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ORIGINAL ARTICLE

Residual Risk of Transmission of Hepatitis B Virus through Blood Transfusion in Ghana: Evaluation of the performance of Rapid Immunochromatographic Assay with Enzyme Linked Immunosorbent Assay

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Blood transfusion necessitates screening of transmissible infectious pathogens such as hepatitis B virus (HBV) to curtail post transfusion risk of infection. The study re-examined this approach by evaluating the efficiency of solely testing for hepatitis B surface antigen (HbsAg) marker for blood transfusion, the efficacy of the various immunochromatographic assays in the screening process and the residual risk of hepatitis B viral transmission through transfusion in Ghana. A convenient purposive sampling technique was used in selecting ten hospitals, from each of the 10 regions. A total of 480 aliquots of blood were collected anonymously, from blood already tested for HbsAg with immunochromatographic assay in the blood banks of the chosen facilities and declared negative. Plasma from the blood was obtained through centrifugation, separated into well labeled microtubes and transported in cold boxes to the Molecular Medicine Department-KNUST. The samples were then re-examined for all six hepatitis B virus (HBV) (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc and IgM anti-HBc) serological markers using ELISA assay. When a total of 480 plasma samples from the blood banks of the ten chosen facilities were re-examined with the ELISA assay, 39(8.13%) samples reacted positive for HBsAg, 60(12.5%) reacted for Anti-HBs, 13(2.71%) reacted for HBeAg, 51(10.63%) for Anti-HBe and 329(68.54%) reacted positively for Anti-HBc. None of the samples reacted positive for IgM anti-HBc. The estimated sero-prevalence for all HBV serological markers is 76.67% whereas the estimated residual risk of HBV infection through blood transfusion caused by the use of immunochromatographic methods in the screening of blood for transfusion was 8.47%(5.98% - 10.94% at 95% CI). An additional risk of 3.10%(1.54% - 4.62% at 95% CI) of HBV infection through transfusion was also estimated for the non-testing of other HBV infectious serological markers. The total residual risk for transfusion transmitted HBV was 11.16%(8.34% - 13.95% at 95% CI). The study revealed that neither the kits in use nor the testing strategy in place now is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to the high residual risk of transmission of HBV. There is therefore an urgent need for a sustainable quality control system on the screening of HBsAg in blood for donation in Ghana.
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Keywords*:* Blood transfusion, HBsAg, immunochromatographic , ELISA, Blood bank, Ghana

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INTRODUCTION

Transfusion transmitted infections are still a major global public health concern confronting the worldwide delivery of transfusion services and this is

even more apparent in the under resourced transfusion centres operating in the third world countries (Aach *et al.*, 1981). Blood transmitted infections involving pathogenic viruses are the most renowned in transfusion medicine (Niederhauser *et al.*, 2005). In spite of all the scientific advancement geared towards blood donation safety, Hepatitis B virus residual risk remains the single most transmitted infectious pathogenic disease passed on from blood donors to their recipients through transfusion (El-Sherif *et al.*, 2007).

Hepatitis B virus infection leads to a sequence of serological markers or viral protein in the bloodstream of infected individuals. Hepatitis B viral deoxyribonucleic acid (DNA) is the first to appear in the blood, then hepatitis B viral surface antigen (HBsAg), followed by viral polymerase DNA and then hepatitis B viral e antigen (HBeAg). Host's response results in production of antibodies to various hepatitis B viral antigens. The first detectable antibody in the serum of an infected person is antibody to the core antigen (Anti-HBc); this antigen is not expressed in the bloodstream and is only found in HBV-infected hepatocytes in the liver. Antibodies to hepatitis B viral e antigen (Anti-HBe) are then secreted into the serum and followed finally by antibody to hepatitis B viral surface antigen (Anti-HBs) (Kumar *et al.*, 2007; Kukka, 2008).

The issues with blood safety are essentially in two parts. First and most important is the identification of tainted or infectious blood units and the onward prevention of its transfusion. In the presence of safeguarding the recipient, steps must also be taken to prevent false positives which could lead to wastage of blood. The donor selection policy adopted by the facility should therefore be able to exclude donors from high risk groups. Also the specificity and sensitivity of the assay technology in use should be high enough to identify all true positive and true negative blood donors or blood units (Allain and Lee, 2005).

Despite the institution of mandatory screening for HBsAg, the issue of transfusion-associated HBV is still a major health problem plaguing most third world and resource poor countries (Dhawan *et al.*, 2008). Blood donors in Ghana are selected on the basis of a health check questionnaire coupled with a visual physical examination and a mandatory microbiological screening which includes Syphilis, HIV, Hepatitis C and HBsAg test (Ampofo *et al.*, 2002).

Among the pathogenic markers which are screened before transfusion in Ghana, human immunodeficiency virus screening is the only marker that has a nationally developed standardised guideline procedure for testing, supervision and centralised provision of test kits (Allain *et al.*, 2003). Unlike HIV, Hepatitis B virus surface antigen (HBsAg) screening in Ghana does not have any nationally developed supervised procedures and thus the choice of assay technology and brand of test kit used is the sole prerogative of health facilities (Ampofo *et al.*, 2002).

Retrospective studies carried out in other countries on blood donors using first generation tests such as immunodiffusion and/or immunochromatography in the detection of HBsAg, found that 52%-69% of recipients of blood developed hepatitis B infection (Alter and Chalmers, 1981). Allain and co-workers in 2003 observed that neither the latex agglutination nor the dipstick assays currently in use for HBsAg screening had sufficient sensitivity in excluding HBV infected blood from transfusion. Latex agglutination method presented a false negative rate of 46%, whilst dipstick recorded a rate of 29% false negative (Allain et al., 2003). HBsAg sero-negative test however does not rule out the risk of transmission of hepatitis B infection (Al-Mekhaizeem *et al.*, 2001; Brechot *et al.*, 2001; Kumar *et al.*, 2007), and this can be as a result of undetected infected donors with early acute infection in the "window period", resolving infection, silent or occult infection or infection with atypical variants or mutation (Reesink *et al.*, 2008).

The accurate estimation of residual risk of transfusion-transmissible infectious diseases are essential for monitoring the safety of blood supply and the evaluation of the potential effect of screening tests and the risk reduction procedures under implementation (Schreiber *et al.*, 1996).The most direct way of estimating the residual risk of viral agents transmissible

through transfusion would be a prospective study that evaluates the rate of viral infection in blood recipients (Aach *et al.*, 1981; Donegan *et al.*, 1990). However such a prospective study would require testing a large patient population for HBV before transfusion and then follow up testing after transfusion for the risk to be measured accurately and this would be both resource and time demanding. There is also an obvious implication of responsibility for health facilities where recipient after transfusion later test positive for HBV and these make such research impracticable in most settings. The alternative therefore is further retesting for the rate of infection in samples of donated blood that test negative on routine screening by the use of more sensitive assays for the viral agents (Schreiber *et al.*, 1996). The primary objective of this study therefore was to evaluate the residual risk of transmission of hepatitis B virus through blood transfusion and thus assess the efficacy of the test methods being used for HBsAg screening and the efficiency of using HBsAg as sole hepatitis B viral marker for blood transfusion in Ghana.

MATERIALS AND METHODS

Sampling

A convenient, purposive cross-sectional study was carried out from October 2007 to June 2008 in ten laboratories, one in each of the ten regions in Ghana. The study was anonymous and non-linked. Aliquots of blood were collected from donated blood units that tested sero-negative for HBsAg upon screening with immunochromatography assays in the facilities. The plasma was separated into well labelled micro-tubes and transported in cold boxes to the Molecular Medicine Laboratory of the Kwame Nkrumah University of Science and Technology in Kumasi and stored at -20 degrees. The types and brand names of the immunochromatogrphic assays used for the initial screening at the various facilities were recorded. Samples were re-examined for HBsAg and the five other serological markers of HBV (Anti-HBs, HBeAg, Anti-HBe, Anti-HBc and IgM Anti-HBc) using an Intec advanced 3rd generation ELISA test kit according to the manufacturers instruction. All blood donors in Ghana are of consenting age and consented to the retesting of HBV. Approval for this study was granted by the research and coordinating units of all ten laboratory facilities involved in the conduct of the study.

Dipstick assay methods

All samples from the Central, Ashanti and Brong Ahafo regions were pre-screened with DiaSpot™ one step test strip (DiaSpot World of Health Biotech Co.; USA). Samples from the Volta and Upper East regions were screened with Virucheck™ dipstick, (Orchid Biomedical Systems, India). Samples from the Greater Accra and the Eastern regions were screened with Clinotech™ HBsAg Dipstick, (Clinotech Diagnostics, Canada). Samples from the Northern regions were screened with one step HBsAg dipstick (Wondfo™ Guangzhou Wondfo Biotech Co., Ltd. China). Samples from the Upper West were screened with Novatec™ dipstick (Novatec Immundiagnostica GmbH, Technologie & Waldpark. Germany) and samples from the Western region were screened with Accull-Tell™ test kits. (AccuBiotech Co. Ltd. China). The strips were dipped in the serum or plasma for about 10 seconds and read for the visibility of one test and one procedural control line after about 10 minutes.

Enzyme Linked Immunosorbent Assay (ELISA)

HBsAg, Anti-HBs and HBeAg were determined by a double sandwich enzyme-linked immunosorbent assay (ELISA) using Advanced™ 3rdgeneration assay kits (InTec Products Inc. China). Anti-HBe and Anti-HBc were determined by competitive immunoassay using Advanced™ 3rd generation assay kits (InTec Products Inc.China). Anti-HBc IgM was tested with an immunoassay using Advanced™ 3rd generation assay kits (InTec Products Inc.China). Reactive samples were retested in duplicate and considered to be reactive if at least 1 of the 2 repetitions also gave a positive result.

Statistical analysis

Statistical analysis was performed using MedCalc Version 10.2.0.0 for Windows 98/NT/Me/2000/XP/Vista (Vienna, Ausra. http://www.medcalc.be). The Bland-Altman analysis was used to compare

the immunochromatographic method for the screening of HBsAg and ELISA screening for HBsAg for each region. The calculation of the residual risk of HBV transmission through blood transfusion was done using the Modified Wald analysis for categorical outcomes as recommended by Agresti and Coull, (1998).

RESULTS

Reactivity of Hepatitis B viral marker in the various regions upon re-examination

As shown in Table 1, out of the total number of 480 samples that had tested sero-negative upon testing with rapid immunochromatographic test kit in the various facilities and thus the blood declared safe for transfusion, 39(8.13%) of the samples reacted sero-positive for HBsAg upon retesting with ELISA. Sixty (12.5%) of the sample showed sero-positivity for Anti-HBs, 13(2.71%) reacted for HBeAg, 51 (10.63%) for Anti-HBe, 329(68.54%) reacting sero-positively for Anti-HBc with 226(47.08%) reacting sero-positive for isolated or only Anti-HBc. None of the samples however reacted sero-positive to IgM anti-HBc. The Volta region 10(20.00%), the Northern region 6(13.95%), the Upper East region 7(13.46%) and the Eastern region 5(10.00%) all showed a higher HBsAg seroreactivity than the total percentage seroreactivity of 39(8.13%) from all centres.

Comparison of dipstick methods with ELISA method using Bland-Altman analysis

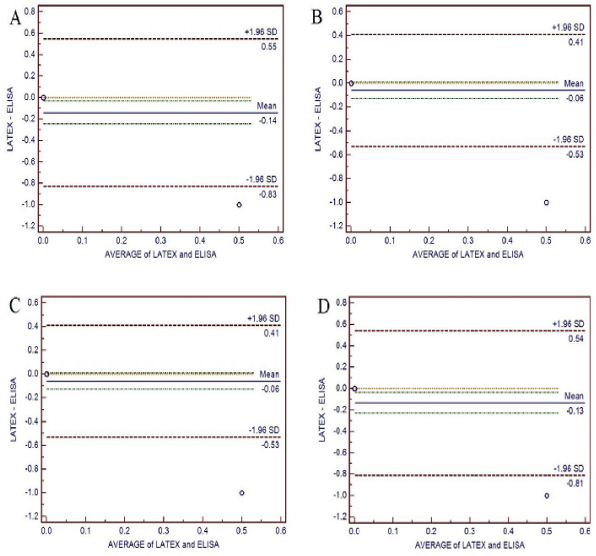
Table 1: Reactivity of Hepatitis B viral marker in the various regions upon re-examination

Region	Sample	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Isolated Anti-HBc
Eastern	50	5(10.00)	10(20.00)	1(2.00)	0(0.00)	40(80.00)	25(50.00)
Volta	50	10(20.00)	1(2.00)	3(6.00)	1(2.00)	19(38.00)	13(26.00)
Central	50	0(0.00)	3(6.00)	0(0.00)	1(2.00)	22(44.00)	19(38.00)
Northern	43	6(13.95)	0(0.00)	3(6.98)	6(13.95)	37(86.05)	29(67.44)
Greater Accra	50	0(0.00)	5(10.00)	1(2.00)	11(22.00)	35(70.00)	22(44.00)
Ashanti	50	3(6.00)	12(24.00)	2(4.00)	8(16.00)	28(56.00)	15(30.00)
Western	42	3(7.14)	6(14.29)	0(0.00)	6(14.29)	31(73.81)	22(52.38)
Upper West	43	2(4.65)	9(20.93)	1(2.33)	5(11.63)	32(74.42)	25(58.14)
Brong Ahafo	50	3(6.00)	8(16.00)	2(4.00)	8(16.00)	40(80.00)	26(52.00)
Upper East	52	7(13.46)	6(11.53)	0(0.00)	5(9.62)	45(86.54)	30(57.69
Total	480	39(8.13)	60(12.50)	13(2.71)	51(10.63)	329(68.54)	226(47.08

Data are presented as number or number (percentage). HBsAg = Hepatitis B virus Surface antigen, Anti-HBs = Antibody to Hepatitis B virus surface antigen, HBeAg = Hepatitis B virus e antigen, Anti-HBe = Antibody to Hepatitis B virus e antigen, Anti-HBc = Antibody to hepatitis B virus core antigen

When the results for HBsAg screening using the rapid latex immunochromatographic method was compared with the ELISA assay using the Bland-Altman analysis and as shown in figures 1 and 2. The Volta region (ViruCheck) showed the highest underestimation with a bias of -0.20 with a 95% limit of agreement of -0.99 to 0.59 (figure 2D). Northern Region (Wondfo), also underestimated presence of HBsAg in the samples (-0.14 and the 95% limit of agreement was -0.83 to 0.55, Figure 1A). Upper East region (Virucheck) generated underestimated HBsAg sero-reactivity with a bias of -0.13 and 95% limit of agreement of -0.81 to 0.54 (Figure 1D). This was followed by Eastern region (Clinotech) generating a bias of -0.10 and the 95% limit of agreement was -0.69 to 0.49 (Figure 2B). Western region (Acull-Tell) showed a bias of -0.07 and the 95% limit of agreement was -0.58 to 0.44 (Figure 2C). The bias was -0.06 and the 95% limit of agreement were -0.53 to 0.41 for both Ashanti and Brong Ahafo regions (DiaSpot) (Figure 1B and 1C respectively). A bias of -0.05 and a 95% limit of

agreement of -0.46 to 0.37 was generated in the Upper West region (Novatec) (Figure 2A). There was perfect agreement between the immunochromatographic methods used in both Central and Greater Accra region facilities and the ELISA method.



A = Northern region, B = Ashanti region, C = Brong Ahafo region and D = Upper east region

Figure 1: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay

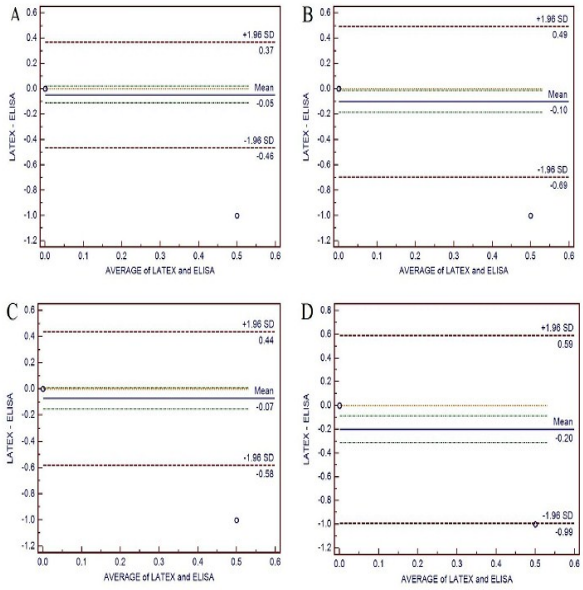


Figure 3: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay. A = Upper West region, B = Eastern region, C = Western region, D = Volta region

Residual risk of transfusion

The modified Wald analysis was used to estimate the residual risk of transmission of HBV through blood transfusion for the use of rapid immunochromatographic test kits in screening for the HBsAg before transfusion. As shown in Table 2, the national mean residual risk of transmission of HBV was 8.47% (5.98% to 10.94% at 95% CI).The facility at the Volta region (VR) generated the highest residual risk of transmission, 22.22% (11.05% to 33.23% at 95% CI) and followed in a descending order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Northern region (NR), was 17.02 % (6.18% to 27.64% at 95% CI), the Upper East region (UE), 16.07% (6.37% to 25.58% at 95% CI), the Eastern region (ER), 12.96 % (3.91% to 21.79% at 95% CI), the Western region (WR), 10.87% (1.77% to 19.70% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region generated a residual risk of 9.26% (1.44% to 16.84% at 95% CI). The facility in the Upper West region (UW) generated a residual risk of 8.51% (0.44% to 16.30% at 95% CI). With the exceptions of the two facilities in the Central and the Greater Accra regions which generated a residual risk of 3.70% (0.00% to 8.52% at 95% CI) each, all the other facilities had a residual risk higher than the national mean residual risk of HBV transmission through transfusion.

To assess the risk of transmission of HBV for solely testing for hepatitis B surface antigen (HBsAg) marker for blood transfusion without testing the other HBV serological markers (anti-HBs, HBeAg,

anti-HBe, anti-HBc and IgM anti-HBc), two serological markers which are indicators of the presence of the virus and thus a risk of transfusion hepatitis infections were considered in the Modified Wald analysis. These were reactivity of HBeAg which is serologically indicative of high HBV replication and IgM anti-HBc which indicates an acute infection of HBV. Thus samples that showed sero-reactivity for HBeAg and IgM anti-HBc without HBsAg were considered.

The total percentage residual risk of HBV transmission for using the HBsAg as the sole HBV marker to transfused blood was 3.10 %(1.54% to 4.62% at 95% CI). The facility at the Northern region (NR) generated the highest residual risk of transmission, 10.64% (1.72% to 19.29% at 95% CI) and followed in a decreasing order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Volta region (VR),was 9.26% (1.44% to 16.84% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA) generated a residual risk of 7.41% (0.34% to 14.22% at 95% CI) for non inclusion of the two markers in the testing of HBV blood transfusion. Again the facilities in the Greater Accra region (GA) and the one in the Eastern region (ER) each generated a residual risk of 5.56% (0.01% to 11.47% at 95% CI). The residual risk for the facility in the Western region (WR) was 4.35% (0.00% to 9.99% at 95% CI) and that for the Central region (CR) was 3.70% (0.00% to 8.52% at 95% CI). A residual risk of 3.57% (0.01% to 13.16% at 95% CI) and 3.57% (0.00% to 8.22% at 95% CI) were obtained for the two facilities in the Upper West region (UW) and the Upper East region (UE) respectively. As shown in table 2. All the individual facilities generated a residual risk higher than the total average.

In the estimation of the total residual risk of HBV transmission through transfusion, the samples that reacted sero-positive for any of the infectious HBV serological markers (HBsAg, HBeAg and IgM anti-HBc) were considered and counted only once. Though the risk of transmission is not nil for isolated anti-HBe sero-positive samples in the absence of HBsAg positivity, however the inability to establish the presence or otherwise of viral DNA in these samples by the test method (ELISA) led to the exclusion of such samples. Also sero-reactivity for anti-HBc was excluded due to the inability of the test method (ELISA) to distinguish between samples that tested sero-positive for anti-HBc and sero -negative for all other HBV markers indicative of infectivity (HBsAg, HBeag, and IgM anti-HBc) but contained viral DNA and thus infectious, from those that exhibited the same serological profile but lack viral DNA.

As shown in Table 2, the total percentage residual risk was estimated as 11.16% (8.34% to 13.95% at 95% CI). The total residual risk of transmission of the individual facilities were 27.78% (15.76% to 39.67% at 95% CI) for VR, 23.40% (11.21% to 35.42% at 95% CI) for NR, 16.07% (6.37 to 25.58% at 95% CI) for UE. A total residual risk of 16.07% (1.72% to 19.29% at 95% CI) and 14.82% (5.25% to 24.17% at 95% CI) for the Upper West (UW) and the Eastern regions (ER) respectively. The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA), each generated a total residual risk of 12.96% (3.91% to 21.79% at 95% CI).The facilities Western, Greater Accra and Central regions all had their total residual risk below the national average, 10.87% (1.77% to 19.70% at 95% CI) for WR, 5.56% (0.01% to 11.47% at 95% CI) for GA and 3.70% (0.00% to 8.52% at 95% CI) for CR.

Reactivity Profile of ELISA upon Retesting

The reactivity profile of the samples upon retesting with enzyme linked immunosorbent assay showed disparity between the number of samples that reacted for a particular antigen and its corresponding antibody. Figure 3A and Figure 3B represents regional reactivity profile of HBV surface antigen and its corresponding regional reactivity profile of surface antibody respectively, whilst Figure 3C and Figure 3D represents regional reactivity for HBV e antigen and e antibody respectively. There were a higher number of samples that reacted for anti-HBc only (Figure 3F) compared to the sample that reacted for anti-HBc and other serological markers (Figure 3E). There was high discordance between HBsAg and HBeAg with only three of the samples

Table 2: Estimated Residual Risk of Hepatitis B virus transmission through blood transfusion

LOCATION	RRT for testing HBsAg with Immunochromato-graphic Assays	RRT for the non testing of other HBV serological markers	Total RRT of HBV through Transfusion
Eastern	12.96(3.91 - 21.79)	5.56(0.01 - 11.47)	14.82(5.25 - 24.17)
Volta	22.22(11.05 - 33.23)	9.26(1.44 - 16.84)	27.78(15.76 - 39.67)
Central	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)
Northern	17.02(6.18 - 27.64)	10.64(1.72 -19.29)	23.40(11.21 - 35.42)
Greater Accra	3.70(0.00 - 8.52)	5.56(0.01 - 11.47)	5.56(0.01 - 11.47)
Ashanti	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Western	10.87(1.77 - 19.70)	4.35(0.00 - 9.99)	10.87(1.77 - 19.70)
Upper West	8.51(0.44 - 16.30)	3.57(0.01 - 13.16)	16.07(1.72 - 19.29)
Brong Ahafo	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Upper East	16.07(6.37 -25.58)	3.57(0.00 - 8.22)	16.07(6.37 - 25.58)
Total	8.47(5.98 - 10.94)	3.10(1.54 - 4.62)	11.16(8.34 - 13.95)

Data are presented in percentage, RRT Residual risk of transmission

from the northern region showing concordance for HBsAg (Figure 3G). There was high reactivity correlation between Anti-HBs to the Anti-HBc (Figure 4D). Anti-HBe also showed higher reactivity correlation to Anti-HBc (Figure 4G) than Anti-HBs.

DISCUSSION

The year 2012 has been targeted as the year for safe blood and the achievement of 100% testing for infectious markers by the World Health Organization (WHO) (Tagny *et al*., 2008). Though these may look achievable in the developed world, sub-

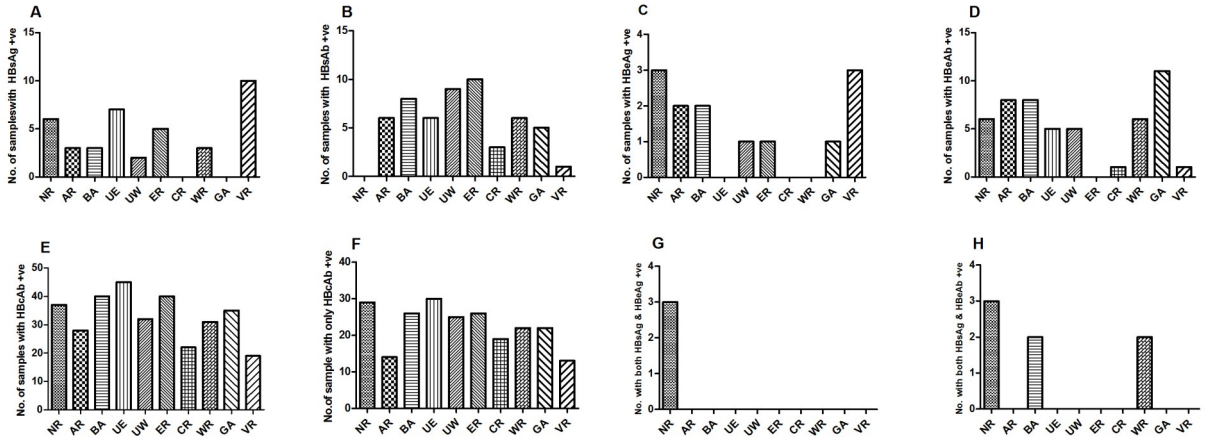


Figure 3: Hepatitis B Viral Serological Marker Reactivity Profile of Enzyme Linked Immuno-Sorbent Assay upon Retesting

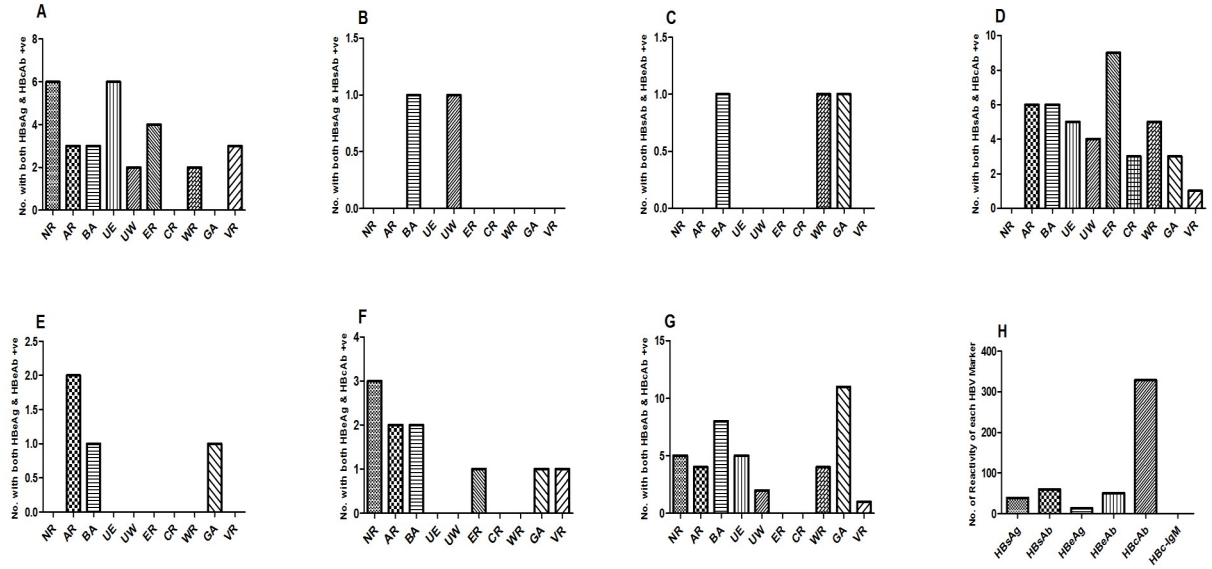


Figure 4: Hepatitis B Viral Serological Marker Reactivity Profile of Enzyme Linked Immuno-Sorbent Assay upon Retesting

Saharan Africa remains drawn back, since it is confronted with multi-factorial issues that compromise blood safety in most of the countries. The most important of these borders on the deficient screening of donor blood for infectious markers like hepatitis B and C virus, in an environment already plagued by a high burden of blood-borne infections and poverty (Tagny *et al.*, 2008).

Even though by the study design the immunochromatographic assay sorted out many of the HBsAg positive samples and its accompanied other HBV serological markers, an estimated sero-prevalence of 76.67% for all HBV serological markers was observed; this is in agreement with previous work by Martinson *et al.,*(1998) who reported a sero-prevalence of 74.7% for all HBV serological markers for a rural community in the Ashanti region. Upon retesting with the ELISA, 8.13% of the samples reacted sero-positive for HBsAg. Amidu *et al.*, (2010) in a four year retrospective study from 2004 to 2007 reported a hepatitis B virus sero-prevalence of 12.64% among blood donors in the upper east region using rapid immunochromatographic assay. Thus it can be said that prevalence of hepatitis B virus among blood donors was under estimated by 8.13% in general and 13.46% in particular to Upper East region.

False negative HBsAg results were obtained by six different brands of rapid latex immunochromatographic assays that were used in all the ten facilities. Torane and Shastri (2008) who recommended the discontinued use of rapid immunochromatographic assays in donor blood screening suggested lack of sensitivity by rapid test may be due to inadequate coating of the antigens, the nature of the antigens used.and genetic heterogeneity of the virus. The study also revealed that in the case where the same type of assay brand were being used in different laboratories, varied degrees of biases were obtained as was evident in the case of DiaSpot which showed a perfect agreement with the ELISA in the Central region, but failed to detect all HBsAg positive samples in both Brong Ahafo and Ashanti regions. This was also true for Clinotech brand whose sensitivity was comparable to the ELISA in Greater Accra, but failed to detect all HBsAg positives in the Eastern region. ViruCheck rapid test on the other hand showed different sensitivity in Upper

East and Volta regions.

Although an attempt to do an inter-laboratory comparison of biases would be limited because different samples were used in each facility, and also the fact that performance of an assay is greatly affected by factors such as the technique and the storage of the test kits. However, the impact of lack of centralised control on standardised guideline on acquisition of test kits, coupled with a non-existence of a national policy geared toward ensuring the quality of pre-screening of HBsAg before transfusion cannot be underestimated (Allain and Lee, 2005). Again even in a facility it was revealed that different brands of the rapid latex immunochromatographic assay were used depending on what was available in the open market. It was estimated that each facility uses an average of four (4) different hepatitis B virus rapid latex immunochromatographic assays within a year, thus the inaccuracy of a low performing assay would be wide spread in different laboratories and this may contribute to the high risk of being infected with HBV through blood transfusion in sub-Sahara Africa estimated by Jayaraman et al., (2010) to be over four times the risk of HIV infection through blood transfusion.

Using the Modified Wald method (Agresti and Coull, 1998), the estimated residual risk of HBV transmission for the use of the rapid immunochromatographic assays for screening HBsAg in Ghana was 8.47%. This estimated risk percentage is similar to what Allain *et al.,* reported in 2003 (9.09%) as the risk of transmission of transfusion borne HBV to recipients under 10 years by the use of immnunochromatographic assay in Kumasi. Thus all these years not much improvement in the quality and reliability of pre-transfusion screening for HBV has been achieved since the screening regimen has not improved to date. The presence of HBeAg in the bloodstream is an indication of active HBV replication associated with large quantity of HBV-DNA in the bloodstream and therefore an enhanced infectivity (Lee, 1997; Yuen *et al.*, 2004; El-Sherif *et al.*, 2007; Kukka, 2008; Zahn *et al.*, 2008). Whilst the positivity for serum Anti-HBe is an indication of seroconversion with a low viral load and unlikely to produce a viable infectivity through transmission portals such as needle stick, sexual contact and household contact, it has a high potential of producing a viable infectivity through blood transfusion because of the large volume of viral DNA that is passed on to the recipient (Kukka, 2008).

HBV infection exhibits a broad spectrum of serologic patterns associated with HBV-DNA persistence. It is rare to find sero-positivity for HBeAg, anti-HBe nor both in the serum of subjects who are sero-negative for HBsAg (Brechot *et al.*, 2001; Allain *et al.*, 2003; Kidd-Ljunggren *et al.*, 2004). But as was evident in this study, out of the 13 samples that reacted sero-positive for HBeAg, only 3 were also sero-positive for HBsAg and out of the 51 samples that reacted sero-positive for Anti-HBe, only 7 also reacted sero-positive for HBsAg. Though rare undetectable HBsAg in the presence of detectable HBeAg has been reported and most of these cases had been attributed to escape mutants with point mutations in the preS1, preS2 and preS regions (Osiowy, 2002; Yang *et al.*, 2003). Chronic hepatitis with ongoing HBV replication in presence of anti-HBe has frequently been observed (Lai *et al.*, 1991), Kidd-Ljunggren et al.,(2004) reported that 65% of anti-HBe sero-positive patients who might be considered most likely to be non viremic had HBV viral DNA. Thus the non-testing of HBeAg/anti-HBe during HBV pre-donation screening may increase the residual risk of HBV transmission through blood transfusion in Ghana.

The study also revealed a high reactivity for Anti-HBc 68.54%, and this was consistent with the findings of Allain, (2006) who estimated that 75% of the Ghanaian population by the age of 16 tested sero-positive for Anti-HBc. Among the Anti-HBc sero-positive samples 8.82% also reacted sero-positive for Anti-HBs and these represent the proportion of donors that had cleared the virus and hence had developed immunity (Al-Mekhaizeem *et al.*, 2001), contrary evidence however suggests this accession may not be the case at all times (Yotsuyanagi *et al.*, 1998; Owiredu *et al.*, 2001; Dhawan *et al.*, 2008; Allain *et al.*, 2009). Among the isolated Anti-HBc sero-positive, two possible donor

groups exist, those chronically infected with persistence of HBV-DNA but had undetectable level of HBsAg and therefore produce viable viraemic infection, and those who had eventually recovered from the infection and no longer carry HBV-DNA but show no detectable Anti-HBs (Reesink *et al.*, 2008).

The ELISA test method could not be used in distinguishing between the two groups of Anti-HBc only positive donors mentioned in the preceding paragraph, however, Zahn et al.,(2008) estimates that the Ghanaian donor population includes 1.5% of the first group. It should be acknowledged however, that despite the indicative potential of this serological marker in identifying donors who have been naturally exposed to HBV, its practical use as a marker for pre-screening of blood in Ghana may not be adequate, essentially because of the high prevalence rate in the donor population (Reesink *et al.*, 2008). Though in estimating the residual risk of transfusion for the non testing of other HBV markers, this group (Anti-HBc sero -positivity) was not considered and was also not included in the total residual risk estimation, the high sero -positivity of Anti-HBc in this study even heightens the risk of getting transfused with HBV infectious blood unit in Ghana.

CONCLUSIONS

This study evaluated the performance of test kits used in pre-donation screening for HBV, and the efficiency of the screening strategy among donors in ten facilities in Ghana. The indication was that neither the kits in used nor the testing strategy in place now, is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to high residual risk of transmission of HBV through blood transfusion. There is therefore an urgent need for an effective quality control system for the pre-donation screening of blood for HBV in Ghana.

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