

# Hepatitis B core antibody testing in Indian blood donors: A double-edged sword!

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## Abstract:

**Background:** Until lately, anti-HBc antibodies were considered an effective marker for occult Hepatitis B virus (HBV) infection and have served their role in improving blood safety. But, with the development of advanced tests for HBV DNA detection, the role of anti-HBc in this regard stands uncertain. **Materials and Methods:** Anti-HBc and HBsAg ELISA and ID-NAT tests were run in parallel on donor blood samples between April 1, 2006 and December 31, 2010 at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. A positive ID-NAT was followed by Discriminatory NAT assay. **Results:** A total of 94 247 samples were tested with a total core positivity rate of 10.22%. We identified nearly 9.17% of donors who were reactive for anti-HBc and negative for HBsAg and HBV DNA. These are the donors who are potentially non-infectious and may be returned to the donor pool. **Conclusion:** Although anti HBc testing has a definite role in improving blood safety, centers that have incorporated NAT testing may not derive any additional benefit by performing anti-HBc testing, especially in resource-limited countries like ours.

## Key words:

Anti-HBc, HBsAg, ID-NAT

## Introduction

Presently, screening for HBsAg is the only mandatory screening test for the detection of Hepatitis B virus (HBV) infection in blood banks in India as per the Drugs and Cosmetics Act, 1940 and the amendments thereof. Screening of blood for the detection of HBsAg, however, does not rule out the risk of transmission of hepatitis B totally, because during the serological response of the host to infection, there is a phase during which the HBsAg cannot be detected in the blood, although hepatitis B infection is present. This phase is called as the “core window period.” During this “window period,” detection of the antibody to the hepatitis B core antigen (anti-HBc) serves as a useful serological marker for hepatitis B infection.<sup>[1]</sup> Evaluating the usefulness of anti-HBc screening is critical, particularly for India and other countries that have intermediate and high hepatitis B endemicity.<sup>[2]</sup> Anti-HBc was initially introduced in blood banking as a surrogate marker for hepatitis C virus (HCV) and to a certain extent for human immunodeficiency virus (HIV) as well. It was also found to be an excellent indicator of occult HBV infection during the “core window” period. Blood units collected from individuals chronically infected with HBV in whom HBsAg is not detectable and from donors with acute hepatitis B who are in the window period following disappearance of HBsAg and prior to the appearance of anti-HBs can effectively be removed from the

inventory. It was for these reasons that a few blood banks in India as well incorporated anti-HBc testing in their donor screening protocols.<sup>[3]</sup> However, with the introduction and institution of more advanced techniques for Nucleic Acid Testing (NAT) like TMA (Transcription Mediated Amplification) or PCR (Polymerase Chain Reaction), those are able to amplify and detect the HBV DNA; the role of anti-HBc antibody in donor screening is being questioned by users across the globe. In addition, testing for anti-HBc results in attrition of large number of donors, causing shrinkage of the effective donor pool.

The present study was undertaken in the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi from April 2006 till December 2010. The aim of this study was to evaluate the usefulness of anti-HBc as routine donor screening tool in a blood bank already equipped with NAT in a resource-limited country which falls in the intermediate zone for Hepatitis B endemicity.

## Materials and Methods

This study was conducted in the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi from April 1, 2006 till December 31, 2010. All donors coming to the Department of Transfusion Medicine and fulfilling the general criteria for blood donation as per the Drugs and Cosmetics Act, 1940 and the amendments thereof, during

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the study period, were included in the study. Donors who did not fulfill these criteria were deferred from donating blood and hence excluded from the study. Donors with medical history of symptoms suggestive of Acquired Immuno Deficiency syndrome, history of jaundice or blood transfusion in last one year, and history of close contact with a patient suffering from hepatitis in the last 6 months were also deferred from donating blood at our center. The detailed history and clinical examination was carried out for all the donors by qualified staff trained to screen donors for blood donation. Consent for blood donation and infectious marker testing was obtained at the time of donor selection. Samples for infectious marker testing were collected at the time of blood donation from the diversion pouch of the primary bag. A 3- to 4-ml sample in a plain vacutainer was collected for ELISA testing. Another 6-ml sample was collected in K2 EDTA vacutainer for NAT. The samples were run in parallel by ELISA for the detection of HBsAg and anti-HBc and Individual Donor NAT (ID-NAT). Third-generation ELISA kits (Monolisa™ HBsAg ULTRA, BIO-RAD and Monolisa™ Anti-HBc PLUS, BIO-RAD) using fully automated EVOLIS walk away system (BIO-RAD) were used for detecting HBsAg and anti-HBc, respectively. All samples testing positive by ELISA for HBsAg and/or anti-HBc were repeat tested in duplicate using the same ELISA kits. ID-NAT was performed using PROCLEIX® ULTRIO® Assay (Gen-Probe Inc.) using a semi-automated platform “eSAS.” All samples testing positive by ID-NAT (initial reactive) were repeat tested to rule out false positivity or contamination. All repeat-reactive samples by ID-NAT were then subjected to discriminatory NAT testing (dNAT) using Procleix discriminatory assay to differentiate between HBV, HIV, and HCV nucleic acids.

Only those samples which were repeat reactive for ELISA or reactive by dNAT using Procleix discriminatory assay ID-NAT were considered as positive/reactive.

## Results

A total of 94 247 samples collected at the time of donation were tested between April 1, 2006 and December 31, 2010. The total core positivity rate (IgG+IgM) observed at our center was 10.22% (9 638/94 247), while 1.2% (1 134/94 247) of donors were positive for HBsAg. The results are given in Table 1.

Of the 8 660 donors reactive by anti-HBc ELISA but negative for HBsAg, only 0.15% (13/8 660) showed the presence of HBV DNA by discriminatory assay, while the remaining 99.85% (8 647/8 660) were HBV DNA negative. These constitute 9.17% (8 647/94 247) of the total donor population.

The cohort of donors testing positive for anti-HBc in the absence of HBsAg and HBV DNA was primarily constituted of male donors (98.39%; 8 508/8 647), with 1.61% (139/8 647) being females. All the donors were between 18 and 60 years of age, with the majority, 40.67% (3 517/8 647), in the 18 to 30 years age group. The second largest category (34.10%, 2 945/8 647) was of donors between

31 and 40 years of age. Nearly 17.76% (1 536/8 647) of donors were between 41 to 50 years of age, the remainder, 7.47% (649/8647) being above 50 years of age.

## Discussion

With the incidence of HBsAg in India being fairly high, there is a definite risk of inadvertently transfusing HBV-infected blood.<sup>[1,4]</sup> Despite testing for HBsAg, post Transfusion HBV infection continues to occur due to the fact that many a times, HBsAg is circulating at very low and undetectable level for screening assays.<sup>[5]</sup> Therefore, it is strongly felt that a marker must be utilized for screening of blood in the Indian population to detect the presence of hepatitis B during the window period.<sup>[1,4]</sup> It was Mosley *et al.* who suggested that anti-HBc screening of blood donations might prevent HBV transmission from HBsAg-negative blood donors.<sup>[6]</sup> Although these sensitive serological tests have shortened the pre-seroconversion window period, they still are not able to identify a number of newly infected donors.<sup>[7]</sup> This residual risk due to donations of blood during the window period has been circumvented by using NAT assays which has considerably shortened the window period.<sup>[8-11]</sup> The window period for HBV infection by ID-NAT is estimated to be 24.6 days as compared with 38.3 days by serological tests (Ag-Ab combo assays).<sup>[12]</sup>

Testing for anti-HBc still remains an unresolved issue across the globe. It is felt that a large fraction of “anti-HBc-alone” patients can be assumed to have an unresolved chronic infection with low-grade, possibly intermittent virus production. Such individuals may have detectable serum HBV DNA and are potentially infectious.<sup>[13]</sup> In our study, 10.22% (9 638/94 247) were positive for anti-HBc, of which, 9.19% (8 660/94 247) of donors were positive for anti-HBc and negative for HBsAg. Varied prevalence of core positivity has been reported by Indian studies. Dhawan *et al.*,<sup>[14]</sup> from Chandigarh reported a core positive rate of 8.4%, whereas a high positivity rate of 18.3% was observed by Bhattacharya *et al.*<sup>[15]</sup> from West Bengal. In a study by Col Kumar *et al.*,<sup>[11]</sup> incidence of anti-HBc total in blood donor population was pegged at 15.9% and it was 11.6% in our previous study<sup>[16]</sup> at New Delhi. Another study from New Delhi by Chaudhuri *et al.*<sup>[17]</sup> revealed the prevalence of anti-HBc to be 10.82%. These observations are comparable with our data.

Amini *et al.*,<sup>[18]</sup> in a study done on 4 930 healthy blood donors, found that 5.1% were positive for anti-HBc without having any detectable HBsAg; however, they did not determine the presence of HBV-DNA. Behzad-Behbahani *et al.*,<sup>[5]</sup> in a study conducted on 2 000 healthy blood donors in Iran, found 6.55% HBsAg-negative blood samples to be positive for anti-HBc antibody. Our results, to a large extent, were in agreement to these studies.

In our study, only 0.15% of anti-HBc-positive and HBsAg-negative donors showed the presence of HBV DNA in their sera. Majority, i.e., 99.85% of these donors were anti-HBc positive and negative for HBsAg and HBV DNA. These constitute 9.17% of the total donor population. Such units may be considered non-infectious and returned to the donor pool. This is supported by the evidence from Poland, where the HBV infection was not transmitted by transfusion of red blood cell and platelet concentrate from donor with ~200 IU HBV DNA/ml and from HBV DNA-negative/anti-HBc-positive donors in which the HBV DNA was detected in the next donation.<sup>[19]</sup> Though a number of other

**Table 1: HBsAg and Anti HBc ELISA results**

HBsAg ELISA	Anti HBc ELISA	Number	Percentage
Positive	Non Reactive	156	0.16
Positive	Reactive	978	1.03
Negative	Reactive	8660	9.19

studies report cases of HBV transmission after the transfusion of anti-HBc-positive blood,<sup>[20]</sup> the most recent of these studies dates back to late nineties.

In countries where HBV infection is endemic, anti-HBc donor screening would result in high discard rate of collected blood,<sup>[21,22]</sup> HBV-DNA screening of blood donors is presumed to be necessary and very beneficial.<sup>[22]</sup>

Usefulness of NAT in detecting early Window Period donations has been accepted by many authors.<sup>[23]</sup> NAT is considered most beneficial in developing countries where the estimated disease burden is high. However, its introduction for routine screening of donated blood has suffered a setback due to high costs, which makes it unaffordable for many centers.

Sawke and Sawke<sup>[24]</sup> feel that as India has high prevalence of anti-HBc, screening of donor blood for total anti-HBc is not practical and should not be used as a criterion to discard blood. Screening for IgM- anti- HBc of blood units negative for HBsAg, on the other hand, could identify potentially infectious units. El-Sherif *et al.*<sup>[25]</sup> have proposed a policy of testing blood donors for anti-HBc in addition to HBsAg and those found positive for anti-HBc to be submitted to HBV DNA testing. This approach would be less expensive, would reduce the risk of transfusion-transmitted HBV infection, and decrease the rejection rate of the precious units of collected blood positive for anti-HBc only. Though this approach would return 9.17% of our donors to the donor pool, it would still fail to identify window period donations.

Allain<sup>[26]</sup> has recommended that the use of anti-HBc screening of blood donors be restricted to areas where HBV seroprevalence is relatively low (<2% anti-HBc reactivity), otherwise, the impact on donor deferral would be unsustainable. Another factor that limits the efficacy of this test is its notorious lack of specificity. According to Laperche,<sup>[10]</sup> HBV DNA screening would be more effective in countries with high or medium endemicity, and where anti-HBc testing is not routinely done.

However, considering the resource limitations in many areas in our country, it may not be possible or practical to provide facilities for advanced tests like NAT. The importance of core antibody testing in such areas cannot be under emphasized.

## Conclusion

Addition of anti-HBc to blood donor screening along with HBsAg improves blood safety, but leads to attrition of donors, especially in India which lies in the intermediate zone of hepatitis B endemicity. The best alternative for improving blood safety in our country is to add NAT testing to donated blood. It will identify most potentially infectious units of blood including window period donations and seronegative infections. Therefore, the applicability of anti-HBc testing in routine screening of donated blood is doubtful.

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