 0.4—3.005 which is not significant.

Table 4.4:- Hepatitis B sero antigen among study populations with history of previous blood donation.

Previous blood donors	Number tested	Reactive	Proportion (%)
No	123	31	27.2
Yes	77	20	25.9
Total	200	51	25.5

The result shows that previous blood donation were 20/77 (25.97**%**) positive and Never blood donation were 31/123 (27.20**%**). Chi-square shows that $x^2 = 0.903$, DF = 1 and p-value 0.015, old ration 1.041and C I 0.543—1.999 is significance.

Table 4.5:- Age distribution of Hepatitis B sero antigen among study populations.

Age	Number Tested	Reactive	Proportion (%)
15-30	75	24	32.0
31-45	65	14	21.5
>45	60	13	21.6
Total	200	51	25.5

The result shows that age range between15 - 30 were 24 (32.0**%**) positive 31 - 45 were 14 (21.5 **%**) while 46 an above 13 (21.5**%**) reactive and percentages.

Table 4.6:- Occupational status distribution of Hepatitis B sero-antigen (HBsAg) among study population.

Occupation	Number tested	Reactive	Proportion (%)
Civil Service	66	16	24.2
Business	93	24	25.8
Others	41	11	26.8
Total	200	51	25.5

The result shows that civil servants were 16 (24.2 **%**), Business were 24 (25.80**%**) and others were 11 (26.8 **%**) reactive and their percentages.

Discussion:-

Hepatitis B virus is considered one of the most common viral infection spread through blood transfusion; therefore, Safety of blood products is a critical factor for successful blood transfusion as it decreases the incidence of post transfusion hepatitis B infection. Out of two hundred (n-200) samples screened using HBsAg rapid strip test, 51 study subjects tested reactive, giving an overall prevalence of 25.5%. This implies that hepatitis B is positive in one out of every four blood donors in the study site. The value obtained in the present study is regarded as high as described by World Health Organisation classification of assessment of the severity sof hepatitis B virus infection in hepatitis B virus endemic countries. WHO defines low prevalence to be less than 2%, moderate 2-8% and high when values are greater than 8% (WHO, 2012). The result obtained in this study is similar to 25.7% obtained by Bada (1996) in Ilorin kwara state Nigeria and 26.5% observed by Mustapha and Jibrin, (2004), in Gombe, Nigeria but it is much higher than the 9.02% that obtained by Mohiadeen*et al.* (2014) yemen. 10.9% reported by Busari*et al.* (2017) Ibadan Nigeria, and 14.0% reported by AlaoOlusayo*et al.*, (2009). 14.0% and 23.4% reported by Musa *et al.*,(2013) in an prevalence of hepatitis B in Nigeria. These differences might be by using difference methods, it might also be related to the fact that, the infection tend to vary from one locality to another and from one country to another and also differ in associated risk factors. The value obtained in this study is far less than 42.1% reported by Kristen *et al.* (1999) and 38.1% reported by Alkali *et al.* (2017), in Sokoto, these could be attributed to the differences in the sampling population and associated risk factors. For instance, Alkali *et al.* (2017) studied in inmates in correctional facilities who were at high risk of harbouring infectious diseases, unlike the study subject individuals sampled in this study who are more likely blood donors. Thus, it is not unexpected if the incidence rate is lower in this population. Gender specific prevalence of hepatitis B virus infection among the study subject blood donors, revealed out the prevalence of 12.0% (03/28) for females and 38.7% (48/172) for male. Therefore, there was tune out of male individuals with high frequency in blood donation than female during the period of this study. The Male might be a carrier of this infection which might be easily transferred during blood donation, in considering they were in high frequency in blood donation than females in the study area. This finding agrees with that of Kouassi*et al.* (2011) and Pennap(2011) in a tertiary institution in Nigeria where results obtained showed a higher prevalence of HBsAg

among male than female. This result is higher than an incidence of 24.7% male and 28.2% incidence obtained by Mustapha and Jibrin (2004) in Gombe, it also deferred from the prevalence of 93.6% male and 6.4% in females reported by Wasfi and Sadek*et al*. (2011) in Alexandria Egypt. This could be attributed to the higher percentage of male to females study subjects in the study. The result obtained in this study is higher observation made by Lavanya*et al.* (2012) WHO reported an incidence of 3.5% among all the study subject male individuals, but no female donors were positive for the virus. In a similar study by Uneka (2005) in Jos Nigeria, 12.9% for female and 14.3% for male were observed to be positive. The differences might be due to differences in the inclusion criteria. Therefore, the finding in this study may be explained by the fact that male preponderance common and more obvious in blood donation than female.

In relation to Marital status, highest prevalence was recorded among Single 27.11% (16/59) compared to married donors with prevalence of 24.82% (35/141) blood donors which was statistically not significant (x^2= 0.115, df=1, p-value=0.734. odd ration 01.127 and at 95%CI=0.5-2.246) respectively. However, this result contradicts to the finding of Okwesili*et al*. (2014) in Minna, Niger State, Nigeria who reported that Married blood donors were significantly as risk for HBV with proportion percentage (78.6%) compared to singles with (26.5%) blood donors at p-value (p=0.0001). This may be explained by the fact that promiscuity and unprotected sexual behaviours among single unmarried individuals might be higher than among the married in the study area. Therefore, increasing the risk of acquiring the viral infection is due to their inability to stick to only one sexual partner. The implication is that, it will be easier for them to transmit the hepatitis B sero antigen during blood donation.

The prevalence of hepatitis B in relation to previous blood donors was 27.97% (20/77) while 25.20% (31/123) for non-previous blood donor with statistically significance, x^2= 0.903, df =1, p-value 0.015, odd ration 1.041 and 95% CI=0.543-1.999 respectively. This finding may be due to the fact that, none previous blood donors had lack of knowledge on the issues concerning the infection. The result may be attributed to the lack of awareness on hepatitis B infection and lack of medical check-up since they never participated on blood donation.

Distribution of hepatitis B virus infection among Intravenous drugs user was 06/22 with incidence 27.27% while 25.28% (45/178) for non-intravenous drug users with x^2= 0.041, df=1and p-value 0.840 at 95% CI = 0.4 – 3.005 respectively. The six number of intravenous drugs user ware drugs editors. It serves as route of transmission of hepatitis B. This may be possible among those who confessed to engage in sharing of injections during drug addiction than none intravenous drug user. Therefore, those intravenous drugs users had ability to acquire hepatitis B sero antigen and might serve as reservoirs of an infection.

The distribution of hepatitis B sero antigen in relation to occupational status, Hepatitis B incidence was significantly higher among others occupational 26.83% (11/41) followed by business 25.81% (24/93) and lowest among civil servant with 24.24% (16/66) (p=0.01). The result is contradicted to Okwesili*et al.* (2014) who's reported that Hepatitis B prevalence was significantly higher among civil servants 6(42.9%) and farmers 4(28.6%) and lowest among traders and students 2(14.3%) (p=0.01) might be due to differences in variable.

The prevalence of Hepatitis B virus in relation to age year, the prevalence was highest among younger donors in the 15-30 years age group compared to study subject in the 31-45 and 45 and above years age groups (p=0.01). The result agrees with the findings by Okwesili*et al*. (2014) who reported the highest incidence among younger donors in the 18-28 years age group, compared to study subjects in the 29-38- and 39-48-years age groups.

The prevalence of twenty-one point zero (21.0%) were detected among 91 non-haemolyse serum after hepatitis B surface antigen (HBsAg) ELISA technics shows that, the infection ware escape the RDT test but it can be detected using ELISA technics as confirmatory test. This finding might be as a result of specificity and sensitivity rate between the RDT and ELISA technics. However, the finding may also be due to other reasons, such as variation in the sensitivity of HBV screening kits and methodological quality of the studies and indeed, in study sample sizes. The result is high than 8% prevalence finding by Offid*et al.* (2011), in Calaba Metropolis, 2.2% Mosab *et al,*.2017 in Atbara, Sudan and 12.3% by Musa *et al,*.2013Hepatitis B infection systematic review in Nigeria. This result shows that, hepatitis b infection transmission in blood transfusion occurs at window period in a study area if depending on strip test RDT during blood donation. Among the variable, higher incidence of Hepatitis B was reported among the male individual and non-intravenous drug users with 81.8%, female and intravenous drugs users with 18.0% and 18.8% in this study. Furthermore, hepatitis b HBsAg were revealed out that, non-previously in

blood donation with 72.7%, previous blood donation with 27.2%. Therefore, using ELISA here may serve as confirmatory test, it will serve as a factor of transferring the infection from blood donors during blood donation.

Three (3) out of the eleven Elisa samples showed HBsAg profile. Two of the samples showed;HBeAb, HBcAb and HBsAg positive, which indicates that the presence of the virus may be as a result of vaccination or the patient is in the recovery state from a recent infection while among all subjected samples viral envelop antigen HBeAg not presence, it shows that no viral in blood circulation while the remaining seven sample were negatives for HBeAg.

Conclusion:-

It is clear from this study we found that sero-proportion percentage of Hepatitis B viral antigen among blood donors to be 25.5% using RDT, it indicate that hepatitis B infection occurs in one out of every four blood donors, and one out of every eight none reactive donors from the RDT screening. The study has also observed that the virus antigen is highly endemic there are many unvaccinated blood donor, based on this, these blood donors can serve as potential reservoir for the transmission of the virus to susceptible host individuals in the study area.

Recommendations:-

From this study, following recommendations are suggested;
General vaccination, mass immunization public health education should be carried out and compulsory blood screening of Hepatitis B virus of blood donors before blood transfusion is very necessary and should be maintained as routine.

Conflict of interest;
The authors declare no conflicts of interest in this work.

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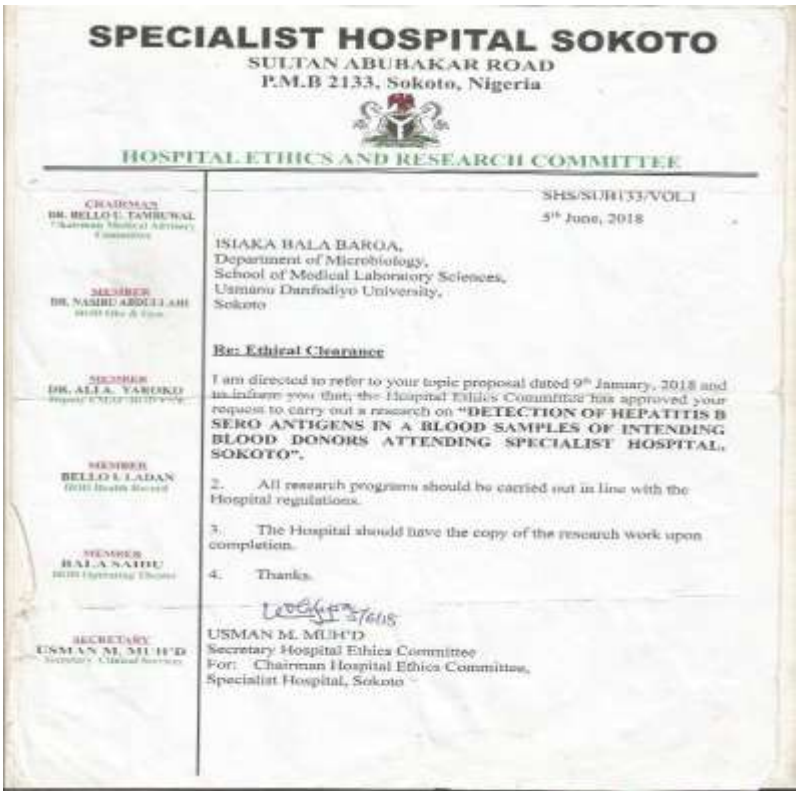
Appendix I:-



SPECIALIST HOSPITAL SOKOTO
SULTAN ABUBAKAR ROAD
P.M.B 2133, Sokoto, Nigeria

HOSPITAL ETHICS AND RESEARCH COMMITTEE

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MEMBER
BALA SAIDU

SECRETARY
USMAN M. MUH'D

SHS/SUB133/VOL.1
5th June, 2018

ISIAKA BALA BAROA,
Department of Microbiology,
School of Medical Laboratory Sciences,
Usmanu Danfodiyo University,
Sokoto

Re: Ethical Clearance

I am directed to refer to your topic proposal dated 9th January, 2018 and to inform you that the Hospital Ethics Committee has approved your request to carry out a research on **"DETECTION OF HEPATITIS B SERO ANTIGENS IN A BLOOD SAMPLES OF INTENDING BLOOD DONORS ATTENDING SPECIALIST HOSPITAL, SOKOTO".**

2. All research programs should be carried out in line with the Hospital regulations.

3. The Hospital should have the copy of the research work upon completion.

4. Thanks.

5/6/18

USMAN M. MUH'D
Secretary Hospital Ethics Committee
For: Chairman Hospital Ethics Committee,
Specialist Hospital, Sokoto

Ethical Approval

Appendix Vi:-

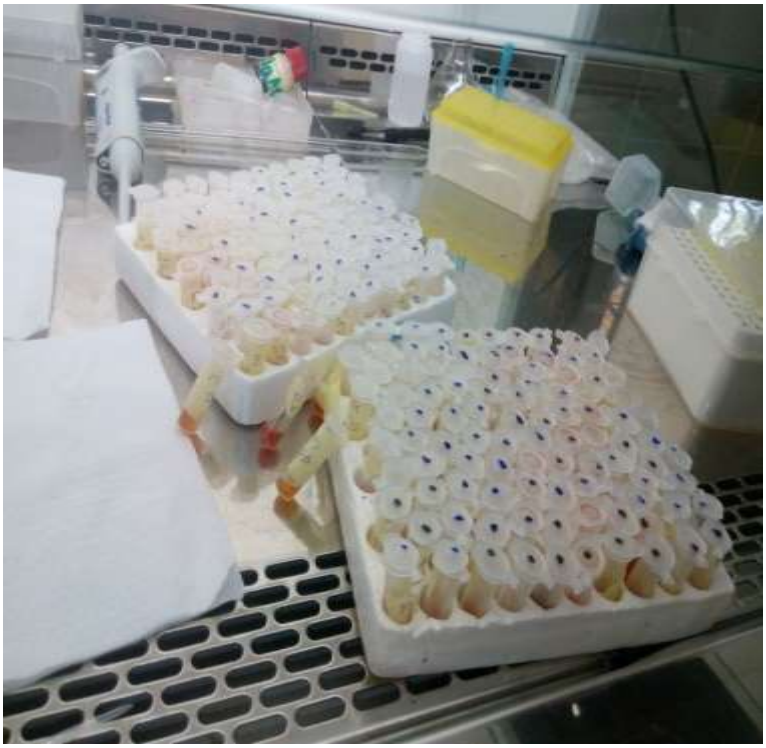

Plate 3:-Two hundred serum samples for RDT test.

Appendix Vii:-