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Evaluation of the Diagnostic Performance of three Rapid Diagnostic Tests for HBsAg screening for blood donation in Cameroon

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Abstract

Background: Rapid Diagnostic Tests have been wildly reported for HBsAg screening in Cameroon. **Aims and Objectives:** The present study aimed at assessing the diagnostic performance and the limit of detection of three Rapid Diagnostic Tests used for HBsAg screening for blood donation in Cameroon. **Study Design:** A hospital-based cross-sectional study involving blood donors who met blood banks requirements was done. **Setting:** The study was carried out at Douala Laquintinie Hospital and Bamenda Regional Hospital. **Materials and Methods:** Ten mL of blood specimen was collected among blood donors who accepted to partake in the study by signing the inform consent. Laboratory processing was performed at the University of Buea. The limit of detection of the assays under evaluation was checked and the diagnostic performance assessed. The automated Architect HBsAg assay and the ELISA Biorex HBsAg were used as the reference standard. **Statistics:** Sensitivity, specificity was obtained by comparing the results of each of the assay to those of the reference standard. The limit of detection (LOD) of the three RDTs compared to the ELISA Biorex was assessed by preparing 14-fold Dilution of known positive control samples. **Results:** The limit of detection of the tests under evaluation was 0.18IU/mL whereas the one of the ELISA Biorex was 0.05IU/mL. Diaspot and Fastep obtained a sensitivity of 88.24% when compared to Architect and respectively 60.53% and 57.89% when compared to Biorex. Abon showed a lower sensitivity of 50.0% as compared to Biorex and 58.82% compare to Architect. Diaspot and Fastep had a specificity > 99% independent on the standard while Fastep had 98.62% using Biorex and 97.54% using Architect. **Conclusion:** Diaspot and Fastep feature the World Health Organization required specificity independent of the standard used while none of the tests reached the expected sensitivity and limit of detection.

Keywords: HBsAg, Rapid Diagnostic Test, Diagnostic Performance, Blood Donation.

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INTRODUCTION

Blood transfusion is an essential component of health care that contributes to saving lives and improving the quality of life for millions of people worldwide. However, despite the availability of effective measures to ensure the quality and safety of blood and blood products, there is still significant risk associated with their clinical use, including adverse reactions and transmission of transfusion-transmitted infection (TTI) [1].

Worldwide, hepatitis B represent the high risk among TTI, for instance, it was estimated at 1 in 43,666 donations in Korea in 2012, 1.86 times higher than the hepatitis C risk and 31.0 times higher than the HIV risk [2]. The same picture was reported in German, Spain, Gabon, Zimbabwe and Burkina Faso, where the risk was respectively 1 in 360,000 (11.9 times higher than HCV and 30.2 times higher than HIV) [3]. 1 in 346,101 (13.6 times higher than HCV and 4.1 times higher than HIV) [4]. 1 in 1871 (2.6 times higher than HCV and 8.3 times higher than HIV) [5]. 1 in 1628 (4.8 times higher than HCV and 3.9 times higher than HIV) [6]. and 1 in 408 (3.3 times higher than HIV) [7]. The high risk of transfusion-transmitted VHB can be justified by the long length of the diagnostic window period couple with the occult hepatitis B.

In order to reduce the window, period must have developed countries introduced Nucleic Acid Test (NAT) in blood donated screening. The introduction of NAT in northern Brazil contributes for example to a reduction of the RR of transmitting HBV from 1 in 14,492 donations to 1 in 29,411 donations [8].

In Sub Saharan African countries in general and Cameroon in particular, NAT is not yet introduced for blood donation screening [9]. and HBV screening strategies are mostly confined to testing for HBsAg only using RDT [10,11].

For the HBsAg in vitro diagnostic test for screening of blood donations, the World Health Organization recommends the use of Enzyme Immuno-Assays having a 100% sensitivity and a minimum of 98% of specificity or Rapid Diagnostic Test with a minimum sensitivity of 99% and specificity of ≥98% both having a Limit of Detection (LOD) ≤0.13IU/mL [12].

The present study aimed at evaluating the diagnostic performance of three Rapid Diagnostic Assays used for blood donation screening, to assess their LOD and determine the risk to transmit the Hepatitis B Virus (HBV) through blood transfusion.

MATERIALS AND METHODS

Study design and settings

This was a hospital-based cross-sectional study carried out among blood donors recruited at the blood banks of Douala Laquintinie Hospital (DLH) and Bamenda Regional Hospital (BdaRH) during the month of July 2018.

Study population and inclusion

The participants included all potential blood donors at the DLH and BdaRH who accepted to take part in the study by signing the consent form. The blood donors have to fulfil blood banks requirements including the age ranging between 18-60 years, a minimum weight of 50 kg and validated the medical selection. Blood donors were made of all gender participating in blood donation for varied reasons.

Data and specimen Collection

Questionnaires were administered to potential blood donors after consenting to take part in the study. The questionnaire took demographic information, type of donor and motivation for donation. According to the blood banks procedure, Transfusion Transmitted Infections (HIV, HBV and HCV) are screened before the blood donation. 10ml of blood was collected from each volunteer participant at that level.

Laboratory processing at blood banks

At the Douala Laquintinie Hospital, the HBsAg pre-test was performed using a rapid diagnostic test (Diaspot or Abon) and if the result was positive, the blood donor was definitively deferred. If negative, the blood collection was done and analysis using Architect HBsAg.

At the Bamenda Regional Hospital, the HBsAg pre-test was performed using a rapid diagnostic test (Diaspot or Fastep) and if the result was positive, the blood donor was definitively deferred. If negative, the blood collection was done and analysis using a second rapid diagnostic test (Abon or Fastep).

Laboratory processing at the Medical Research and Applied Biochemistry Laboratory

Samples were centrifuged, aliquoted, store at -20 degrees Celsius and transported from collection sites (DLH, BdaRH) to the Medical Research and Applied Biochemistry Laboratory (MRABL) of the University of Buea where analyses was performed. Samples were analyzed in an assurance quality manner using the rapid diagnostic tests (RDTs) Fastep, Diaspot, ABON and ELISA Biorex. The ELISA Biorex and Architect HBsAg were both used as the reference standard for the evaluation of the rapid diagnostic test.

Determination of the Limit of Detection

The limit of detection (LOD) of the three RDTs compared to the ELISA Biorex was assessed by preparing 14-fold Dilution of known positive control samples. The dilution was done by adding 200ul of sample into 200ml of distilled water. Successive dilutions were made by transferring 200ul of the mixture from the first tube into the second, mixed and 200ul transferred from the second tube into the 3rd tube and so on till the 12th tube corresponding on 1/4096 dilution. The positive control sample had a concentration of 188.54 IU/mL. The final concentration was obtained by multiplying the initial concentration by the dilution factor. Each dilution was tested for the detection of HBsAg simultaneously by the three evaluated RDTs and ELISA Biorex.

Statistical analysis

Excel sheet was used to perform data entry for the two study sites. GraphPad Prism 8.4.3 was used to perform statistical analysis. Results of each of the assays were compared to those of the reference standard assays to calculate sensitivity, specificity and predictive values. The chi-square test was used to measure the association between groups and a p-value < 0.05 was considered significant.

Ethical Clearance

Ethical clearance was obtained from the National Ethical Committee for Human Health Research Review Board N°2018/04/996/CE/CNERSH/SP and the participation in the study was based on a consent form duly signed by participants.

RESULTS

Socio-demographic characteristics of participants

A total of 374 blood donors were included in the study, 301 (63.50%) from Douala Laquintinie Hospital and 173 (36.50%) from Bamenda Regional Hospital. The participants were young with 65.82% having less than 35 years and the ages ranged from 18-60 years with a mean age of 31.51(SD=8.51). Pertaining to gender, a majority of participants were males with 88.19% (Table1). A majority of the participants were single totalling up to 67.5% and 46.6% have received at least secondary education. 54.43% of participants had donated at least more than once and the major motivation for donation was family-oriented as over 94.73% responded to this, with just 4.43% voluntary.

Diagnostic performance of the three rapid diagnostic assays

Variation of performance of the three rapid diagnostic assays with a diluted positive sample using ELISA Biorex as standard.

The variation of performance after dilution of a positive control sample showed that all immunochromatographic methods (ABON, Fastep, Diaspot) had a positive outcome until a dilution of 1/1024 corresponding to 0.18 IU/mL whereas the ELISA Biorex showed a positive result of up to a dilution of 1/4096 corresponding to 0.05IU/mL (Table 2).

Reactivity of HBsAg per screening assays

The reactivity of hepatitis B varied between different individual screenings tools ranging from 4.43% to 10.13% (Table 3).

Diagnostic performance of the three rapid diagnostic assays using Biorex as the reference standard

The sensitivities recorded by the different assays were less than 61% with Abon showing the minimum sensitivity of 50.0% (Table 4).

Diagnostic performance of the three rapid diagnostic assays using Architect as the reference standard

Biorex recorded a higher sensitivity of 94.12% while Abon recorded the less sensitivity of 58.82% (Table 5).

Among the RDT under evaluation, the RDT Abon had a higher risk of transmission with an average of 13 (4.5-21.5) per thousand of donations (Figure 1).

Fig1= Fastep; 2= Diaspot, 3=Abon; 4=Biorex. Proportion of missed out positive cases per tool which were positive for the reference test were considered as the proportion per thousand which could have been transmitted by the use of that particular tool.

Table 1: Socio-demographic data of blood donors

Characteristics	No (%)	[95% CI]
Gender		
Females	56 (11.81)	[9.21-15.03]
Males	418 (88.19)	[84.97-90.79]
Age (years)		
18-24	110 (23.21)	[19.63-27.21]
25-34	202 (42.62)	[38.24-47.11]
35-44	119 (25.11)	[21.41-29.20]
≥45	43 (9.07)	[6.80-12.00]
Education		
None	8 (1.69)	[0.86-3.29]
Primary	72 (15.19)	[12.24-18.70]
Secondary	221 (46.62)	[42.18-51.12]
University	173 (36.50)	[32.29-40.92]
Marital status		
Single	322 (67.93)	[63.60-71.98]
Married	14 (29.96)	[1.15-34.23]
Cohabiting	10 (2.11)	[32.29-3.84]
Type of donors		
Family	449 (94.73)	[92.33-96.40]
Voluntary	21 (4.43)	[2.92-6.68]
Paid	4 (0.84)	[0.33-2.15]
Frequency of donation		
First time donors	216 (45.57)	[41.14-50.07]
Regular donors	258 (54.43)	[49.93-58.86]

Table 2: Variation of dilution vis-à-vis reference test ELISA

Dilution	½	¼	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
Concentration (IU/mL)	94.3	47.1	23.6	11.8	5.9	3.0	1.5	0.74	0.37	0.18	0.09	0.05
Sample 1: HBsAg =188.54 IU/Ml												
Reference assay	+*	+	+	+	+	+	+	+	+	+	+	+
Abon	+	+	+	+	+	+	+	+	+	+	-#	-
Diaspot	+	+	+	+	+	+	+	+	+	+	-	-
Fastep	+	+	+	+	+	+	+	+	+	+	-	-

*reactive, #non-reactive

Table 3: Results of reactivity of HBsAg screening per assay

Assays (n)	Reactivity (%)	[95% CI]
Abon (474)	21 (4.43)	[2.92-6.68]
Diaspot (474)	25 (5.27)	[3.60-7.67]
Fastep (474)	28 (5.91)	[4.12-8.41]
Biorex (474)	48 (10.13)	[7.72-13.17]
Architect (301)	17 (5.65)	[3.82-9.25]

Table 4: Diagnostic performance of the three rapid diagnostic assays using Biorex as the reference standard

Performance	Fastep [95% CI]	Diaspot [95% CI]	Abon [95% CI]
Sensitivity	57.89 [42.19- 72.15]	60.53 [44.72- 74.40]	50.0 [34.85-65.15]
Specificity	98.62 [97.03-99.37]	99.54 [98.34- 99.92]	99.54 [98.34- 99.92]
PPV*	78.57 [60.46-89.79]	92.0 [75.03- 98.58]	90.48 [0.7109-0.9831]
NPV#	96.41 [94.25-97.78]	96.66 [94.56- 97.97]	95.81 [93.54-97.30]
LR&	42.07	131.9	109

*Positive Predictive Value; #Negative Predictive Value; &Likelihood Ratio

Table 5: Diagnostic performance of the three rapid diagnostic assays using Architect as the reference standard

Performance	Fastep [95% CI]	Diaspot [95% CI]	Abon [95% CI]	Biorex [95% CI]
Sensitivity	88.24	88.24	58.82	94.12
	[65.66- 97.91]	[65.66- 97.91]	[36.01-78.39]	[73.02-99.70]
Specificity	97.54	99.3	99.3	95.42

	[95.00-98.80]	[97.47-99.87]	[97.47-99.87]	[92.33-97.31]
PPV*	68.18	88.24	83.33	55.17
	[47.32-83.64]	[65.66- 97.91]	[55.20-97.04]	[37.55-71.59]
NPV#	99.28	99.3	97.58	99.63
	[97.42-99.87]	[97.47- 99.87]	[95.09-98.82]	[97.95-99.98]
LR&	35.8	125.3	83.53	20.56

*Positive Predictive Value; #Negative Predictive Value; &Likelihood Ratio

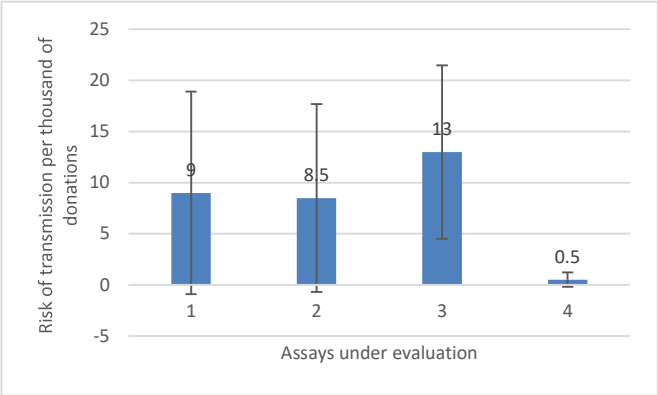

Figure 1: Representation of the risk of transmitting hepatitis through transfusion per assay depending on the gold standard used and express per thousand of donation

DISCUSSION

This study aimed at evaluating rapid diagnostic tests used for HBsAg screening for blood donation in two blood banks in Cameroon.

Demographics of blood donors

Blood donors were young with about 66% having less than 35 years with a mean age of 31.51±8.5 and males were the major participants totalling up to 88.19% confirming findings previously observed within the country, 91.54% at Douala General Hospital in 2019 [13]. 86.2% at Yaounde University Teaching Hospital in 2019 [14]. and 75.5% among six African countries in 2021 [15]. Regular blood donors represented 54.43% of participants and the major motivation for donation was family-oriented for about 94.73%. Compared to previous data within the country, family donors represented 81% of blood donors at Yaounde Jamot Hospital in 2020 [16]. and up to 98.72% at Yaounde Central Hospital in 2021 [17]. Concerning the frequency of donating blood, the 53.43% of regular blood donors obtained in the present study differ from the 8.94% reported in five blood banks in Cameroon in 2020 [18]. and with the 1.88% revealed in four blood banks [19]. The difference in the proportion of regular blood donors can be explained by the fact that in the present study difference was not made between lapsed and regular donors. In general, donors do not easily return for more donations. Thus, Ndoumba and co-workers in 2020 reported that the barriers to donor return of more donations were mainly lack of information on blood needs in 35.60% and time constraint for blood donation in 26.73% [20].

Reactivity of HBsAg per assay

HBsAg reactivity widely varied from one test kit to another 4.43%, 5.27%, 5.65%, 5.91%, and 10.13% respectively Abon HBsAg, Diaspot HBsAg, Architect HBsAg, Fastep HBsAg and Biorex HBsAg.

The variation in reactivity may be justified by the various performance characteristics of an assay that varies markedly with the prevalence of the disease in the population [21]. and moreover by the variation reported on the limit of detection of HBsAg depending on assays format and even in the same assay format [22]. These variations of reactivity can explain the difference in prevalence reported among

blood donors within the country, for instance, a meta-analysis conducted on seroprevalence of hepatitis B virus infection in Cameroon in 2017 reported among 12 studies a variation of prevalence from 3.5% to 16.9% [11]. among blood donors with a mean of 10.5% (8.7-12.4).

Diagnostic accuracy of the assays

The verification of the limit of detection of assays was performed using a known positive sample diluted 14-fold in distilled water, all the RDTs showed a reactive result till the dilution of 1 in 1024 corresponding to 0.18 IU/mL. The limit of detection obtained by the RDTs under evaluation was low compared to ≤0.13 IU/mL required by the World Health Organization. The limit of detection of the reference standard made of ELISA principle features the ≤0.13 IU/mL recommends by the World Health Organization.

In this study, the evaluation of the three commonly used HbsAg rapid diagnostic assays for blood donated screening in Cameroon was performed using both HbsAg ELISA Biorex and HbsAg CMIA Architect as gold standards. None of the three assays under evaluation featured the sensitivity of ≥ 99% as recommends by WHO [12]. The sensitivity of 50% obtained in the present study for the HBsAg Abon with ELISA Biorex as the reference standard is equal to the 50% of sensitivity obtained in Ghana among blood donors using the same assay compare to an ELISA assay as standard [10]. The sensitivity of 88.24% observed for the RDT Diaspot in the present study was low as compared to 98.9% reported by Afolabi and collaborators in Nigeria in 2018 using the same assay with an automated EIA as reference [23]. and also low as compared to the 100% obtained by Fokam and coworkers in 2019 in Cameroon among HIV positives children using an ELISA as Gold standard [24]. The difference observed in sensitivity can be explained by the difference in the studied population, the limit of detection for HBsAg for diagnostic is up to 4IU/mL [12].

The low sensitivity of the rapid diagnostic assays observed can be explained by their low limit of detection on one hand but can also be the results of circulation of mutant's strains of hepatitis B among blood donors. The circulation of 1.2% of mutant's strains and 3.1% of potential mutant's strains were reported in rural South Cameroon [25].

The risk of transmitting HBV using the assays under evaluation varies from 6.64 per thousand of donations to 40.8 per thousand donations. The mean risk of 35.2 per thousand is obtained when using the ELISA Biorex as standard. The present risk rate is comparable to the 30 per thousand obtained in Ghana among five hospital-based blood banks [26]. In both studies, RDTs was compared using ELISA-based principle assays. The mean risk obtained using Architect a CMIA based principle was 12.18% 2.88 times lower than the one using an ELISA assay as reference. This could be explained by the fact that CMIA based assays are reported to be more accurate with a low limit of detection than ELISA assays [22,10].

CONCLUSION

None of the assays under evaluation fulfilled the WHO recommendation for HBsAg in vitro diagnostic for screening of blood donations of ≥ 99% of sensitivity and the limit of detection of 0.13IU/mL but feature the limit of detection for a diagnostic assay. The limit of detection of assays for blood donation screening propose should be check before their use.

Authorship contributions

Conceived the study: GDE; NAJ; ZD; FJ. Collected the data: GDE; TPB; TD. Analyzed the data: GDE; ZD; NAJ; IL, AF. Drafted the manuscript: GDE; ZD; TPB. Revised the manuscript: GDE; ZD, NAJ, FJ. Approved the final version of the submitted manuscript: All.

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Conflict of Interest

None declared.

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None declared.

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