# EVALUATION OF HEPATITIS B CORE ANTIBODY (TOTAL) IN THE PRESENCE OF ID-NAT

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## ABSTRACT

***Background & Aim**: The testing for HBcAb is done by different blood centres to ensure the blood safety for transfusion. However, In India, HBcAb is not compulsory test as per Drugs and Cosmetics Act, 1940. Introduction of ID-NAT (Nucleic Acid Testing) for detection of HBV DNA have proven to be of great success but on the contrary increase the cost of blood transfusion. Therefore, the present study was undertaken to find the possibility of obviating the need of screening of HBcAb with existing ID-NAT, so as to optimize the resource utilization. **Materials & Methods:** Donor blood samples between October 2008 and April 2010 covering 8221 samples were collected and tested simultaneously for Anti-HBc and HBsAg ELISA and ID-NAT tests. **Results**: Nearly 7% (581) were reactive for HBV marker. Further the samples reactive to serological markers HBsAg & HBcAb (Total), were compared with ID-NAT. Of all the samples reactive for HBV, 7.6% was reactive by ID- NAT. About 2.07% of solitary HBcAb reactive samples were reactive by ID-NAT (Table 1). However, none of the non- reactive samples was reactive to ID-NAT. **Conclusion**: Still, HBcAb has a definitive role in ruling out the transmission of HBV among HBsAg non-reactive blood (2.07%; Table 1). On the contrary, not even one sample was NAT reactive and not reactive by CLIA. Screening of blood by Anti- HBcAb didn't enhance the blood safety. In addition, centers that have incorporated NAT testing may not derive any additional benefit as this cannot replace anti-HBc testing, especially in developing country like ours.*


KEY WORDS: Hepatitis core antibody, ID-NAT- Individual donor- Nucleic acid Test, HBsAg- Hepatitis B Surface Antigen, TTI- Transfusion Transmitted Infection

**خلاصة: الخلفية والهدف:** يتم اختبار الاجسام المضادة لنواة فيروس الالتهاب الكبدي نوع ب (HBcAb) من قبل مراكز الدم المختلفة لضمان سلامة نقل الدم. ومع ذلك فإن اختبار (HBcAb) في الهند ليس إجباريا حسب قانون الادوية و مستحضرات التجميل للعام ١٩٤٠. أثبت إدخال اختبار الحمض النووي {ID-NAT (Nucleic Acid Testing)} للكشف عن الحمض النووي لفيروس التهاب الكبد نوع ب نجاح كبير ولكنه يزيد من تكلفة عملية نقل الدم. لذلك أجريت هذه الدراسة لاختبار إمكانية تفادي او انتفاء الحاجة لفحص (HBcAb) بواسطة اختبار {ID-NAT (Nucleic Acid Testing)} , وذلك لتحسين استخدام الموارد. **منهج الدراسة**: تم جمع العينات من المتبرعين بالدم ما بين أكتوبر ٢٠٠٨ وأبريل ٢٠١٠ وبلغت العينات ٨٢٢١ عينة واختبرت في وقت واحد لمعرفة أولا: الاجسام المضادة لنواة فيروس الالتهاب الكبدي نوع ب (Anti-HBc) و ثانيا: المستضد السطحي لفيروس الالتهاب الكبدي نوع ب ( HbsAg ELISA ) وثالثا: اختبار الحمض النووي . {(ID-NAT (Nucleic Acid Testing)} **النتائج:** حوالي ٧٪ (٥٨١) كانوا ايجابيين لعلامات فيروس الكبد نوع ب. وعلاوة على ذلك كانت العينات ايجابية لعلامات المستضد السطحي وايضا الاجسام المضادة للنواة المصلية (HBsAg ) و ( HBcAb)عند مقارنتهم جميعا مع اختبار الحمض النووي ( ID-NAT (Nucleic Acid Testing). من كل العينات الايجابية لفيروس التهاب الكبد البائي فقط ٧,٦٪ اعطي نتيجة ايجابية بواسطة اختبار الحمض النووي (ID-NAT (Nucleic Acid Testing). و حوالي ٢,٠٧٪ من العينات الايجابية ل HBcAb كانت ايجابية بواسطة اختبار (ID-NAT (Nucleic Acid Testing). ومع ذلك، لم تكن أي من العينات السلبية النتيجة ايجابية مع اختبار (ID-NAT ,Nucleic Acid Testing)

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**الاستنتاج**: لا يزال اختبار ، HBcAb له دور حاسم في استبعاد انتقال فيروس الكبد نوع ب من الدم السلبي النتيجة للمستضد السطحي للفيروس (HBsAg) (2.07٪). و على العكس من ذلك، لم يكن حتى عينة واحدة من اختبار الحمض النووي ( NAT ) ايجابية ولم تكن كذلك بواسطة فحص التوهج المناعي (CLIA). فحص الدم عن طريق الاجسام المضادة للنواة للالتهاب الكبدي الفيروسي نوع ب ( HBcAb ) لا يعزز سلامة نقل الدم. وبالإضافة إلى ذلك، المراكز التي أدرجت اختبار الحمض النووي ( NAT ) قد لا تستمد أي فائدة إضافية لأنه لا يمكن أن يحل محل اختبار الاجسام المضادة للنواة للالتهاب الكبدي الفيروسي نوع ب ( HBcAb )، وخاصة في البلدان النامية مثل بلدنا.

## INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health problem which is affecting two billion people worldwide, and 350 million people suffer from chronic HBV infection. This is approximately 5% of the world population.[1] There are over 4 million new cases of acute clinical hepatitis yearly. About one million of those infected die annually from chronic hepatitis B infection or one of its complications-cirrhosis and hepatocellular carcinoma.[2] Transmission of hepatitis through blood transfusion was first reported in 1943.[3,4]

In India, Hepatitis B virus (HBV) infection via blood transfusion is a serious health problem. After an incubation period that can range between 50 to 180 days, this infection has an insidious onset. The viruses are detectable in the blood for several months or even years and about 5-10% of individuals become chronic carriers. Chronic infection by HBV could lead to the development of cirrhosis and hepatic malignancy.[5] Screening for hepatitis B infection by surface antigen (HBsAg) started in 1971.[6] Usually testing for HBsAg is performed using EIA, radioimmunoassay or other immunological based assays. The latest, most sensitive HBsAg detection method uses chemiluminescence and this method is widely used in Canada, Europe, and some parts of Asia.[7] Method for detection of HBsAg is not yet standardized. The only requirement now for HBsAg testing is licensing or accreditation of the test in this country became selection of the test different in different countries.

In some tropical countries where blood donor centres have limited resources, rapid and less expensive immuno filtration, latex based, or immuno chromatographic methods are used, without any confirmatory testing techniques.[8]

The mandatory screening for HBsAg by ELISA for over 20 years, still transfusion-associated HBV (TAHBV) continues to be a major problem in India, and more so in patients receiving repeated blood transfusions.[9] However, it has been observed that some HBsAg-negative individuals continue to replicate HBV.[10,11] Hence, the non reactive result of HBsAg in the blood can not reflect that the person is completely free from Hepatitis B Virus. Blood containing anti-HBc with or without detectable presence of HBsAg might be infectious. In 1992, antibody to the core protein of hepatitis B (anti HBc total) was introduced in the screening process as a surrogate marker for post-transfusion non A non B hepatitis.[12] With introducing of anti HBc (total) screening, a phenomenal reduction target has been achieved in the number of hepatitis B viral infectious units from entering the donor pool. Therefore, routine blood donor screening for anti-HBc was implemented to reduce the risk of post-transfusion HBV infection.[13] Screening practices of Transfusion Transmitted Infections (TTI) depends on pathogenicity, morbidity, mortality, endemicity of pathogen. Ethics vs. financial viability have led to varied practices across the globe.

Hepatitis B core antibody screening is controversial in blood banks worldwide, because Hepatitis B core antigen is found only in liver cell and not in blood circulation, it is true that blood circulation is detected mainly with anti HB core total (HBcAb IgM & IgG). Recently introduced ID- NAT testing has reduced the window period but does not completely eliminate HBV. Hepatitis B virus infection has a worldwide prevalence due to its infectivity, chronicity and ability to cause substantial morbidity and mortality, in the form of cirrhosis and Hepatocellular Carcinoma (HCC).[14]

However, current development of ID-NAT technology can be proved more sensitive testing for safe blood supply by HBV infection, but it expensive. It can also be an effective test to rule out occult hepatitis, HBsAg negative and anti-HBc positive infections with low levels of viraemia. The complete elimination of traditional testing methods in the near future is difficult.[15,16,6]

Screening of HBsAg and HBV DNA by NAT would appear preeminent in the countries with a normal to high prevalence of HBsAg. On the other hand, HBV DNA by NAT and anti-HBc testing may be superior to HBV DNA by NAT and HBsAg testing in countries with low prevalence rate. Pathogen inactivation will possibly have a role in increasing the chances of the safety of blood products reducing the risk of transfusion-transmitted HBV.[17-19]

In India, screening for HIV 1 & 2, HCV and HBsAg is mandatory by law. However, the screening for Anti HBV core antibodies IgM & IgG is employed by various blood banks to ensure the decrease of transmission of HBV in case of HBsAg negative blood.

In Recent time, the use of nucleic acid testing (NAT) of blood donors has been adopted to detect the presence of occult hepatitis B virus in blood donors which is considered a Potential risk for transfusion of hepatitis B virus.[9] Though the use of NAT to prevent transfusion of hepatitis B virus may offer a new opportunity to blood screening programmes, the feasibility of implementing it should be fully considered, since the requirements for infrastructure, financing, staffing levels, training and quality systems and the overall costs of implementation may far outweigh any potential benefit in terms of increased blood safety. The present study was carried out to look into the possibility of obviating the need of screening HBV core if a facility of NAT exists, so as to optimize the resource utilization.

## MATERIALS & METHODS

Donated blood was evaluated and collected between October 2008 and April 2010 covering 8221 samples. The hospital caters to patient population from different parts of country and world such as USA, Canada, Iraq, Nigeria, Oman, Kazakhstan etc. All samples were screened for serology and ID- NAT. Serological screening was performed by random access Chemiluminescence Immuno Assay (CLIA) for TTI including HBsAg & HBcAb – Total (IgM & IgG), (Vitros ECI; OCD; JNJ from USA). Nucleic acid screening (Supplementary test) Individual donor ID-NAT was performed by Transcription Mediated Amplification (TMA) technology (Novartis Diagnostic from USA).

## RESULTS

Of the 8221 blood donors, 581 (7%) were reactive for HBV marker. All the samples screened reactive by serological markers- HBsAg & HBcAb (Total), were compared with ID-NAT. Of all the samples reactive for HBV, 7.6% is reactive by ID- NAT. About 2.07% of solitary HBcAb reactive samples were positive by ID-NAT (Table 1 & Figure 1). However, no non- reactive

sample was observed to be reactive by ID-NAT, over the study period.

## DISCUSSION

In the present study, out of 8221, 581(7%) reactive samples for HBV marker, 07(1.21%) samples were HBsAg reactive/anti HB core Total non reactive /ID-NAT non reactive, 17(2.93%) samples were HBsAg reactive/anti HB core Total reactive - /ID-NAT non reactive, 13(5.34%) samples were found to be HBsAg reactive/anti-HB core total reactive/ID-NAT reactive, 513(88.3%) samples were found to be HBsAg non reactive/anti-HB core total reactive/ID-NAT non reactive, 12(2.07%) samples were HBsAg non reactive/anti-HB core total reactive/ID-NAT reactive, 0(0%) samples were found to be HBsAg non reactive/anti-HB core total non reactive/ID-NAT reactive, and only 1(0.2%) samples were found to be HBsAg reactive/anti-HB core total non reactive/ID-NAT reactive. The result shows that there was no sample which was neither ID-NAT reactive nor reactive by CLIA.

***Table 1 Distribution of HBV markers among all samples***

| HBsAg | HBcAb | NAT | Total | Percentage (%) |
|---|---|---|---|---|
| R | NR | NR | 7 | 1.21 |
| R | R | NR | 17 | 2.93 |
| R | R | R | 31 | 5.34 |
| NR | R | NR | 513 | 88.3 |
| NR | R | R | 12 | 2.07 |
| NR | NR | R | 0 | 0 |
| R | NR | R | 1 | 0.2 |
| NR | NR | NR | 7640 | |

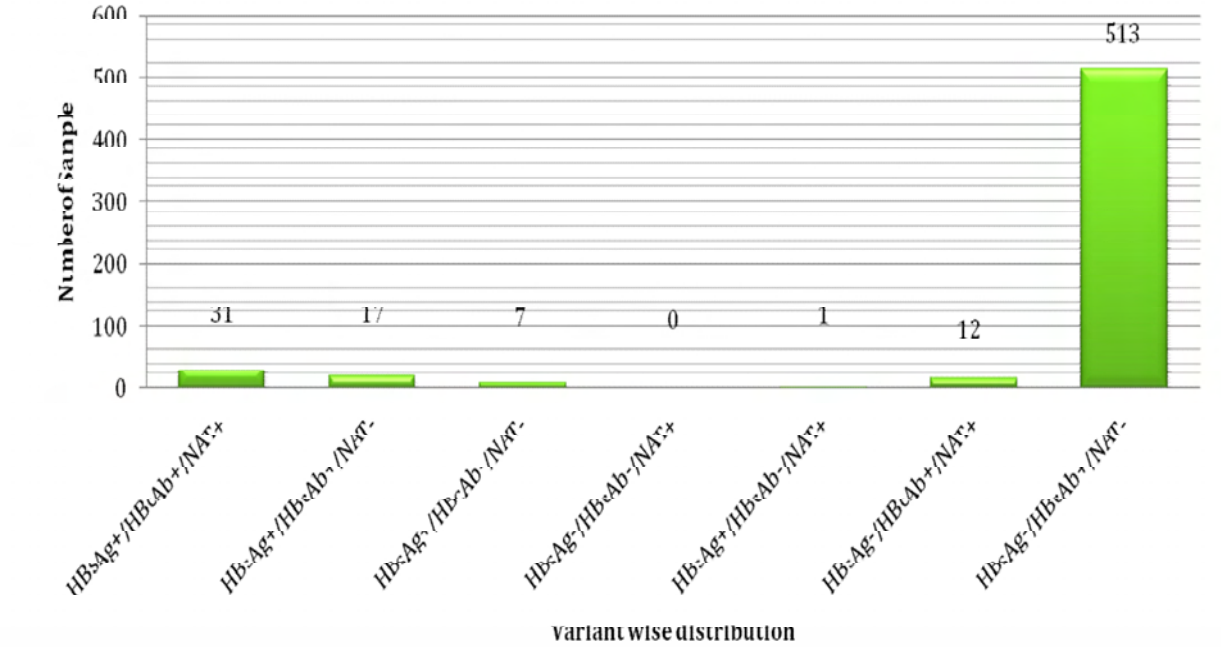
The above mentioned fact suggests that though ID-NAT is not supporting to eliminate anti HB core but still is required validity based upon time to time, because mutation of the viral pathogen.

Safety of blood is a challenge in India because of the high prevalence of HBV, HCV and HIV, the relatively low percentage of volunteer donors and the lack of standardization of screening procedures among the multitude of blood collection centres.[20] The potential of NAT yield in India is staggering when compared to other countries that have already implemented the technology. The study showed that only 0.2% of the positive samples were HBsAg reactive/ ant-HB core non reactive/ ID-NAT reactive and none of the sample (0%) were found to be HBsAg non reactive/ anti-HB core non reactive/ ID-NAT reactive. These findings clearly indicate that NAT is capital extensive in the present scenario. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units versus considering ID NAT needs to be assessed.

Another Indian study on large section of blood donors demonstrated 4.2 per cent anti-HBc only positivity.[9] Hence, we have observed 6.24% (513 out of 8221) prevalence of anti-HBc positivity, which corroborates with our finding. The other study supports the presence of occult HBV infections in blood donors positive for antibodies against hepatitis B core antigen and emphasizes on the need for establishing sensitive screening modalities for blood transfusion.[32] In our study, we also find that the presence of occult HBV infections in blood donor's positive hepatitis B core antibody and focuses on the need for establishing sensitive screening modalities for blood transfusion.

It is commonly followed that the diagnosis of infection by HBV is based on the presence of the HBsAg in the bloodstream.[21] However, screening of blood bank donors for HBsAg does not totally minimize the chances of the risk of HBV

infection through blood transfusion,[22] since the absence of HBV marker in the serum does not exclude the presence of HBV DNA.[23,24] It is in this context it can be say that, donors with occult HBV infection, who lacked detectable HBsAg but whose exposure to HBV infection was indicated by a positive anti-HBc and HBV DNA, may be a potential source of HBV infection.




In India, anti-HBc screening is not mandatory. However, higher prevalence of only anti-HBc (58.8%) cases with 22.8% HBV DNA reactivity from Kolkata.[25] Similarly, other studies also substantiate with Pakistani and Egyptian studies showing 17.2 and 21.47 per cent anti-HBc prevalence in HBsAg-negative, HBV DNA-negative blood donors.[26,27] In India where the anti-HBc reactivity ranges from 4.2% to 18.3 % in healthy donors.[9,28] In this study also found that between the above findings.

Most studies on occult hepatitis B infection have reported higher rates of HBV- DNA detection in liver or peripheral mononuclear cells compared with serum or plasma.[22] The result of study on only serum or plasma sample showed that only 0.2% of the positive samples were HBsAg reactive/ HB core non reactive/ ID-NAT reactive and none of the sample (0%) were found to be HBsAg non reactive/ HB core non reactive/ ID-NAT reactive, these finding also indicate the HBV-DNA detection is very low. Bre´chot et al.(2001) Reported that occult HBV infection exists and that most cases are related to very low levels of HBV rather than to HBV mutants that do not express or produce abnormal surface proteins and consequently are undetected by standard method.[29] Because HBV-DNA detection is the gold standard method to diagnosis of occult HBV infection, the type of assay used and its sensitivity must be specified. The sensitivity of PCR assays for HBV DNA in studies on occult HBV infection varies from 101 to 103 copies/mL.[30] However, most of the PCR assays including commercially available assays are not standardized.[31]

Our earlier study shows out of 2740 samples (n= 186) 6.7% sample reactive by HBV marker. There was no sample which was non reactive by serological markers but observed to be ID-NAT reactive. This study shows that about 6% of HB core antibody were reactive and the other related study shows the percentage of anti HB core

reactivity is 4.2% to 18.3 % in healthy donors in our country.[9,28,31] Our study also shows no non- reactive sample was observed to be reactive by ID-NAT, during the study period. Further the study finds that the presence of various races in NCR increases the chances of genetic diversity or mutation of genes and this tendency makes difficult for ID-NAT. Hence, it is suggested that the reliability of NAT should be verified and assessed timely.

## CONCLUSION

Anti-HB core is the most common cause of blood discarding by TTI screening. Still, HBcAb should be carried out to rule out transmission of HBV among HBsAg non-reactive blood (2.07%: Table 1). On contrary, no sample was observed, which was NAT reactive and is not reactive by CLIA. Screening of blood by Anti- HBcAb does enhance the blood safety. In addition, NAT facility can enhance the blood safety but cannot replace Anti HB core Screening.

The facility for HBV core screening is same as that of HBsAg, HIV & HCV, whereas, NAT is capital extensive requiring dedicated space and staff. Practical benefits random access CLIA such as minimal use of rapid methods, high sensitivity, documentation and reproducibility should be taken in to consideration for formulating policies of TTI screening. Therefore, the screening for core antibody seems to be a better strategy than NAT in case of resource limitation. The policy should be based on available resources, potential human economic value in the target population, technical competence and presence of viral strains that can be detected by the NAT.

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