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Evaluation of a rapid test kit for detection of HBsAg/eAg in whole blood: a possible method for pre-donation testing

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Summary

In many developing countries, where hepatitis B is endemic, positivity rate for HBsAg in donor blood is high, and in some places, up to 20% of donated blood has to be discarded for being HBsAg positive. This degree of wastage may be financially crippling for some developing countries. Pre-donation testing may be useful, so that donors who test HBsAg positive are deferred and wastage of costly blood bags is reduced. The study is to evaluate the suitability of the AMRAD kit, for pre-donation testing,for HBsAg. One hundred and one (101) healthy blood donors were screened for HBsAg/eAg using the test kit. The same specimens were screened using Monolisa (ELISA) kits for HBsAg and eAg as the standard. True positive (TrP), False negative (FN), True negative (TrN), and false positive (FP) values were then found, from which, sensitivity, and specificity were derived. The AMRAD test kit detected 93 specimens as negative and 8 specimens as positive for HBsAg, as against 94 negatives (TrN) and 7 positives (TrP) by monolisa. Thus, one false positive (FP) result was found in using the kit while no false negative (FN) occurred. The findings in this preliminary study suggest that AMRAD kit may be a useful pre-donation screening test for HBsAg.

Keywords: *AMRAD kit, Hbs Ag, testing, blood safety, donor blood*

Résumé

Dans plusieurs pays sous développés ou l hépatite B est endémique,une prevalence de HBsAg des donneurs de sang était élevé, et sur certaine places plus de 20% du sang récu doit être rejecté pour le fait d être positive au HBsAg. Cette perte peut financiérement paralysée certains pays sous-dévelopés. Un test de depistage du HBsAg avant le don de sang pourrait etre utile. L objectif de cette étude avait pour but d évaluer l utilisation du AMRAD kit pour le depistage.Cent-un donneurs sain participaient au dépistage du HbsAg/eAg. Ces meme échantillons étaient aussi évalués utilisant Monlisa(ELISA) kit comme standard pour HbsAg et eAg. Les résultats positive, négative,vrai positive et vrai négative étaient enrégistrées, la sensitivité et la spécificité étaient dérivées. The AMRAD test kit détectait 93 échantillons comme négative et 8 positive au HBsAg contre 94 négative(TrN) et 7 positive(TrP) au Monolisa kit. Ainsi un échantillon vrai négative était enregistré. Ces résultats préliminaires suggérent que AMRAD kit peut être utile pour le test de depistage du HBsAg avant les dons de sang.

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Introduction

There are many factors contributing to poor blood safety in Africa [1]. Important among them is the near absence of organized voluntary blood donation programmes. A 5-year report (1990-1994) at the University of Ilorin Teaching Hospital in Nigeria showed that only 5% of donors were truly voluntary and altruistic [2]. A 5-year review (1995-2000) of types of blood donors at the Lagos University Teaching Hospital also showed that only 1,203 (5.6%) of 21,568 donors were voluntary donors. (Akanmu, unpublished). Proportion of true voluntary donors at the University College Hospital, Ibadan, is also reported to be about 1.8% (Shokunbi, personal communication). These figures are in sharp contrast to those from developed countries of Europe, for example, where 100% of donations are voluntary. Majority of donors in many developing countries are family replacement donors, some of whom may be paid donors who disguise as patients' relatives. Paid, and some family-replacement donors, for fear of rejection, may hide relevant information for which they may be deferred as donors [3]. Consequently, there is often a high prevalence of Transfusion Transmissible Infections (T.T.I.) among blood donors in the developing countries, in spite of all attempts to screen off unsuitable donors from medical history alone. This situation leads to a high discard rate, by which, as much as 20% of donated units of blood may be discarded after post-donation testing for HBsAg [4]. This amount of wastage, and the subjection of the donors to unnecessary donation procedure, may not only be unethical, but also economically self-destructive [3]. Therefore a pre-donation test may be desirable, by which prospective donors who are positive for the HBsAg are deferred. In this way the time and energy of donor clinic staff are not wasted, the prospective donor is spared unnecessary inconvenience and savings are made on costly donation materials, such as imported blood bags.

Currently available methods of screening for Hepatitis B are not convenient for pre-donation testing. With standard ELISA screening method for example, the donor would have to wait for about two hours for results of screening to become available. This waiting time would be unacceptable; it would dampen donors' enthusiasm and

give transfusion service a negative image [3] Quick latex agglutination, and dipstick methods, which are designed to use serum or plasma are available, but not always very sensitive, and may miss some truly HBsAg positive donors. They also require time for blood to clot or be spun, and serum or plasma harvested. For these reasons, Adewuyi has suggested that it would help to find quick screening tests for HBsAg, which can use drops of whole blood, perhaps from a finger prick [3].

This desirable specification appears to be met in a new rapid HBsAg/eAg diagnostic test method. It is important, however to ascertain that any new test kit is capable of detecting small concentrations of HBsAg in whole blood and that samples negative for HBsAg are not falsely detected as positive.

The kit under test also claims to detect HBeAg in whole blood. HBeAg is a degradation product of HbcAg. [5,6] and is found as a soluble antigen freely in the plasma. It is seen early in the course of infection and disappears before the appearance of anti-HBs. Presence of e antigen coexisting with HBsAg indicates active replication of HBV, and patients with chronic hepatitis B who are s and e antigen positive tend to have more severe inflammatory disease than those who are s antigen positive but e antigen negative [7]. The presence of the e antigen also correlates with other markers of replication of the virus such as presence of Dane particles [8] viral DNA polymerase [9], high titre of HBsAg and HBV DNA sequences [10,11]. The combined HBsAg/eAg test may therefore help in the diagnosis and assessment of the severity of HBV infection. The rapid diagnostic test (which takes about three minutes to perform) for the combined antigen detection, if found reliable, could facilitate quick patient evaluation at the clinic while awaiting detail laboratory reports. The aim of the present study is to evaluate the AMRAD kit, as a rapid pre-donation test for the presence of HBsAg in prospective blood donors.

Materials and methods

Subjects

The subjects were apparently healthy Nigerian adult male prospective blood donors presenting at the Lagos University Teaching Hospital (LUTH) and the General Hospital Lagos (GHL). One hundred and one of the prospective donors were recruited randomly for the exercise, after giving informed consent Blood samples were drawn from the donors into EDTA specimen bottles at the end of blood donation.

Thirty-six patients, with confirmed chronic liver disease, were also recruited and bled for testing. Samples were tested immediately for HBsAg/eAg, with the AMRAD rapid diagnostic test kit, as described by the manufacturer, (AMRAD ICT, 13 Rodborough Road, Frenchs Forest, AUSTRALIA). Testing in all cases was completed within three minutes. Subjects' plasma was then extracted and frozen at –20°C, and later tested in batches for HBsAg and the eAg using Monolisa (ELISA) screening kits. (Sanofi Diagnostic, Pasteur, France).

Principle of the Assays

1. AMRAD HBsAg/eAg rapid diagnostic test

The test is performed on whole blood. The test card has two leaves that can be apposed to one another. On the inner surface of the left leaf is a pad made up of a lower whitish part and an upper pinkish part. The pinkish part is impregnated with a set of antibodies to both the s and e antigens. These antibodies are coated onto colloidal gold particles. The inner part of the right leaf has a thin strip (membrane) attached to it. This strip is impregnated with another set of antibodies to the s and e antigens. The anti-s and anti-e on the strip occupy different bands. There is a third band, which serves as a control to show that the procedure is correct. Whole blood is applied to the white part of the pad on the left leaf. The red cells are retained in this part while plasma moves to the pinkish part where the s and e antigens in the plasma interact with the colloidal gold carrying anti HBs and anti HBe respectively. When the two leaves are apposed, the colloidal gold carrying the s and e antigens moves by capillary action along the membrane so that the s-antigen-carrying colloid particles are immobilized at the s band, while those carrying e antigens are immobilized at the e band. Colour development at either or both bands indicates the presence of s or e or both antigens in the plasma respectively. The procedure was carried out as directed by the manufacturer.

2. Monolisa^{R} HBsAg and Monolisa^{R} HBeAg test

Monolisa is a standard sandwich ELISA test and was carried out according to manufacturer's specification.

Analysis of results.

From the results (numbers of specimens positive and negative for HBsAg using Monolisa and AMRAD Kits), the sensitivity and specificity of the AMRAD method were calculated using the sandwich ELISA technique (Monolisa) as the reference method (Table 1).

Results

HbsAg

The sandwich ELISA (standard) method detected seven (7) of the 101 blood donor specimens as positive (approximately 6.99%) but the AMRAD picked up eight (8) specimens as positive – (Table 1). Ninety-four and 93 specimens were HBsAg negative by the standard and test methods respectively. For the population of patients with chronic liver disease, 4 of the 36 subjects were identified as HBsAg positive by the standard ELISA method. All the 4 were also identified as positive by the AMRAD test kit (Table 2).

Table 1: Prevalence of HBsAg in blood donors using Monolisa (ELISA) and AMRAD (Kit) methods

	Monolisa		
	Positive	Negative	Total
Positive (AMRAD)	7 (TeP)	1 (FP)	8
Negative (AMRAD)	0 (FN)	93 (TeN)	93
Total	7 (TrP)	94 (TrN)	101

Key:
TrP = True positives: samples positive by standard ELISA(Monolisa)
FP = False positives: samples negative by standard ELISA but positive by AMRAD
TrN = True negatives: samples negative by standard ELISA (Monolisa)
FN = False negatives: samples positive by standard ELISA but negative by AMRAD
TeP = Test positives: samples positive by AMRAD and Monolisa
TeN = Test negatives: samples negative by AMRAD and Monolisa

Table 2: Prevalence of HBsAg in patients with chronic liver disease, using monolisa (ELISA) and amrad (kit) methods

	Monolisa		
	Positive	Negative	Total
Positive (AMRAD)	4	0	4
Negative (AMRAD)	0	32	32
Total	4	32	36

Sensitivity of AMRAD HBsAg method for blood donors
Sensitivity is defined as the proportion of positives by the trial method, which are also reference positive, compared with total reference positives. In this study, the sensitivity of the AMRAD method may be calculated as follows (Table 1):

$$\frac{\text{7(no. positive by both AMRAD and ELISA i.e.TeP) x 100}}{\text{7 (total number positive b y ELISA i.e. TrP.)}} = 100\%$$

Specificity of AMRAD HBsAg method for blood donors
Specificity is defined as the proportion of negatives by the trial method, which are also reference negative, compared with the total reference negatives. The specificity of the AMRAD method using Monolisa ELISA as the reference, may be calculated from Table I as

$$\frac{\text{93 (no. negative by bothAMRAD and ELISA i.e.TeN 100}}{\text{94 (total number negative by ELISA i.e. TrN.)}} = 98.94\%$$

Sensitivity and Specificity of AMRAD method for HBsAg screening of patients
For the 36 patients with chronic liver disease who were screened for HBsAg, 4 positive and 32 negative results obtained by the ELISA method came up correctly as positive and negative respectively by the AMRAD test kit. The sensitivity and specificity of the test kit in these patients were therefore 100% each.

HBeAg
Only one of the 101 blood donors was positive for HBeAg. The donor was positive by both the test and standard methods. None of the patients with chronic liver disease was positive for HBeAg. The sensitivity and specificity of AMRAD HBeAg assay were 100% in this study. The HBeAg-reactive specimen was also positive for HBsAg using the test kit but negative for HBsAg by the standard Elisa method. It was the only specimen that showed discordant HBsAg result by the AMRAD and the Monolisa methods.

Discussion
The criteria for the licensure of reliable HBsAg ELISA screening kits require that sensitivity and specificity should be over 99% [12]. A satisfactorily sensitive kit, according to the World Health Organisation (WHO), should be able to detect as low as 1 British standard unit, equivalent to 1ng of HBsAg/ml of blood [13]. The manufacturer of the AMRAD kit claims that the kit is capable of detecting HBsAg at a minimum concentration of between 15ng/ml of blood.

Although the manufacturer's cut-off value seems higher than that demanded by the W.H.O, most asymptomatic patients positive for HbsAg tend to have much higher levels of surface antigen particles [14].

This study has attempted to test the ability of the AMRAD kit to correctly detect HB surface antigens where they are present, and to correctly identify as negative, samples in which they are truly absent. To achieve this objective, the kit has been evaluated against a highly sensitive sandwich ELISA method (Monolisa).
The sensitivity of the kit for the HBsAg test is 100%, implying that it is capable of identifying all the blood donors with HBsAg in their blood. This is good, as it may provide a method by which blood donors may be rapidly screened prior to blood donation, without fear of missing any positives.

In this study, the prevalence of HBsAg by standard ELISA testing was 7%. Thus, 7 out of every hundred blood bags would have been saved if pre-donation HBsAg was done and the man-hours expended unnecessarily by blood donor clinic staff would also have been saved. Environmental safety may also be enhanced, as less quantity of infected blood would require disposal, when pre-donation screening is adopted.

The specificity of the AMRAD kit for HBsAg testing in blood donors was found to be 98.94%. which is satisfactory for blood donor screening. The single false positive test which brought the specificity to less than 100% means that the donor would not have been bled and so would not pose danger to a recipient as a false negative would. The results of this preliminary study would suggest that the AMRAD kit might provide a useful pre-donation screening test for HBsAg. A larger study will be required to confirm this impression.

Other findings from this study showed the sensitivity and specificity of the AMRAD kit for HBcAg testing to be 100%. when compared with the Monolisa ELISA technique. This suggests that the AMRAD kit may also be useful in the rapid investigation of patients with chronic liver disease. for the presence and infectivity of HB. However the number of patients in this study was small and there was no HBeAg positive case among them. A larger study will therefore be required for firm conclusions on the usefulness of the AMRAD kit test for investigating chronic liver disease patients for their HBeAg status.

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