

SJMLS-3(4)-001

Compromise in Rapid Test Kit Makes It Unsuitable for Blood Transfusion SafetyMuhibi, M.A.^{*1,2}, Ifeanyiichukwu, M.O.², Oluogun, W.A.³, Jeremiah, Z.A.⁴

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Abstract

In developing nations, blood safety continues to be a major dilemma due to high prevalence of infectious agents among blood donors compounded with the problem of limited resources that preclude the use of sensitive and specific technologies for screening of blood and blood products. The aim of this study was to compare the performances of rapid diagnostic kits to stringent enzyme-linked immunosorbent assays (ELISA) in the detection of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) among prospective blood donors in Osun State, Southwest Nigeria. A total of 321 prospective blood donors from 3 senatorial districts in Osun State were recruited for the study after obtaining ethical clearance from LAUTECH Teaching Hospital Ethics Review Committee. Oral informed consent was obtained from each participant. The modified National Blood Transfusion Services (NBTS) questionnaire was administered to every participant to obtain information on exposure to risky behaviors, among others. Venous blood was collected and used for blood typing and viral screenings. Screening for HBsAg, HCV and HIV was done using rapid test kits (RTK) and ELISA. Data obtained were subjected to statistical analysis using SPSS version 20.0 to generate frequency and central tendencies while Pearson's Chi square was used for comparison of variables. Sensitivity, specificity, efficiency, positive predictive value and negative predictive value of the rapid test kits were obtained against ELISA. Prevalence of HBsAg, HCV and HIV by RTK were 8.65%, 0.64% and 1.6% respectively; while

ELISA revealed prevalence of 8.97%, 0.64% and 2.24% for HBsAg, HCV and HIV respectively. The results obtained by RTK showed a compromised sensitivity, efficiency and negative predictive value. Gender and exposure to risky behaviours have no effect on the prevalence of all the Transfusion Transmissible Infections (TTIs) in the population studied. HBsAg rapid test kit revealed 100% specificity with sensitivity of 96.55% and HCV rapid kits had the same efficiency with third generation ELISA kit. With respect to HIV, the sensitivity of RTK was 77.78%, when validated against fourth generation ELISA kit. It could be concluded from this study that the prevalence of TTI's as determined by ELISA is higher, when compared to results generated by RTK method. Thus, there is residual risk of transmitting HBV and HIV to blood recipients, if blood units are screened using RTK only. Stringent ELISA procedures will bring the risk to the best achievable minimum when combined with administration of standard donor's questionnaire.

Keywords: Blood Safety, ELISA, RTK, Sensitivity, Blood Donors.

Introduction

It is undisputable that the First World War acted as a catalyst for the rapid development of blood banking and transfusion services. Canadian Lieutenant Lawrence Bruce Robertson was instrumental in persuading the Royal Army Medical Corps to adopt the use of blood transfusion at the Casualty Clearing Stations for the wounded. In October 1915, he performed his first wartime transfusion with a syringe to a

patient suffering from multiple shrapnel wounds. He followed this up with four subsequent transfusions in the following months and his success was reported to Sir Walter Morley Fletcher, director of the Medical Research Committee. Robertson published his findings in the *British Medical Journal* in 1916 and, with the help of his colleagues, was able to persuade the British authorities of the merits of blood transfusion. Robertson went on to establish the first blood transfusion apparatus at a Casualty Clearing Station on the Western Front in the spring of 1917 (Kim, 2001). All these practices went on without screening for viral diseases that could be transmitted through transfusion, as little was known about most transfusion-transmissible agents then (Koate *et al.*, 2015; Salawu *et al.*, 2010; Halim and Ajayi, 2000).

Transfusion-transmissible infections (TTIs) are infections transmissible from person-to-person through parenteral administration of blood or blood products (CDC, 2004). Examples of known TTIs include: Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), Human Immunodeficiency Virus (HIV), *Treponema pallidum* and *Plasmodium species*. Transfusion may also transmit the Human T-Lymphotropic Virus-1 and II (HTLV-1 and II) in some places like, the French West Indies, the Cytomegalovirus (CMV) but only in patients with weak immunity, and West Nile Virus (Terry *et al.*, 2011; Rouet *et al.*, 2004). A TTI may also result from new or emerging infectious agents, for which there is biologic plausibility of person-to-person transmission. Such an example is the agent responsible for new variant Creutzfeldt-Jacob Disease (Peden *et al.*, 2004).

Blood and organ donation constitute an essential element in modern health care system. While donation exercises are intended to be life saving, a qualitative in vitro screening test for individual human donors, including donors of whole blood and blood components, and other living tissues is needed in order to prevent disease propagation through transfusion and transplantation. Inadequacies in blood safety and supply contribute significantly to the burden of disease and loss of life. Safety of blood transfusion is of extreme importance in order to avoid any serious

morbidity or mortality of the recipient of blood units and products. It has been established that screening of blood donors for TTIs during donors' assessment stage will help to reduce the spread of TTIs through blood transfusion (Muhibi *et al.*, 2017). It remains a question, whether the widely used rapid test kits are efficient enough to achieve the goal of identifying all positive blood donors for deferment.

Aim

The aim of this study was to assess the performances of rapid diagnostic kits in the detection of transfusion-transmissible viruses; using stringent ELISA techniques as the gold standards.

Methodology

Study Population

This study was carried out in Osun State which is an inland State in South-Western Nigeria with Osogbo as the capital city. It is bounded in the north by Kwara State, in the east partly by Ekiti State and partly by Ondo State, in the south by Ogun State and in the west by Oyo State. The major sub-ethnic groups in Osun are Ife, Ijesha, Oyo, Ibolo and Igbomina of the Yoruba people, although there are also people from other parts of Nigeria, with the presence of tribes like Hausas, Ibos, Edos and nationals of countries like Ghana and Togo who have settled there. According to the 2006 National Population Census, the population of the state was put at 3,423,535 (NPC, 2007).

Study Design and Subjects

The study was an epidemiological study which captured major Blood Banks where blood donation exercises and blood units and products storage are being carried out in large scale in Osun State. Prospective apparently healthy blood donors were randomly selected during pre-donation screening exercise at the blood donation centers. After securing ethical clearance from Ladoke Akintola University of Technology Teaching Hospital Research Ethics Committee, the biodata of all consenting blood donors were obtained by administering validated questionnaire adapted from National Blood Transfusion Services (NBTS) template.

Sample Size

The sample size was determined using the statistical formula $(n=pq(z)^2/e^2)$ (Hulley and Cummings, 2001) and with prevalence of HBsAg reported by Salawu *et al.* (2010), adopted for all the TTIs.

The sample size for the 3 centers involved was 321. The centers enrolled were Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ladoke Akintola University of Technology Teaching Hospital and Witness Medical Laboratory, Ede.

Sample Collection

Five milliliters (5ml) of venous blood was collected from each participant by venepuncture into vacuum EDTA tube. The ABO and Rh 'D' blood types of each blood donor were determined by tube method. Within 2 hours of collection, the samples were centrifuged at 3000rpm for 5 minutes to harvest plasma which was then transported at a temperature of $<10^{\circ}\text{C}$ and frozen immediately on arrival at -20 to -25°C . These samples were later tested for HIV, HCV and hepatitis B surface antigen (HBsAg) by rapid testing and ELISA methods.

TTIs Screening

The national algorithm for HIV screening, which involves the use of rapid test kits serially (Determine HIV 1/2 test kit and Unigold test kit) for serological testing, was adhered to. This was followed by the use of fourth generation ELISA kit, as gold standard in this research work. The Manufacturers' Standard Operating Procedures (SOPs) were adhered to.

Hepatitis B surface Antigen (HBsAg) and anti-HCV rapid test kits supplied by the Global Diagnostic were used for HBV and HCV screenings respectively. The kits employ a specific monoclonal and polyclonal antibody directed towards the virus by double sandwich principle in a solid phase membrane. Chromogen embedded moves by diffusion to bring about colour to be read visually at test band region where there is Ab-Ag-Ab complex. Simultaneously, human immunoglobulins present in the plasma are captured by anti-human

globulin antibodies on the control region and the complex.

HBsAg and HCV ELISA kits (DIAPRO, Italy) using antibody “sandwich” ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg and HCV respectively. Participants' sera were added to the microwell together with a second antibody conjugated with the enzyme horse radish peroxidase (the HRP-Conjugate) and directed against a different epitope of HBsAg and HCV. After washing to remove non-specific plasma proteins and unbound HRP-conjugate antibody, Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide were added to the wells. In presence of the antibody-antigen-antibody (HRP) “sandwich” immuno-complex, the colorless TMB Solution are hydrolyzed by the bound HRP-conjugate antibody to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity was measured and it is proportional to the amount of antigen captured in the wells. Wells containing samples negative for the virus remain colorless.

Statistical Methods

The data generated were coded, entered, validated and analyzed using Statistical Package for Social Science (SPSS) version 20.0. The sero-epidemiology of HBsAg, HCV and HIV were expressed for the entire study group and by social demographic variables and risky behaviors of participants. Comparisons between the groups were done using Pearson Chi-Square. P-values <0.05 were considered significant. The sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for the RTKs were calculated.

Results

A total of 312 blood donors who consented to participate in this study made up of 264 males adults (84.6%) and 48 females (15.4%). A total of 144 (46.2%) participants in the age bracket (28-37) years constituted the majority in the age group categorization. There was no Rh-negative subject among the participants but the AB Rh 'D' positive subjects were 6 (1.9%). Two hundred

and four (65.4%) of the donors were O Rh 'D' positive and this group constituted the majority in blood types categorization (Table 1). Prevalence of HBsAg, HCV and HIV by rapid test kits (RTK) were 8.65%, 0.64% and 1.6% respectively; while ELISA revealed prevalence of 8.97%, 0.64% and 2.24% for HBsAg, HCV and HIV, in that order. There was no concomitant infection detected among the participants (Table 2).

When subjected to Pearson's chi square test, HIV screening by forth generation ELISA method

showed a significant difference ($p=0.001$) in prevalence, when age group is considered as independent variable (Table 3).

HBsAg rapid test kit revealed 100% specificity while the sensitivity of 96.55% was reported (Table 4). HCV rapid kits had the same efficiency with 3rd generation EIA kit with sensitivity, specificity and predictive values as 100% (Table 5). With respect to HIV, the sensitivity of RTK is severely compromised to 77.78%, when validated against ELISA (Table 6).

Table 1: Socio-demographic variables of blood donors

Variable	Frequency	Percentage
Age group		
18-27yrs	100	32.1
28-37yrs	144	46.2
38-47yrs	46	14.7
48-57yrs	13	4.2
58-65yrs	09	2.9
Gender		
Male	264	84.6
Female	48	15.4
Marital Status		
Single	114	36.5
Married	188	60.3
Divorced	8	2.6
Widowed	2	0.6
Educational Status		
Illiterate	17	5.4
Primary Education	55	17.6
Secondary Education	143	45.8
Tertiary Education	97	31.1
Occupation		
Engaged	252	80.8
Not engaged	60	19.2
Blood Type		
A Rh 'D' Positive	50	16.0
B Rh 'D' Positive	52	16.7
AB Rh 'D' Positive	06	1.9
O Rh 'D' Positive	204	65.4
Total	312	100.0

Table 2: Prevalence of TTIs among Blood Donors in Osun State

	HBsAg-RTK	HBsAg-EIA	HCV-RTK	HCV-EIA	HIV-RTK	HIV-EIA
Positive	27	28	2	2	5	7
Negative	285	284	310	310	307	305
Total	312	312	312	312	312	312
Percent	8.65	8.97	0.64	0.64	1.60	2.24

Key:

TTIs = Transfusion Transmissible Infections
 HBsAg = Hepatitis B surface Antigen
 RTK = Rapid Test Kit
 EIA = Enzyme Immuno Assay
 HCV = Hepatitis C Virus
 HIV = Human Immunodeficiency Virus

Table 3: Influence of age group on prevalence of TTIs by different methods

Parameter	N	Df	Relative standard	p-value
HBsAg-RTK	312	4	.05	.213
HBsAg-EIA	312	4	.05	.296
HCV-RTK	312	4	.05	.646
HCV-EIA	312	4	.05	.646
HIV-RTK	312	4	.05	.219
HIV-EIA	312	4	.05	.001

Key:

Df = Degree of freedom

Table 4: Evaluation of the HBsAg RTK against ELISA

Test	HBsAg-RTK Method		Total
	Positive	Negative	
HBsAg-EIA Positive	27	1(c)	28(a)
HBsAg-EIA Negative	0(b)	284	284(d)
Total	27	285	312

Key:

a= number of true positives
b= number of false positives
c= number of false negatives
d= number of true negatives

Therefore;

Variable	Percentage
Sensitivity	96.5
Specificity	100.0
Efficiency	99.68
PPV	100.0
NPV	99.65

Table 5: Evaluation of the HCV- RTK against ELISA

Test	HCV-RTK Method		
	Positive	Negative	Total
HCV-EIA Positive	2	0(c)	2(a)
HCV-EIA Negative	0(b)	310	310(d)
Total	2	310	312

Key:

a= number of true positives
b= number of false positives
c= number of false negatives
d= number of true negatives

Therefore;

Variable	Percentage
Sensitivity	100.0
Specificity	100.0
Efficiency	100.0
PPV	100.0
NPV	100.0

Table 6: Evaluation of the HIV- RTK against ELISA

Test	HIV-RTK Method		
	Positive	Negative	Total
HIV-EIA Positive	5	2(c)	7(a)
HIV-EIA Negative	0(b)	305	305(d)
Total	5	307	312

Key:

a= number of true positives

b= number of false positives

c= number of false negatives

d= number of true negatives

PPV = Positive predictive value

NPV = Negative predictive value

Therefore;

Parameter	Percentage
Sensitivity	77.78
Specificity	100.0
Efficiency	99.36
PPV	100.0
NPV	99.35

Discussion

This study revealed high prevalence of transfusion-transmissible infectious agents by rapid test kits (RTK) –HBsAg: 8.65%, HCV: 0.64%, HIV: 1.6%- and higher frequencies by enzyme immuno assay (EIA) yielding 8.97% and 2.24% for HBsAg and HIV respectively. We say the prevalence is high because it shows high probability of blood recipients being transfused with TTV positive blood if blood units are not properly screened before transfusion.

These findings partly agree with the findings of Baba *et al.* (2000) in Maiduguri; Mustapha *et al.* (2002) in Gombe; Chukwurah and Nneli (2005) in Enugu and Buseri *et al.* (2009) in Osun State of Nigeria where high prevalence of TTVs were reported. WHO estimated that 5% to 10% of HIV transmission resulted from transfusion of contaminated blood and blood products (WHO, 2000 and 2007).

The higher prevalence of HBsAg observed in this study where majority (>70%) of the positive subjects are youth in the age range 18-37 is alarming. This is in contrast to the findings of Mustapha and Jibrin, (2004) who found the highest rate of HBsAg seropositivity (41.6%) in the 40-49 years age group. The result agrees with the observation that majority of carriers of HBV in sub-Sahara Africa are youths (Kiire,1996).

The findings from this study indicates that the conventional RTK-based assays revealed prevalence rates lower than that obtained by

stringent ELISA techniques. This compromised efficiency seen with the conventional RTK-based assays is similar to what was reported previously by other researchers (Erhabor *et al.*, 2014; Comanor and Holland, 2006; Heyns *et al.*, 2006; Zanetti *et al.*, 2006; Velati *et al.*, 2005; Satku,2004; Allain *et al.*, 2003; Giachetti *et al.*,2002; Stramer *et al.*, 2000). That sensitivity, efficiency and percentage negative predictive value in respect of HBsAg were compromised to 96.55%, 99.68 and 99.65 respectively. This means that the kit may miss a positive sample in error. The sensitivity of HIV- RTK (77.78%) is seriously compromised in screening blood donors, when validated against forth generation EIA kit which has potential to detect p-24 antigens in addition to the antibodies against HIV 1& 2. Majority of the donors in this society are not voluntary donors whose previous statuses are known, therefore, there is a greater likelihood that first timer donor in this society might be in window period.

Conclusion

It could be concluded from this study that the prevalence rates for HBsAg were 8.65% and 8.97% by RTK and ELISA respectively. The prevalence of HCV is 0.64% by the 2 methods employed; while HIV by RTK and ELISA yielded 1.6% and 2.24% respectively. Results obtained by RTK show a compromised sensitivity, efficiency and negative predictive value. Thus, when donors are certified suitable

by RTK, there is still residual risk of transmitting the TTIs through transfusion.

The use of ELISA based techniques for screening blood donors for TTIs should be enforced in all stand alone and hospital-based blood banks. Efforts should be geared towards evolving a sustainable register of voluntary, non-remunerated donors for the nation through strengthening of National Blood Transfusion Services (NBTS). Consistent sentinel survey should be sustained and reviewed with the focus on efficiency of technologies being employed in the sentinel survey.

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Citation: Muhibi, M.A., Ifeanyiichukwu, M.O., Oluogun, W.A., Jeremiah, Z.A. Compromise in Rapid Test Kit Makes It Unsuitable for Blood Transfusion Safety. *Sokoto Journal of Medical Laboratory Science*;3(4):5 - 13

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