# A Comparison of an Immunochromatographic Technique with Enzyme-Linked Immunosorbent Assay for the Detection of Hepatitis B Surface Antigen in Calabar.

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***Abstract:*** *Background /Aims: Hepatitis B viral (HBV) infection has been reported by the World Health Organization (WHO) to have attained hyperendemic proportions in sub-Saharan Africa. Routine screening of the general population in Nigerian laboratories for hepatitis B surface antigen (HBsAg) frequently uses rapid techniques such as the immunochromatographic assay (ICA). This study aimed to compare an immunochromatographic technique with the more widely accepted enzyme-linked immunosorbent assay (ELISA) technique for the detection of (HBsAg) in Calabar, southern Nigeria.*
***Material and Methods:*** *This was a prospective observational study of 72 adults attending the gastroenterology clinic of the Department of Medicine, University of Calabar Teaching Hospital (UCTH) between February and May 2013. Each participant was tested for hepatitis B surface antigen (HBs Ag) using both ICA test strip and ELISA methods.*
***Results:*** *The study determined that the negative predictive value of the ICA test was 80%, with a negative likelihood ratio of 0.27.*
***Conclusion:*** *This study revealed that using the ICA test alone was not effective in excluding HBV infection.*
***Recommendation:*** *With the high burden of HBV infection in Nigeria and the grave consequences of contracting the infection, there is an urgent need for nationwide policy change to encourage improved screening methods.*
***Keywords****: Calabar, ELISA, hepatitis B virus, Immunochromatography.*

## I. Introduction

Viral hepatitis is a systemic disease that primarily affects the liver. Viral hepatitis is caused by hepatotrophic viruses (Hepatitis A-G) but hepatitis A, B and C account for a significant proportion of all cases of acute viral hepatitis [1]. Hepatitis B infection is one of the most common causes of chronic liver disease, with up to 25% of chronic carriers of hepatitis B developing serious liver disease, chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma worldwide [2].

Hepatitis B viral (HBV) infection is a global public health problem, with 2 billion of the world's population being infected with the virus, with an estimated 400 million chronic carriers worldwide [3]. In developed countries of America and Europe, HBV prevalence is relatively low (≤2%), whereas in less developed countries of Asia, Africa and the Middle East, HBV prevalence rates are much higher, reaching 5 – 20% of the general population [4].

Hepatitis B viral infection in Nigeria is said to have reached hyperendemic levels (i.e. Hepatitis B surface antigen greater than 7% of an adult population) [5]. It has been estimated that 20 million Nigerians have HBV infection [6].The sero-prevalence of hepatitis B surface antigen (HBsAg) in Nigeria ranges between 9-39%, depending on the population evaluated [7].

HBV infection is a silent illness especially among those infected at an early stage of their life. Adults with chronic hepatitis B (CHB) may be asymptomatic and are unlikely to get tested, with up to 80% of these individuals unaware that they have been infected with HBV [8]. Therefore it is imperative to accurately

diagnose or screen for HBV infection in at risk populations or prior to blood donation. All these measures are aimed to minimize the spread of HBV and its resultant complications [8].

Hepatitis B surface antigen appears in serum 2 to 10 weeks after exposure to HBV and before the onset of symptoms or elevation of serum aminotransferase levels [9]. In self-limiting acute HBV infection, HBsAg usually becomes undetectable after four to six months. Persistence of HBsAg for more than six months implies progression to chronic HBV infection [9]. Consequently, HBsAg has been found to be a useful viral marker for both population screening and diagnosis of acute HBV infection or CHB [9].

There are several serological methods available to detect HBsAg, including enzyme immunoassays (EIA), radioimmunoassays (RIA), immunochromatographic assays (ICA) and haemagglutination assays [8]. In Nigeria, blood banks commonly use ICA test strips to screen for HBsAg in blood donors [10]. These test strips are relatively cheap and have neither storage requirements nor special training before use. Also, no equipment is required for the ICA testing [8]. In spite of claims of high sensitivity and specificity by the manufacturers of the ICA test strips, there are worries about the potential risk of missing cases of HBV infection in clinical settings [10, 11]. These concerns may have been allayed somewhat with the emergence of very sensitive tests such as the enzyme linked immunosorbent assay (ELISA). The ELISA kit is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma which requires no special preparation of the patient [12]. ELISA testing has been adopted by blood bank services in several countries but this has not been the case in Nigeria. Head to head comparisons of both screening methods may form the basis for policy change with respect to HBV screening in Nigeria and other countries where ICA is still being utilized for this purpose.

This study compared an ICA technique with ELISA for detection of HBsAg antibodies among a cohort of patients attending the gastroenterology clinic of the University of Calabar Teaching Hospital (UCTH).

## II.    Materials and Methods

The selection of participants for this study was done using convenience sampling of patients attending the gastroenterology clinic of the Department of Medicine, UCTH between February and May 2013. The UCTH is the only tertiary health facility in Cross River State which is in south eastern Nigeria. Located in the capital city of Calabar, the UCTH has 410 beds and provides specialist care for people of Cross River and the neighboring states [13].

The study was on observational cross sectional study involving individuals attending the gastroenterology clinic. The objectives of the study were first explained to all intending participants. Thereafter, written informed consent was obtained from consenting participants.

Three (3) mls of blood was collected from 72 participants in the Department of Chemical Pathology, UCTH. The samples were allowed to clot at room temperature for 20 minutes. The serum was then separated by centrifuging at 4000 rpm for 10 minutes and stored in sample bottles at -20°C until tested. The sera were allowed to thaw and brought to room temperature just before testing. Each serum sample was tested using Wondfo Biotech one step HBsAg rapid screen test and the DRG ®in -vitro enzyme-linked immunosorbent assay kit. All the tests were carried out on the same day with strict adherence to the manufacturer's directions.

The Wondfo Biotech one step HBsAg rapid screen test is a lateral flow, immunochromatographic screening test for HBsAg. The test is based on the principle of sandwich immunoassay for determination that utilises two monoclonal antibodies to identify HBsAg specifically. The duration of this one step test was about 15-20 minutes and test results were subsequently read visually without any instrument. Negative results appeared as only one colour band on the control region, while positive results appeared as distinct colour bands on the control and region tests.

The DRG ®kit is an ELISA kit for the qualitative detection of HBsAg in human serum or plasma samples. The principle here involved the coating of the solid phase multi wells with anti-HBsAg antibodies (primary antibody). Serum samples (containing HBsAg) and enzyme (horseradish peroxidase, HRP) conjugated antibody (secondary antibody, HRP-conjugated; "Enzyme conjugate") were added to the coated wells. After incubation, a complex formed between the primary antibody (anti-HBsAg), the antigen (HBsAg) and the HRP-conjugated antibody. Following a final washing step to remove unbound components, the substrate (TMB) was added to the wells which, after an incubation period resulted in the formation of a coloured product (blue colour). After the addition of stop solution, a yellow coloured product was formed. The presence of HBsAg was then detected at 450 nm in an ELISA reader.

Ethical clearance for this study was obtained from the health research ethics committee of UCTH assigned number; UCTH/HREC/33/92.

## III.    Statistical analyses

Data was entered into the Predictive Analytics Software (PASW) version 18.0 IBM New York USA and subsequently analyzed. Categorical groupings were generated for gender, place of origin (based on geographical zone) and age of the participants. A receiver operating characteristic (ROC) curve was generated

(using PASW) to compare two operating characteristics i.e. the true positive rate (TPR) and false positive rate (FPR) and ultimately obtain the diagnostic efficiency. In addition, the positive predictive value [true positive (TP) divided by the sum of TP and false positive (FP)], negative predictive value [true negative (TN) divided by the sum of TN and FN] of RIA were calculated. Positive likelihood ratio (sensitivity/ 1- specificity), negative likelihood ratio (1-sensitivity / specificity) and diagnostic accuracy (TP+ TN / TP + FP + FN + TN) were also calculated.

# IV.    Results

Three-quarter (54, 75%) of the participants were males, while the remaining one quarter (18, 25%) were females. Their age ranged from 19 to 76 years, with the majority (34.7%) of the participants belonging to the 30-39 year age category (Table 1). Most (34.7%) of the participants were from the Northern part of Cross River State with those from outside of Cross River also constituting a significant proportion (29.2%) as shown in Table 2.

Twenty five of the participants had positive results from both ICA and ELISA tests while 36 had negative results from both tests (Table 3). Using ELISA as gold standard, the sensitivity of the ICA test was 73.5% with specificity of 94.7% (Table 4). The positive predictive value of the ICA test was 92.6% and its negative predictive value was 80%. The positive likelihood ratio of the test was 14.8, while the negative likelihood ratio was 0.27. The overall diagnostic accuracy of the ICA test was 84.7% (Table 4).

While the receiver operating characteristic (ROC) curve, revealed a modest true positive rate of the ICA test (i.e. diagnostic efficiency similar to that calculated above) with the area under the curve value of 0.863 (86.3%). See figure 1.

# V.      Figures and Tables

**Table 1:** Age category of participants

| Age category | Frequency | Percent (%) |
|---|---|---|
| < 20 | 2 | 2.8 |
| 20-29 | 19 | 26.4 |
| 30-39 | 25 | 34.7 |
| 40-49 | 14 | 19.4 |
| 50-59 | 5 | 6.9 |
| >60 | 7 | 9.7 |
| Total | 72 | 100.0 |

**Table 2:** Area of origin of participants

| Area of origin | Frequency | Percent (%) |
|---|---|---|
| Northern district of Cross River | 25 | 34.7 |
| Central district of Cross River | 16 | 22.2 |
| Southern district of Cross River | 10 | 13.9 |
| Others (Hausa, Yoruba, Ibo etc) | 21 | 29.2 |

**Table 3:** Comparison of Immunochromatography assay to ELISA

| | | ELISA ( gold standard) | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| ICA | Positive | 25 (true positive) | 2 (false positive) | 27 |
| | Negative | 9 (false negative) | 36(true negative) | 45 |
| Total | | 34 | 38 | 72 |

**Table 4:** Quality evaluation parameters of immunochromatography diagnostic assay

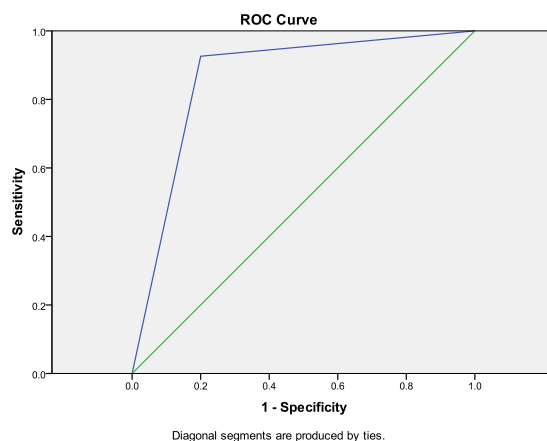| | Sensitivity | Specificity | PPV | NPV | Diagnostic accuracy | Negative likelihood ratio | Positive likelihood ratio |
|---|---|---|---|---|---|---|---|
| HBsAg by rapid immunochromatography | 73.5% | 94.7% | 92.6% | 80% | 84.7% | 0.27 | 14.8 |

Fig 1: Receiver operating characteristic (ROC) curve

**Table 5:** Area Under the Curve

| Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|------|-----------|----------------|--------------------------------|---|
| | | | Lower Bound | Upper Bound |
| .863 | .046 | .000 | .772 | .954 |

## VI.  Discussion

Hepatitis B is a highly infectious virus which leads to a long term insidious infection. It remains a major blood borne infection with grave implications for blood recipients if infected blood is not identified during screening. In Nigeria, the ICA test continues to be widely used as the sole test to detect HBsAg for both screening purposes and diagnosis of acute and chronic HBV infections [10]. The manufacturers of these kits lay claims to high sensitivity and specificity of the test kits.

However, studies have shown that ELISA tests are more sensitive (up to 100%) and accurate for blood screening, but it is yet to be widely used by blood bank services in Nigeria [10]. This is probably due to the relatively higher cost of the ELISA test kits, lack of expertise and stringent storage requirements. Concerns have been raised regarding the low sensitivity of the ICA kit when compared to ELISA (the gold standard) [8, 10, 11] Our study has demonstrated that the ICA kit may not be an efficient screening test based on its relatively low sensitivity (73.5%). Furthermore with a large number of false negative cases and few false positive cases  a negative screening test is in itself poor at excluding HBsAg (NPV =80%). These findings are similar to the work carried out by Chameera et al [8].

However, a positive result reveals that the ICA test is likely to be a good screening tool (PPV=92.6%) in detecting HBsAg in a population. It is important to note that reporting only sensitivity and specificity hides the true performance of a test. Unlike sensitivity and specificity, predictive values are largely dependent on disease prevalence in the population [14].

A NPV of 80% would infer that among 100 patients with a negative result, only 80 cases are actually negative, with 20 potentially false negative tests. On the other hand, the ICA test proves to be quite efficient in detecting HBsAg in a population based on its high PPV .In addition, when using the ICA test the likelihood of the test 'ruling in' or diagnosing HBsAg is good (i.e. positive likelihood ratio = 14.8), while its ability to 'rule out' or exclude the infection is poor (negative likelihood ratio = 0.27).

The ROC curve revealed a qualitative cutoff value (0.863, p= 0.001) that represents a modest diagnostic efficiency (86.3%) of the ICA test. Though our study revealed that the ICA was efficient in screening for HBsAg based on its high PPV, good positive likelihood ratio and modest diagnostic efficiency, it was poor in excluding HBsAg infection (based on its poor NPV). Furthermore, studies have shown that HBV genotype can influence the ICA test outcome and that not all ICA kits have the equal ability to identify all the HBV sub groups (8 and 9 different genotypes and serological subtypes respectively). This would mean that some products have less ability to detect HBsAg from a certain HBV sub type [8].

With the chronic and often indolent nature of HBV infection, the implication of having such a large false negative result in a given population is worrying. Quantitative ELISA methods are considered to be more sensitive tests for HBsAg detection. This is why ELISA is widely used at well-equipped reference centre's or central blood banks in most developed countries [8].In resource poor settings, serious consideration needs to be given as to whether the high rates of false negative results does not nullify any perceived benefits of ICA use.

## VII. Conclusion

The finding of our study reflects the magnitude of the problem that would arise when the ICA method is used as the sole screening test for HBV infection. The wider implication of negative results from ICA tests is of grave concern bearing in mind the high false negative results displayed in this study. Arising from this, it is imperative that methods of HBV screening in Nigeria and other countries with similar circumstances be urgently reviewed in a bid to providing more sensitive tests. This will offer better guarantees for persons accessing blood transfusion services in countries were ICA is still the sole screening test. This study had some limitations. One was the relatively small sample size. Another was the lack of confirmatory testing for HBsAg. Polymerase chain reaction is the gold standard for HBsAg detection.

## Acknowledgement

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