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## Hepatitis C Virus Diagnosis in Prospective Blood Donors: Epidemiology, Optimal Testing Approach and Treatment Cut-offs

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### Abstract

Hepatitis C virus (HCV) infection has been noted a major public health problem with no vaccines available currently. This study aims at optimizing 'safe blood' practice by advancing HCV testing beyond Diaspot rapid enzyme immunoassay in current use in Nigerian Health Institutions. Between August, 2014 and November, 2015, a total of 300 blood donors' plasma samples were screened with both Diaspot and HCV Ag-Ab enzyme-linked immunosorbent assay techniques. HCV-RNA confirmation and quantification were performed with real-time polymerase chain reaction (PCR). Alanine aminotransferase (ALT) was performed on confirmed positive blood donor for treatment purpose. The overall gender ratio and mean age of blood donors screened for HCV were 1.5:1 and  $27.67 \pm 7.77$  years respectively. Of the 300 blood donors screened, 5 (1.67%) and 1 (0.33%) were seropositive for HCV on the basis of Diaspot and enzyme-linked immunosorbent assay (ELISA) techniques respectively. Diagnostic odds ratio showed that ELISA is nearly 9-fold a better diagnostic tool compared to Diaspot technique. Real-time PCR assay confirmed positivity of 1 (0.33%) of the blood donor for hepatitis C. HCV-RNA viral load and plasma ALT of the lone sample were 133, 209 IU/mL and 11.1 IU/L respectively. HCV prevalence among blood donors in Ekiti state is low, 0.33%. Enzyme-linked immunosorbent assay technique should be the starting point of HCV serologic screening and a surrogate technique for real-time PCR. The development of workable algorithm to reduce risks associated with blood transfusion and enhances both blood donors' and recipients' safety is highly imperative.

**Keywords:** Hepatitis C virus, prospective blood donors, Diagnosis, Diaspot, Real-time PCR

## 1. Introduction

Hepatitis C Virus (HCV) is a major public health problem and a leading cause of chronic liver disease worldwide [1-2]. It is a single-stranded positive-sense RNA virus with a diameter of about 50nm, and a genome of approximately 9.6 kilobases [3]. HCV belongs to the genus Hepacivirus in the family of Flaviridae. HCV is more than four-fold infectious compared to human immunodeficiency (HIV) virus and requires less exposure than HIV to cause infection [4]. Studies revealed that more than 10 types and 70 subtypes of HCV genotype has been described [5] and recent study reported type 1 and 2 in Nigerian subjects [6]. HCV has seven non-structural proteins which include p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. The structural proteins form the viral particle and include the core protein and the envelope glycoproteins E1 and E2 [7]. Owing to its rapid replication and the high rate of error insertion of the RNA-dependent RNA polymerase, HCV spontaneously mutates within a given infected individual to form a related but distinct “quasispecies” [8]. The generation of these mutants appears to be one of the key mechanisms by which HCV escape the host’s immune response, maintaining persistent infection [9]. Very importantly, the replication cycle of the HCV occurs totally in the cytoplasm and - once the replication is stopped - the virus can be cleared from the cells and thus the infection definitively cured.

### 1.1 HCV epidemiology and mortality reports

An estimated 170 million people are infected with HCV globally and annually, 3-4 million new HCV infections are reported yearly. Almost 500,000 individuals were estimated to have died from HCV-related liver disease in 2010 [10]. 75-85% of newly infected persons develop chronic infections and 60-70% of chronically infected persons develop chronic liver disease, 5-20% develops cirrhosis, 1-5% dies from liver cirrhosis or cancer [11]. In many of the developed countries, HCV prevalence is < 2% including the United States of America and ≥ 2% in some countries in Africa. An overall regional HCV prevalence of 5.3% and approximately 32 million people have

been reported in Africa according to the World Health Organization with Egypt being the leading country with HCV prevalence of 17.5% [13]. In 25% of liver cancer patients, the underlying cause is HCV. HCV in the Western region of Africa including Nigeria is considered to be highly endemic.

### 1.2 HCV transmission routes and blood transfusion risks

Direct percutaneous inoculation has been recognized as the most efficient mode of transmission of HCV in regions with ≥ 2% HCV prevalence [12]. Transfusion of blood and blood products, sexual and vertical transmission, tattooing as well as medical and surgical risk factors has been reported [13-17]. In Africa for instance, only 19% of blood is being screened for blood transfusion due to limited resources and cost of laboratory tests and posts a lot of risks for recipients of blood transfusion [18]. Intravenous drug injection is the most common route of HCV transmission in regions with < 2% HCV prevalence. Despite the fact that blood transfusion is known as a risk factor of acquiring HCV infection, it is still being used as a means of life-saving therapy for myriads of transfusion-dependent patients to reduce morbidity and mortality and save thousands of lives. Although introduction of screening tests for HCV in blood donors has led to further reduction in HCV transmission through blood and blood products, it still remains a public health problem. Lack of vaccine, the inconsistency in screening procedures, lack of confirmatory diagnosis in blood donors due to associated costs and long turnaround time, and non-availability of workable algorithm for HCV as for HIV testing have led to possible variations in reports including under-diagnosis and under-reporting as well as over-estimated HCV endemicity among blood donors from country to country [7, 19-23]. Most hepatitis C viral diagnosis in Nigeria has been on the basis of detectable antibodies to the virus in human serum or plasma by Diaspot technique with varied conclusions [19, 24-26]. This study is aimed at advancing HCV screening beyond Diaspot rapid technique by testing with the third generation Genscreen HCV Ag-Ab Monolisa kit by ELISA technique and confirming diagnosis

with Real-time PCR. Alanine aminotransferase (ALT) was performed for confirmed blood donors for treatment purpose.

## 2. Materials and Methods

### 2.1 Sample collection, storage and transportation

Five millilitres (5mL) of K<sub>3</sub>EDTA anticoagulated samples were collected from prospective blood donors following an informed consent. Plasma was separated according to Clinical and Laboratory Standard Institute's guideline [26] and aliquot into three separate plain containers. Samples not run immediately were frozen at -40 to -70°C and transported in cold chain till analysis.

### 2.2 Study design

This is a descriptive cross-sectional study meant to evaluate the epidemiology of hepatitis C virus among prospective blood donors and establish diagnosis through confirmation of HCV status with advanced techniques.

### 2.3 HCV diagnosis

Anti-HCV antibody screening was performed with Diaspot rapid technique and subsequently repeated with ELISA technique using Genscreen HCV Ag-Ab Monolisa kit (Biorad, Marnes-la-Coquette-France) according to the manufacturers' instructions. Positive/discordant results were confirmed for HCV-RNA with real-time PCR assay. HCV-RNA quantification and plasma ALT were performed for positive sample using real-time PCR quantitative assay and Randox technique respectively. These were performed using the third aliquot plasma sample.

### 2.4 Informed consent and ethical clearance

Prospective blood donors who gave informed consent were administered questionnaires to prospective blood donors to obtain their social demographic characteristics and research information. Ethical clearance was obtained from the Ethics and Research Committee of the Federal Teaching Hospital, Ido Ekiti.

## 2.5 Statistical analysis

Results were analyzed using mean and standard deviation, percentage, t-test of the Statistical Package for Social Sciences (SPSS Inc., Chicago, USA)

## 3. Results

### 3.1 Gender ratios, age groups, religious and educational status of prospective blood donors screened for HCV antibody

Between August 2014 and November, 2015, 300 of the prospective blood donors were screened for hepatitis C virus antibody using Diaspot one-step enzyme immunoassay (EIA) and repeated with Genscreen HCV Ag-Ab technique. 222 (74.0%), 69 (23.0%) and 9 (3.0%) of the population screened were voluntary, replacement and paid blood donors respectively. The overall mean age ( $\pm$ SD) and male: female ratio of PBD w  $27.67 \pm 7.77$  and 1.5:1. Based on the population of each category of blood donors screened, the mean age ( $\pm$ SD) and male: female ratios of VBD, RBD and PBD were  $26.77 \pm 7.79$  and 1:1;  $30.70 \pm 7.39$  and 10.5:1; and  $21.22 \pm 0.44$  and 3.5:1 respectively. However, comparing the mean age and male: female ratios based on blood donor categories, replacement blood donors had the highest ( $30.70 \pm 7.39$  and 10.5:1). Similarly, of the 300 PBD screened, 252 (84.0%) of prospective blood donors were Christians. According to blood donor categories, 185 (83.3%), 61 (88.4%) and 7 (77.8%) of VBD, RBD and PBD respectively were Christians while the rest were Islamic faithfuls. Over 84% (84.3%) of the total number of PBD had tertiary education. Only 13.3% had secondary education. The rest were less than 5%. Comparing the blood donor categories, predominantly, most VBD (90.5%) had tertiary education compared to two-thirds (66.7%) of RBD and PBD. While there were 3 (1.4%) of PBD without formal education among VBD, the least level of education among RBD and PBD were primary and secondary schools respectively. Table 1 below presented the results.

### 3.2 Marital status and blood donation history of prospective blood donors screened for HCV antibody

Table 2 showed that, overall, nearly 60% (58.0%) of the PBD were singles while 48 (16.0%), 6(2.0%) and 1(0.3%) were married, widowed and divorced/separated respectively. 134 (60.4%), 84 (37.8%), 3 (1.4%) and 1 (0.5%) of VBD were singles, married, widowed and divorced/separated

respectively. RBD had a different pattern showing more married individuals than singles (49.3% compared to 46.4%). Only, 3 (4.3%) were widows. Nearly 90.0% (88.9%) of PDBD were singles and 1 (11.1%) was married. Of the overall PBD, 168 (56.0%) were first timers while 132 (44.0%) were previous donors and these were distributed among VBD, RBD and PDBD categories.

**Table 1:** Gender Ratios, age groups, religious and educational status of blood donors screened for HCV antibody

Social Demog	Overall PBD n (%)	VBD n (%)	RBD n (%)	PDBD n (%)
<b>Sex</b>				
Male	181	111	63	7
Female	119	111	6	2
<b>Male: Female Ratio</b>	1.5:1	1:1	10.5:1	3.5:1
Mean age (Mean ± SD)	27.67 ± 7.77	26.77 ± 7.79	30.70 ± 7.39	21.22 ± 0.44
<b>Age Range</b>				
16-25	133 (44.3)	108 (48.6)	20 (29.0)	5 (55.6)
26-35	112 (37.3)	78 (35.1)	30 (43.5)	4 (44.4)
36-45	48 (16.0)	31 (14.0)	17 (24.6)	0 (0.0)
46-55	5 (1.7)	3 (1.4)	2 (2.9)	0 (0.0)
56-59	2 (0.7)	2 (0.9)	0 (0.0)	0 (0.0)
<b>Religion:</b>				
Christianity	252 (84.0)	185 (83.3)	61 (88.4)	7 (77.8)
Islam	48 (16.0)	37 (16.7)	8 (11.6)	2 (22.2)
<b>Education:</b>				
None	3(1.0)	3(1.4)	0(0.0)	0(0.0)
Primary	4(1.3)	1(0.5)	3(4.3)	0(0.0)
Secondary	40(13.3)	17(7.7)	20(29.0)	3(33.3)
Tertiary	253(84.3)	201(90.5)	46(66.7)	6(66.7)

Abbreviation: VBD, Voluntary Blood Donors; RBD, Replacement Blood Donors; PDBD, Paid Blood Donors

### 3.3 Statistical comparison of the diagnostic accuracy of HCV serologic techniques using AmpliPrep/COBAS TaqMan PCR as the Gold Standard

Table 3 showed the comparison of HCV antibody results of Diaspot one-step rapid EIA and Biorad HCV Ag-Ab Monolisa assay using statistical indices including diagnostic odds ratio which is known as the single indicator of test performance as

well as a measure of the effectiveness of a diagnostic test<sup>[27]</sup>. Diaspot showed that 5 (1.67%) of the PBD were seropositive for HCV antibody. When repeated with Genscreen HCV Ag-Ab Monolisa kit, the result was 1(0.33%), thus resulting in inconclusive results. Real-time PCR assay was used as the gold standard technique. Sensitivities and negative predictive values of both Diaspot rapid one-step EIA assay and sandwich ELISA assay

were 100.0% but they differ in specificities and positive predictive values. The latter had 100.0% specificity while the former had 99.70%. Similarly, the latter had PPV of 100.0% while the former technique had 20.0%. The result was further validated with the use of diagnostic odds ratio which was independence of prevalence. Diagnostic odds ratios (DORs) for both techniques were undefined but both false positives and false negatives were zero for Genscreen HCV Ag-Ab Monolisa assay (indicating a perfect test) while only false negatives were zero for Diaspot one step EIA. On adding 0.5 to all cells in the contingency table

(to minimize bias that might be introduced), Sandwich ELISA showed similar sensitivity with Diaspot (75.00%), higher specificity (99.66% compared to 97.20%), higher positive predictive value, negative predictive value (99.20%, 89.71% and 99.89% respectively) compared to Diaspot (97.20%, 25.00% and 98.50% respectively) and nine-fold DOR (1764.7) compared to Diaspot rapid enzyme immunoassay (196.1). The statistical findings of the ELISA assay were similar to those of COBAS AmpliPrep/COBAS TaqMan PCR (CAP/CTM).

**Table 2.** Marital status and blood donation history of prospective blood donors screened for HCV antibody

Social Demog	Overall PBD n (%)	VBD n (%)	RBD n (%)	PDBD n (%)
<b>Marital Status:</b>				
Single	174(58.0)	134 (60.4)	32 (46.4)	8 (88.9)
Married	48 (16.0)	84 (37.8)	34 (49.3)	1 (11.1)
Widowed	6 (2.0)	3 (1.4)	3 (4.3)	0 (0.0)
Divorced/ Separated	1 (0.3)	1 (0.5)	0 (0.0)	0 (0.0)
<b>Blood Donation History:</b>				
Never Donated (First timers)	168 (56.0)	132 (59.5)	31 (44.9)	4 (44.4)
Donated (Previous Donors)	132 (44.0)	90 (40.5)	38 (55.1)	5 (55.6)

Abbreviation: VBD, Voluntary Blood Donors; RBD, Replacement Blood Donors; PDBD, Paid Blood Donors

**Table 3.** Statistical comparison of HCV antibody serologic screening techniques using COBAS Ampliprep/COBAS TaqMan PCR (Real-time PCR) as the gold standard

Technique	TP	FP	TN	FN	SS	SP	PPV	NPV	DOR	Prevalence (%)
DIASPOT	1.0	4.0	295.0	0.0	100.0	98.7	20.0	100.0	Infinity	1.7
Sandwich ELISA	1.0	0.0	299.0	0.0	100.0	100.0	100.0	100.0	Infinity*	0.3
CAP/CTM	1.0	0.0	299.0	0.0	100.0	100.0	100.0	100.0	Infinity*	0.3
Computed Data for Adjusted DOR										
Technique	TP	FP	TN	FN	SS	SP	PPV	NPV	DOR	Prevalence (%)
DIASPOT	1.5	4.5	295.5	0.5	75.0	98.5	25.0	98.5	196.1	1.7
Sandwich ELISA	1.5	0.5	299.5	0.5	75.0	99.2	89.7	99.9	1764.7	0.3
CAP/CTM	1.5	0.5	299.5	0.5	75.0	99.2	89.7	99.9	1764.7	0.3

\*Infinity means perfect test

Abbreviation: TP, True positives; TN, True Negatives; SS, Sensitivity; SP, Specificity; FP, False Positives; FN, False Negatives; PPV, Positive Predictive Value; NPV, Negative Predictive Value; DOR, Diagnostic Odds Ratio

**Table 4.** Confirmatory diagnosis hepatitis C virus with Real-time PCR

Technique (Ser/Mol)	Overall PBD Screened n(%)	HCVAb <sup>+</sup> n (%)	HCVAb <sup>-</sup> n (%)	HCV-RNA, DT n (%)	HCV-RNA, NDT N (%)	HCV-RNA VL (IU/mL)
Diaspot EIA	300 (100.0)	5	295(98.33)	-	-	-
ELISA Assay	300(100.0)	1(0.33)	299(99.67)	-	-	-
CAP/CTM PCR (QL)	300(100.0)	-	-	1(0.33)	299(99.67)	-
CAP/CTM PCR (QQ)	1(0.33)	-	-	-	-	133,209

Abbreviation: Ser, Serology; Mol, Molecular; PBD, Prospective Blood Donors; n (%), Absolute number (Percentage); -, Not performed; DT, Detected; NDT, Non-detected; HCVAb<sup>+</sup>, Seropositive Hepatitis C Virus antibody; HCVAb<sup>-</sup>; Sero negative Hepatitis C Virus antibody; HCV-RNA, Hepatitis C Virus ribonucleic acid; PCR, Polymerase Chain Reaction; CAM/CTM, COBAS Ampliprep/ COBAS TaqMan; VL, Viral load; QL, Qualitative Technique; QQ, Quantification Technique; IU/mL, International Units/ millilitre

### 3.4 Confirmatory Diagnosis Hepatitis C Virus with Real-time PCR

Of the 300 PBD screened for HCV within the study period, 5 (1.67%) were seropositive with Diaspot rapid one-step enzyme immunoassay. HCV seroprevalence by ELISA technique using Genscreen HCV Ag-Ab Monolisa kit was 0.33% (i.e. only one seropositive case). Hence, there were four discordant results. Real-time PCR is recognized as the confirmatory test for HCV by detecting HCV-RNA in serum/plasma of infected persons. Further analysis of the donors' samples with COBAS Ampliprep/COBAS TaqMan PCR showed that 1 (0.33%) was positive for HCV-RNA. The only HCV positive blood donor was a male VBD. Quantification of the HCV-RNA (viral load) of the confirmed donor sample showed HCV viraemia of 133,209 IU/mL. Serum ALT of the blood donor was 11.1IU/L (normal range: Up to 20IU/mL). The results of the confirmatory and quantification assay were presented in Table 4 below.

## 4. Discussion

Of the three hundred PBD screened during the study period for HCV antibody, the results showed that almost three-quarter 222 (74.0%) of the PBD were voluntary blood donors. This is line with the current advocacy of the World Health Organization for promotion of 100% voluntary blood donation [28-29]. The strong collaboration between Federal Teaching Hospital and functional NBTS was a

contributing factor. The male: female ratio of 1:1 observed among the VBD in this study compared to RBD (10.5:1) and PDBD (3.5:1) could, beyond other factors, be traceable to more female first timers among the population of VBD due to the knowledge of the PBD on giving blood to save lives and recruitment benefits of having HCV antibody screened and confirmed without cost burden. Study finding on male: female ratio of 1:1 among VBD differed from the research outcomes of Olokoba *et al.* [30]. where most of the blood donors (> 98.0%) were males. It is slightly lower than 1.1:1 reported by Mavenyengwa *et al.* (2014) in Namibia VBD [31]. However, male: female ratio among the RBD (10.5:1), or 91.1% male blood donors conform to those of other researchers. Discovery of lower male: female ratio (3.5) among paid blood donors compared to RBD showed some females also engaged in commercialization of blood.

The most common age group of PBD was 16-25 years (44.3%) while the least was 56-59 years (0.7%). Age groups of VBD followed similar pattern observed in the overall PBD screened while the pattern in RBD was slightly different. The most common age group in VBD was 16-25 years (48.6%) in contrast to 26-35 years (43.5%) among the RBD. The outcomes of Mohammed and Beleke's study which was predominantly among RBD corroborated our observations among RBD in this study while it was in contrast to the patterns among VBD [32].

Furthermore, study showed that higher percentage of Christians than Muslims participated

irrespective of blood donor category and that reflected the predominance of Christians in Ekiti state and their corresponding interest in giving blood to save lives. Similar study in Ilorin showed that higher percentage of Christians (70.8%) gave blood than Muslims [33]. Most of the PBD had formal education. High percentage of educated individuals enrolled in this study irrespective of blood donor category similarly reflected the focus of the NBTS in targeted population, underlying superstitious and cultural beliefs among individuals without formal education and low public enlightenment which hinder participation in blood donation. With respect to educational levels of PBD, Shenga *et al* (2010) and Alfouzan *et al* (2014) published corroborative results showing that history of blood donation was steadily increasing with the increase in the educational level of participants [34-35].

Moreover, more singles (60.4% and 88.9%) participated as VBD and PDBD respectively compared to married persons (37.8% and 11.1%). These were mostly students in tertiary institutions. However, percentage of married persons among RBD was slightly higher (49.3%) compared to that of singles (46.4%). Overall, 58.0% were singles while 16.0% were married. This is in contrast to Alfouzan's study in Saudi Arabia who reported that married individuals had higher blood donation knowledge level compared to singles [35]. Other authors reported varied conclusions [36-37]. The outcomes of this study based on donation status revealed that more first timers than previous donors participated as VBD. However, more previous donors enrolled as RBD (55.1%) and PDBD (55.6%) compared to first timers (44.9%) and 44.4%) in RBD and PDBD respectively.

The consistent observations of higher percentages of VBD among the overall population screened as well as higher percentages of younger age groups (16-25 years in VBD), nearly 1:1 male:female ratio and those with tertiary education showed that achieving 100% voluntary blood donation in Nigeria is a great possibility if each health institution can have a workable collaborative policy with established functional and well-funded NBTS in each state in Nigeria [29]. A study in an Indian blood bank showed achievement of 88.0%

compared to the national average of 39.3% voluntary blood donation [38]. Their success was similarly attributed to active motivational outreach to educational institutes through public lectures, presentations, posters and pamphlets thus recruiting long-term young donors. Several studies have demonstrated recruitment of more male donors than females [38-39,30]. Reports by the World Health Organization also stated that less than 10% of blood is given by women in resource-poor countries [28].

The observed false positive results, hence, higher HCV antibody seroprevalence (1.67%) by Diaspot rapid enzyme immunoassay compared to that of Biorad Genscreen HCV Ag-Ab Monolisa assay (0.33%) based on 'sandwich' ELISA technique in this study contrast the outcomes of studies by other researchers who found false negative results using Diaspot rapid one-step enzyme immunoassay [18]. Current trends in transfusion service demand that initial screening of blood and blood products be based on ELISA technique using kit that both screen for the HCV core antigen and anti-HCV antibodies that improves early detection of HCV infection during the window period and subsequent confirmation of HCV status with recombinant immunoblotting assay (RIBA) or nucleic acid testing (NAT) by real-time PCR [40-42]. Literatures have showed that continued development of newer testing techniques have helped to significantly improve early detection and reduce the window period from 82 days to 66 days and even lower [43]. Genscreen HCV Ag-Ab Monolisa assay has been proved in a study by Laperche to reduce the window period of detection by 21.6 days and 30.1 days when compared to the most sensitive immunoassays and Monolisa HCV Ab. Assay [42]. It is interesting to note that combined detection of HCV core antigen and anti-HCV antibodies has significantly closed the gap of detection between most sensitive HCV antibody assays and NAT thus making it a useful alternative to costly real-time PCR procedure in resource-limited countries for enhancing 'safe blood' practice. This is the first time in Ekiti state that HCV testing in PBD was advanced to include nucleic acid testing by real-time PCR to enhance safe blood practice and give evidence-based report on HCV endemicity in Ekiti state. HCV

seroprevalence of 0.33% reported in this study based on Monolisa HCV Ag-Ab technique, ELISA assay (in contrast to 1.67% by Diaspot) was confirmed with real-time PCR. None of the seronegative samples was positive by the reference technique. Based on its lower specificity and positive predictive value (98.7% and 20.0% respectively) compared to 100.0% for both specificity and positive predictive value by ELISA technique, Diaspot one step EIA cannot be depended upon as the sole technique for diagnosing HCV. This study outcomes corroborated the manufacturer's claims of sensitivity > 99.0% and specificity of 98.6%.

Undefined DOR by Genscreen HCV Ag-Ab Monolisa assay (Biorad HCV Ag-Ab ULTRA Monolisa assay, Marnes la Coquette, France) with both false positives and false negatives being zero implied a perfect test. However, similar statistical finding by Diaspot kit with only false positives showed the need for improvement [44, 47]. Adjusted DOR further elucidated the need to avoid using rapid test kits as sole diagnostic tool for screening blood as they are not specific enough. Genscreen HCV Ag-Ab Monolisa assay showed 9-fold better performance than Diaspot one-step enzyme immunoassay based on adjusted DOR. The implication is that blood donors that would have otherwise been certified fit for blood donation would be excluded on the basis of false positive anti-HCV result leading to more shortage of blood and sometimes psychological and emotional trauma in the blood donors. Other researchers found it was not sensitive enough and should not be used for blood screening [45-46].

HCV diagnosis was advanced to molecular level with the use of real-time PCR assay to resolve serodiscordant findings from Diaspot and ELISA. Elevated (1.67%) HCV antibody seroprevalence given by Diaspot one-step rapid immunochromatographic technique compared to 0.33% by ELISA procedure validated by real-time PCR assay revealed the challenges associated with rapid techniques without a well-defined algorithm such as that available for HIV diagnosis [47-48]. Majority of laboratories in Nigeria are without ELISA facility and as such depend on rapid enzyme immunoassay procedure (especially Diaspot one-

step EIA) for HCV screening in blood donors [18,49]. While Adeyemi *et al* reported less sensitivity of Diaspot EIA in their article, this study showed the lower specificity of the procedure compared to ELISA technique. The reason for the differences in this study outcome and theirs remain unclear but may not be far from the deterioration of the kit due to improper storage in previous studies. Findings in this study corroborated the manufacturer's claim of its sensitivity (99.7%) and specificity (98.6%). Confirmation with real-time PCR validated the seropositivity of the lone sample (0.33%) while the rest (ran in minipools of two samples) showed undetected HCV-RNA. Nucleic acid testing with highly sensitive molecular technique such as the real-time PCR which can detect as low as 5-30 IU/mL of HCV-RNA has been considered the preferred and gold standard technique for HCV confirmation and remain the mainstay in HCV diagnosis but it is very costly [47-50]. NAT for HCV-RNA in blood donors has dramatically reduced the incidence of post-transfusion hepatitis C with the risk of HCV acquisition dropping from 1 per 276,000 to 1 per 2 million donations [51-52]. HCV prevalence in lone VBD confirmed in this study was 0.33%. This was higher than 0.1% reported by Mavengwa and his co-researchers in similar study in Namibia [31] but lower than 0.4% in an Ethiopian study [32] and significantly lower than 6.1% reported by Damulak among blood donors in Jos, Nigeria [53]. Differences in HCV prevalence by authors in various part of the world showed that geographical locations, mode of infections, cultural practices, and prevalent risk factors might all be responsible [12].

Furthermore, HCV quantification and serum alanine aminotransferase results of the blood donor (133,209 IU/mL and 11.1IU/L respectively) demonstrated a typical diagnosis of chronic carrier of hepatitis C virus.

The VBD's status history revealed that the HCV infection was first diagnosed in an asymptomatic phase with rapid enzyme immunoassay technique 7-8 years ago during a routine medical test. Serum ALT was within normal limit (<30U/L). Due to limited resources, HCV-RNA confirmation and quantification could not be performed then. A study documented a cut-off of



800,000IU/mL HCV viraemia [54] irrespective of serum ALT values for initiating antiviral therapy in infected patient/donors while other studies recommended pre-treatment baseline HCV-RNA level of 400, 000 IU/mL [55-56]. Serum alanine aminotransferase >19U/L in female and > 30 U/L in male donors together with elevated HCV-RNA levels (>400,000IU/mL) is an essential condition for initiating therapy. While therapy might not be initiated in the VBD based on the aforementioned recommendations, quarterly monitoring of serum ALT and HCV viral load may be necessary. In contrast, current guidelines by the American Association for the Study of Liver (AASLD) in collaboration with Infectious Diseases Society of America (IDSA) on “When and in whom to initiate HCV therapy” recommended that antiviral therapy be initiated for all categories of patients [57]. Sustained virologic response (SVR), evidence of virologic cure, is the end point of successful hepatitis C treatment. An SVR is defined as the continued absence of detectable HCV-RNA at least 12 weeks after completion of therapy [58-60]. Detection of anti-HCV antibodies is still a possibility when SVR has been achieved in a blood donor treated for HCV infection but such blood donor will no longer have detectable HCV-RNA in serum, peripheral blood mononuclear cells (PBMC) or liver tissue and achieve significant improvement in liver architecture [60]. Actually, treatment decisions by the physicians are based on some vital pieces of information including (but not limited to) unique patient populations (e.g. patients with decompensated cirrhosis, renal impairment, HIV/HCV or HBV/HCV co-infections and those who develop recurrent HCV infection following liver transplantation) and pre-treatment assessment of patient’s understanding of the goals of therapy as well as the need for adherence and follow up. Studies have shown that reduction in severity of disease and eventual cure for HCV contribute to dramatic reduction in all-cause mortality (including hepatic decompensation events, hepatocellular carcinoma and liver-related mortality) and improve quality of life [61-66]. Dhawan documented various drug regimens for the treatment of hepatitis C [67].

## 5. Conclusion

HCV prevalence among prospective blood donors in Ekiti state is low and optimal diagnosis of HCV infection in PBD should consider testing approach that screen simultaneously for HCV core antigen and anti-HCV antibodies as the starting point and subsequent HCV-RNA confirmation and quantification for donors’ healthcare benefits. For blood safety purpose in resource-limited setting, HCV Ag/Ab testing can be the starting and stopping point. Diaspot rapid EIA cannot be depended upon as a diagnostic tool due to lower specificity. To enhance blood safety practice, policy formulation that target 100% voluntary blood donation is strongly recommended for blood transfusion laboratories so as to conform to international standards.

## 6. Authorship Contributions

AGI designed the work, revised the manuscripts, and made scientific inputs. FKA wrote the manuscript, sourced for literatures and performed the analyses. IMO made strong statistical input, corrected the manuscripts and was also involved in the design of the study.

## 7. Disclosure of Conflict of interests

Authors declare that there is no conflict of interest with regard to this study.

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