

HbsAg Rapid Testing of Donor Blood Prior to ELISA Testing in North Central Nigeria: The Implications on Limited Blood Bank Resource Management

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Introduction: Where blood donors are mobilized in resource poor setting like ours, the inadequacy and irregular supply of reagents for the screening of transfusion transmissible pathogens become the major limitation. **Aim:** This study was to determine the implication of pre ELISA rapid testing of HBsAg on the management of blood bank resources. **Method:** Donated blood units from suitable first time voluntary blood donors were screened in two groups for hepatitis B surface antigen. One group was screened by ELISA method straight, while the other was first screened by skytec rapid test strip and the only units that reacted negatively were subjected further to ELISA test. **Results:** Of the two thousand units screened straight with ELISA, 282 (14.1%) were positive for HBsAg. One hundred and eighty (10.1%) donor units of 1784 were rapid test positive for HBsAg. Thirty nine (2.4%) of the 1604 rapid test negative units were positive for HBsAg when further subjected to ELISA screening. A total of 219 (12.3%) units of the 1784 units screened in the second group reacted HBsAg; 180 (82.2%) at strip test and 39 (18.8%) at the ELISA screening of rapid tested negative units. **Conclusion:** A test protocol that applies ELISA screening on the pre rapid strip screened negative donor blood for HbsAg could optimize the utilization of scarce blood bank resource

Keywords: Screening, Donor blood, Resource management.

INTRODUCTION

The sustainable supply of quality blood by the National Blood Transfusion Service, in Nigeria is limited by many factors, most importantly the paucity of informed voluntary non-remunerated blood donors with the attendant poor retention of safe blood givers. Where blood donors are mobilized, the inadequacy and irregular supply of reagents for the screening of transfusion transmissible pathogens become the major limiting factors. The World Health Organisation (WHO) recommends enzyme-linked immunoassay as the minimum screening test for the Human Immunodeficiency, Hepatitis B and C Viruses and *Treponema pallidum*.¹ The WHO further recommended member countries to transfuse only blood components and products that are at least tested negative for the four pathogens.¹

Hepatitis B virus is endemic worldwide, contracted by percutaneous contact with infected blood products, oral and genital contacts or vertically at birth.² Hepatitis B surface antigen is the first serologic marker of HBV detected within 6-16 weeks of infection.³ The antigen usually disappears in 1-2 months after the onset of symptoms, persistence beyond six months indicates the development of chronic carrier or chronic

HBV infection.^{2,3} The use of enzyme linked immuno-sorbent assay screening test for blood transfusion is not for diagnostic confirmation of hepatitis B surface antigen positive donors but for the segregation of sero negative blood units for transfusion.^{3,4}

Nigeria has a high prevalence of HBV infection, detected by hepatitis B surface antigen (HBsAg) test, ranging from 15.5% among low risk group blood donors reported by Egah et al (2007) and 13.8% documented by Damulak et al (2013) among first time voluntary blood donors screened with rapid test kit and ELISA respectively in Jos.^{5,6} A high 27% HBsAg was documented among blood who donated at the hospital service blood bank in Jos University Teaching Hospital, Jos, (Vem et al 2012) exceeding 25.9% earlier observed by Uneke et al (2005) among HIV positive patients in the same setting.^{7,8} Opaleye et al (2013) using immunochromatographic mini strip, found a prevalence of 19.9% HBsAg among blood donors in Osogbo, South Western Nigeria.⁹ The prevalence of HBsAg in our setting is similar to 10.79% and 11.59% respectively among the voluntary and replacement blood donors in Tamale teaching hospital, Ghana.¹⁰

The rate of positivity to markers of HBV infection among blood givers is lower in Turkey where Emekdas and others

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(2006) reported 4.19% in a 1989-2004 study.¹¹ Hazmi and Malak reported a 1.5% rate of HBsAg sero-positivity in the central region of Saudi Arabia.¹² In India, 1.28% and 1.66% were the prevalence of HBsAg among blood donors in West Bengal in 2004 and 2005 respectively while a 2.23% was documented in a four year study in Delhi.^{13,14}

The high rate of HBsAg among our blood donors and dependency on dwindling donor funds for the operation of the blood service requires the determination of affordable and sustainable ways of making blood available for transfusion without compromising quality. This study seeks to determine the benefits of pre-screening blood donor samples with rapid test kit and subjecting negative units to ELISA. The conclusion of this study would enable recommendation for a change in the screening protocol for blood that could optimize the management of scarce blood bank resources in our setting.

METHODOLOGY

All the subjects recruited into this study were newly enrolled voluntary non-remunerated blood donors who donated to the North Central Zonal Centre of the National Blood Transfusion Service in Jos. Three thousand six hundred and eighty four units of blood donated by first time voluntary blood donors who consented to donate and participate in research between August 2015 and October 2015 were screened in this cross sectional study. Samples were screened in two groups of; two thousand donor blood units by - RAD BIO Monolisa HBsAg ULTRA ELISA kit in one group, following the manufacturer's guide.¹⁵ One thousand seven hundred and eighty four units in another group were first screened using Skytech immunochromatographic rapid HBsAg test kit according to the manufacturers guide and then subjected to ELISA if sero negative.¹⁶ Rapid tested positive samples were withdrawn from subsequent ELISA testing. The results were analysed using the epi info 2007 statistical software and p value <0.05 was significant.

LIMITATION

Inadvertent inclusion of donors with concealed risk of contracting HBV infection wishing to access free screening

RESULTS

Three thousand seven hundred and eighty four donor blood units were screened in two groups between August and October 2015 at the National Blood Transfusion Service, North Central zonal Centre, Jos. Two thousand units were screened straight with ELISA kits while 1784 were first screened for HBsAg with Skytec rapid test kit and only negative units were further tested with ELISA.

Of the two thousand units screened straight with ELISA, 282 (14.1%) were positive for HBsAg while 1718 (85.8%) were negative. Screening of 1784 donor units using the Skytec rapid test strip showed that, one hundred and eighty (10.1%) donor units were rapid test positive for HBsAg, while 1604 (89.9%) were negative. Thirty nine (2.4%) of the 1604 rapid test negative units were positive for HBsAg when further subjected to ELISA screening. The differences in the rate of positive reactions picked at an ELISA test done straight and ELISA on rapid tested negative samples was significant ($p=0.0001$), table 1. A total of 219 (12.3%) units of the 1784 units screened in the second group reacted at rapid [180 (82.2%)] and at the ELISA screening of rapid tested negative units [39 (17.8%)]

DISCUSSION

The rate of positive HBsAg reactions of 14.1% and 10.1% by ELISA and Skytec rapid strip tests carried out separately on samples of donated blood units from low risk volunteers are similar to, but lower than 15.5% and 20.8% earlier reported among similar groups in Jos (Egah et al; 2007 and Adekeye et al; 2013).^{5,17} The values in this study are lower than 27% documented by Vem and others among hospital based blood donors in Jos, confirming the superior safety of blood collected from voluntary donors.⁷ The high rate of HBsAg, in our donated blood detected by both methods in our centre is similar to that in Ghana (Dongdem JT et al, 2012), and Cameroon (Jean et al, 2013) implying a possible hyper-endemic regional continuum.^{10,18} Reports of lower rate of HBsAg among donors in Turkey, Saudi Arabia and India were at variance with ours.¹¹⁻¹⁴ This difference suggests regional variability in the transmission of hepatitis B virus infection, which may be linked to cultural ways of life, susceptibility to infection or both.

The need for public enlightenment campaigns, massive immunization, drugs and immuno-stimulatory therapy advocated by Emechebe et al (2001) are still required to reduce the HBV infection rate.¹⁹ We call for free immunization of sero-negative blood donors not only for reward but also to maintain a safe pool of retained blood givers who are potential donor-donor recruiters. The detection of HBsAg in the serum by any method would await its expression, which occurs 6-16 weeks from infection.³ The detectable concentration of HBsAg required for ELISA (measured quantitatively by optical density) is however lower than for rapid test methods (qualitative assay).^{15,16} The World Health Organization for member countries to adopt the ELISA screening for all the mandatory transfusion transmissible infections, as the minimum required to declare any unit of blood fit for transfusion.¹

Despite the endemic state of HBV infection in Nigeria, demonstrated in this study and supported by previous reports, there is no nationally designed sustainable screening protocol for the transfusion service to reduce or eliminate transfusion acquired HBV infections. The establishment of the National Blood Transfusion Service came with the introduction of ELISA screening for markers of Transfusion Transmissible infections including HBsAg for hepatitis B virus. The logistic requirement for running the blood service is however heavily dependent on donor funding, confirming the need for a sustainable local less costly but effective testing protocol. The hyper-endemic state of HBV infection among blood donors in our setting, observed in this study, calls for an initial application of the cheaper rapid strip screening test that would detect and remove 82.2% of reactive units leaving only about 17.8% to be detected by ELISA at a screening of rapid strip tested negative units.

This would reduce the financial resource spent on ELISA kits used in screening a huge sample quantity with HBsAg concentration detectable by rapid test kits. The saved fund could be channelled into the provision of further quality testing such as hepatitis B core antibody and DNA polymerase chain reaction as well as other services to attain higher quality blood transfusion. The implications of screening blood units with semi-automated ELISA straight also include the generation of false positive reactions due to contamination of wells by droplets and aerosols from positive samples, which might occur during sample pipetting and washing stages. The suspicion of false positive reaction is raised by the observed clustering of reactive wells at ELISA straight screening, (picture 1).

Table 1: Outcome of HBsAg screenings of donor blood

Screening Method	Total	No Pos (%)	No Neg (%)	p value
ELISA straight	2000	282 (14.1)	1718 (85.9)	
Pre ELISA Rapid Test	1784	180 (10.1)	1604 (89.9)	0.0001
ELISA on Rapid Test Neg	1604	39 (2.4)	1565 (97.6)	

Pictures of interest



Picture 1: Plate after ELISA straight run for HBsAg
First column show reactive positive control while positive samples are seen within close range to one another

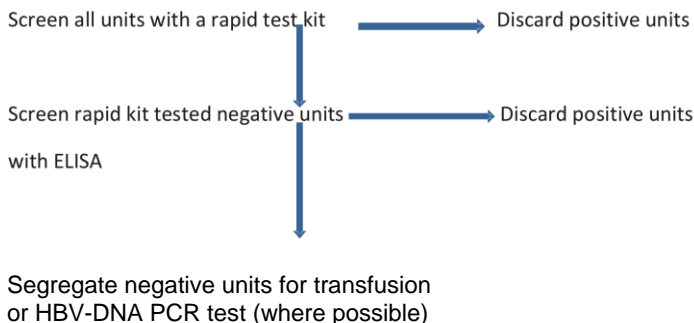


Picture 2: Plate showing the outcome of HBsAg ELISA run on samples earlier tested negative for HBsAg by immunochromatographic Skytec rapid strip. The first column shows the positive control reaction

The false positive reaction generated would necessitate rerunning the screening of such units at the risk of further contaminations and definite utilization of more kits, increase equipment wear and tear, maintenance servicing and repair, human resource fatigue and more errors. Semi-automated ELISA generated the highest positive reaction to hepatitis B virus surface antigen.

This rate of reaction implies the generation of the high number of units for discard which may include false positive and further divert resources into managing error discards. The burden of reactive unit in blood discard is supported by an earlier study when we reported TTIs being responsible for 22.8% of 1854 (25.58%) of all blood collections discarded in our centre (Damulak et al, 2010).²⁰ The false positive reaction leads to donor loss as all blood givers whose blood sample tested positive are permanently differed from subsequent donation with possible accompanying stigmatization.¹ Further investigations and follow-up of the donors whose blood were falsely positive also exert additional strains on the scarce health care resources. The post rapid test ELISA screening exemplified in picture 2 is evidence of the advantages of the pre ELISA rapid test. There is however the need to replicate this study in another centre to confirm our findings.

We recommend a flow chart guide for the screening for HBsAg among blood donors in our setting with high rate of sero-positivity. This guide is sustainable without compromising the quality of blood eventually selected for transfusion. First screen with a rapid test kit and removed all positive units for discard. All negative units are further screened with ELISA, and positive unit segregated for discard while negative ones are issued for transfusion. Screening for hepatitis B virus surface antigen in this staggered protocol would ensure that all units for transfusion are ELISA negative for this marker while reducing the consumption of expensive ELISA kit.



CONCLUSION

We conclude that the rate of HBsAg sero positivity is high for both Immuno-chromatography rapid test kit and ELISA methods. We further conclude that staggered HBsAg screening progressing from rapid strip to ELISA would reduce the consumption of ELISA kit and maximize the utilization of blood bank in settings with high infection rates and lean resources.

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