# Correlation of Serological and Molecular Methods in Hepatitis B & C Reactive Blood Donors

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## Abstract

**Background:** The Standards for Blood Banks & Blood Transfusion Services, issued by NACO mention that all collected blood units should be tested for the mandatory five tests before issuing to any patient. The choice of method may depend upon the number of units collected by the blood centre, availability of trained staff and equipment apart from cost feasibility. The aim of this study was to determine the frequency and viral load of HBV DNA and HCV RNA in Hepatitis B & C reactive donors respectively, and hence it was intended to contribute in determining whether routine HBsAg and HCV screening of blood donors, using ELISA method alone, provide any concrete benefits with regard to HBV and HCV risk reduction.

**Material & Methods:** A total of 20,917 blood donors were screened for HBV, HCV, HIV, Syphilis and Malarial parasite as part of routine blood donation screening at a tertiary care teaching hospital blood centre in Western India. 110 donors having reactive report for HBV and 11 donors having reactive report for HCV were used for the present study.

**Results:** 110 donors were found to be reactive for HBsAg by ELISA testing. Out of these 110 reactive HBsAg donors, only 72 (65%) showed positive results in HBV DNA test. 11 tested anti-HCV positive in ELISA. Out of 11, only 7 (63%) showed positive results in HCV RNA test.

**Conclusion:** In the present study, approximately 35% of the screened units which were reactive for ELISA were negative for viral load. The factors like deterioration of sample, low viral load, false positive ELISA results may account for the same.


**Keywords:** Blood Donors, Hepatitis B, Hepatitis C, Serology, Molecular markers

**Source(s) of Support:** Nil

**Conflicting Interest:** None

## Introduction

According to the Drugs and Cosmetics Rules, 1945, screening of Hepatitis B & C in Blood Donors is mandatory for Blood Centres, by the serological assays.[1] However, this does not rule out the risk of transmission of Hepatitis B or C, through blood products due to the window period infection.

Though many Blood Centres in India have adopted Nucleic Acid Testing (NAT), which reduces the Window period detection, cost constraint is a major factor in deterring it as a mandatory test.

Many blood centres in India are supported by the National AIDS Control Organization (NACO) which issue guidelines from time to time for the safe blood transfusion services across the country. The Standards for Blood Banks & Blood Transfusion Services, issued by NACO mention that all collected blood units should be tested for the mandatory five tests before issuing to any patient. For HIV, HBsAg and HCV, ELISA or Rapid may be used.[2] The choice of method may depend upon the

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number of units collected by the blood centre, availability of trained staff and equipment apart from cost feasibility.

The aim of this study is to determine the frequency and viral load of HBV DNA and HCV RNA in Hepatitis B & C reactive donors respectively, and hence it was intended to contribute in determining whether routine HBsAg and HCV screening of blood donors, using ELISA method alone, provide any concrete benefits with regard to HBV and HCV risk reduction or whether implementation of NAT will be of greater benefit to low resource country like India, which has higher prevalence of HBV and HCV. The study was conducted with the aim to compare Hepatitis B virus (HBV) and Hepatitis C virus (HCV) serological test results with viral load in blood donors screened from June 2019 to December 2019.

## Material and Methods

A total of 20,917 blood donors were screened for HBV, HCV, HIV, Syphilis and Malarial parasite as part of routine blood donation screening at a tertiary care teaching hospital blood centre in Western India; between June 2019 to December 2019. Blood donors who fulfilled the eligibility criteria as per the National Guidelines for donation were included. 110 donors having reactive report for HBV and 11 donors having reactive report for HCV were used for the present study and serum samples obtained from these donors coded with unique identifier number and stored at -80 degree Celsius until use. HBV DNA and HCV RNA quantification were done using Real-time Polymerase chain reaction (Real-time PCR) by Cobas Taqman.

## Blood Specimens

Whole blood samples were collected from 20,917 blood donors in 3 ml tube containing potassium-EDTA (Ethylenediamine tetraacetic acid) at a concentration of 1.6 mg EDTA per millilitre of blood (J K Diagnostics: Rajkot, Gujarat, India) and 4 ml plain tube containing clot activator (J K Diagnostics: Rajkot, Gujarat, India). Such samples were centrifuged at 3000 rpm (3200 g) for 3 minutes, and EDTA-plasma was separated within 24 hours. Plasma specimens were stored at 4 degree Celsius to 8 degree Celsius for no longer than 72 hours or for longer at -80 degree Celsius until further processing.

## Hepatitis B and C Serology

Anti-HBs antigen screening was performed using HbsAg Ab coated microplate (Meril Diagnostics, Vapi, Gujarat, India). Anti-HCV screening was performed using HCVAg coated microplate (Meril Diagnostics, Vapi, Gujarat, India) using Enzyme Linked Immunosorbent assay (ELISA) on an automated machine (EVOLIS, BIO RAD, California, United States). Reactive samples were retested in duplicate and considered to be reactive if at least one of the two repeated samples gave positive results. Only samples that were positive for anti-HBs/anti-HCV in both tests were included in this study.

## HBV DNA and HCV RNA Quantification

The methodology was Real Time Polymerase Chain Reaction performed on COBAS Taqman-48.

The lower limit of detection of HBV DNA was 100 IU/ml and for HCV RNA was 90 IU/ml.

## Results

A total of 20,917 donors were included in the study. The median age of the study participants was 30 years, with a range of 18 to 55 years.

**Table 1: Results of HBsAg and HBV DNA**

| Status of HBV DNA | Number of Donors reactive for HBsAg |
|---|---|
| HBV DNA detected | 72 |
| HBV DNA not detected | 38 |
| Total | 110 |

As shown in Table 1, Out of 20,917, 110 donors were found to be reactive for HBsAg by ELISA testing. Out of these 110 reactive HBsAg donors, only 72 (65%) showed positive results in HBV DNA test.

**Table 2: Results of HCV and HCV RNA**

| Status of HCV RNA | Number of Donors reactive for HCV |
|---|---|
| HCV RNA detected | 07 |
| HCV RNA not detected | 04 |
| Total | 11 |

As shown in Table 2, Out of 20,917 blood donors, 11 tested anti-HCV positive in ELISA. Out of 11, only 7 (63%) showed positive results in HCV RNA test.

## Discussion

NAT for blood screening is still not mandatory in India. Due to high sensitivity and specificity of NAT it could be applied for donor blood screening as the complementary detection in combination with ELISA, which would increase the safety of blood products. The result of NAT can assist the physicians to make judgment in a shorter time and prevent unnecessary medicolegal disputes.

The prevalence of Hepatitis B in our blood donor population was markedly lower (0.52 %) than that recently described (1.0 -13%) for an adult Indian population. This finding may have been due to the blood donor selection prior to testing, to our criteria that called for reactivity in two assays, and to regional differences in the prevalence of HBV infection.

Regarding the Hepatitis reactivity by ELISA and virus DNA/RNA levels, there is lack of correlation and hence ELISA testing cannot be totally omitted. In a study of 200 HBsAg positive blood donors, by Mary C Kuhns et al, on-

ly 128(64%) showed positive results in HBV DNA test.[3] This was similar to our findings of 65%.

Nabuco LC *et al* studied the HBV DNA levels in 78 HBsAg positive blood donors and found HBV DNA detection in only 47 (60%).[4] The authors concluded that most of the HBsAg positive blood donors showed low viral load.

Holger *et al* conducted study of 14251 first time volunteer blood donors and compared anti-HBc and HBsAg with viral load.[5] In 87.5% of the HBsAg positive donors, HBV DNA was detected.

False negative results could be because of viral load lesser than the assay lower limit of detection or presence of rare genotypes or mutations. False positive report may result due to background contamination.

Serological assays are used for the screening of HBV and HCV detection due to its simplicity, automation and convenience, though they can be time-consuming, expensive and require trained staff. Molecular techniques are useful to diagnose chronic infection; to identify HBV occult cases; to evaluate the prognosis of disease; to help in treatment decisions and monitor the antiviral treatment efficacy; and to identify resistance mutants to antiviral treatment. Molecular methods present higher specificity and sensitivity and larger dynamic range of detection compared to other diagnostic assays like serological assays. But these methods are relatively expensive and require special instruments and specialized techniques. The choice of each method should be done according to advantages and disadvantages, and the purpose.[6]

The molecular methods are useful in detecting genotypes which is helpful inpatient management. Genotypes A and D are prevalent in the Indian subcontinent but a changing trend is being witnessed with emergence of genotypes B, E, F and G, which could be attributed to immigration, trafficking and use based on geographical locations, type of transmission and genotypes. Evidence clearly indicates significance of HBV genotypes on disease prognosis. Genotype B being associated with less progressive liver disease than genotype C while genotype D having a less favourable prognosis than genotype A. Association of genotype A and D with disease severity and poor response was also observed in Indian patients.[7]

In a study in Kashmir valley by Ahmed *et al*,[8] where HBsAg negative voluntary blood donors were screened for anti-HBc and HBV DNA, it was found that HBV DNA was highest in anti-HBc positive and anti-HBsAg negative individuals. Further, the authors concluded that the risk of transmitting HBV infection from IgG anti-HBc positive donors was 2.1-8.6%. Hence, the importance of screening donors for anti-HBc was emphasized. However, Sawke and Sawke[9] 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.

Makroo *et al*[10] concluded from their study that although anti-HBc testing has a definite role in improving blood safety, centres that have incorporated NAT testing may not derive any additional benefit by performing anti-HBc testing, especially in resource-limited countries like ours.

## Conclusion

Though ELISA is the most important popular testing method for screening blood units, the reagent and kits used for the same in India needs a thorough review. In the present study, approximately 35% of the screened units which were reactive for ELISA were negative for viral load. The factors like deterioration of sample, low viral load, false positive ELISA results may account for the same. Still it is a waste of resources, time, energy and precious blood if all false positive ELISA results are taken into account.

**References:**


1. The Drugs & Cosmetics Act, 1940 and Rules, 1945.
2. National AIDS Control Organization, Ministry of Health & Family Welfare, Government of India, New Delhi. *Standards for Blood Banks & Blood Transfusion Services. 2007*.
3. Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP; REDS Study Group. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion. 2004* Sep;44(9):1332-9.
4. Nabuco LC, Villela-Nogueira CA, Perez RM, Ceci L, Pannain VL, Nogueira CM, Segadas-Soares JA, Coelho HS. HBV-DNA levels in HBsAg-positive blood donors and its relationship with liver histology. *J Clin Gastroenterol. 2007* Feb;41(2):194-8.
5. Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood. 2002* Oct 1;100(7):2637-41.
6. Villar LM, Cruz HM, Barbosa JR, Bezerra CS, Portilho MM, Scalioni Lde P. Update on hepatitis B and C virus diagnosis. *World J Virol. 2015* Nov 12;4(4):323-42.
7. Amarapurkar DN, Mada K, Kapoor D. Management of Chronic Hepatitis B Infection in India. *J Assoc Physicians India. 2015* Nov;63(11):43-52.
8. Yousuf AW, Kadla SA, Saqib N, Yousuf AM, Lone S, Wani B. Prevalence of hepatitis B DNA and serological markers of hepatitis B infection among HBsAg negative voluntary blood donors in Kashmir valley. International *Journal of Contemporary Medical Research 2017*;4(10):2052-56.
9. Sawke NG, Sawke G. Preventing Post-Transfusion Hepatitis by screening blood donors for IgM antibody to Hepatitis B core antigen. *J Glob Infect Dis. 2010* Sep;2(3):246-7.
10. Makroo RN, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Hepatitis B core antibody testing in Indian blood donors: A double-edged sword! *Asian J Transfus Sci. 2012* Jan;6(1):10-3.