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# Seroprevalence of Australian antigen (HBsAg) among blood donors in the local population at standalone blood center by ELISA and rapid test kit

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**Keywords:** Seroprevalence, Hepatitis B surface antigen, Blood donors

**Abstract**

**Introduction:** Hepatitis B virus (HBV) is highly infectious and can be transmitted by both percutaneous routes and by blood transfusion. Laboratory diagnosis of HBV infection is made by detecting Hepatitis B virus surface antigen (HBsAg), the earliest serological marker of active HBV infection (acute and chronic). In the case of diagnosis of infectious disease, discordant results may have serious consequences among the patients as it causes unnecessary mental stress and tension. For proper diagnosis of infection, as well as Disease management and prevention, and identification of appropriate test kits is necessary.

**Objectives: 1.** To determine the Sero-Prevalence of HBsAg among blood donors in a standalone blood center in Ahmedabad, Gujarat. **2.** To compare the diagnostic kits efficiency of ELISA and Rapid Test Kits in detecting HBs Ag.

**Materials and Methods:** The study was conducted on apparently healthy blood donors for 3 years from January 2021 to December 2023 at the Blood Center, to assess the prevalence of hepatitis B virus infection. A total number of 90754 blood donors were included in this study. HBsAg ELISA test was used for this study. For Initial reactive donors, a second test was done by HBsAg Hepacard Rapid kit to confirm True Reactive (TR).

**Result:** Out of 90754 donors, 85959 (94.81%) were males & 4795(5.17%) were females. Out of blood units, 526(0.57%) were discarded and out of them, 271 were Male and 5 were Female Total of 276(0.30%) were HBsAg reactive. The Seroprevalence of HBsAg was found to be 0.30%.

**Conclusion:** Blood Donors are often found to be reactive to hepatitis B surface antigen and others. In order to reduce this Seroprevalence, more sensitive screening assays and appropriate donor selection are a must.

## Introduction

Hepatitis B virus (HBV) infection is a serious global health problem worldwide. HBV infection accounts for 5,00,000 to 1.2 million deaths each year and is the tenth leading cause of death.[1] HBV is the major cause of chronic liver disease with around 350 million people suffering from chronic HBV infection such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)[2] HBV has a double-stranded DNA encoding for P, X core, and surface proteins. The complex antigen found on the surface of HBV is called Hepatitis B surface antigen (HBsAg). Antibodies against HBV proteins are other immunological markers of infection. Consequently, HBsAg is a useful viral marker for both population screening and diagnosis of acute HBV infection or Chronic Hepatitis B infection.[3] Transfusion-associated hepatitis B viral infection (TAHBV) continues to be a major problem in India even after the adoption of mandatory screening of hepatitis B surface antigen (HBsAg) by enzyme-linked immunosorbent assay (ELISA).[4] These endogenous microbial agents transmitted by blood transfusion have the following characteristics.

* Long incubation period
* Carrier or latent state
* Ability to cause asymptomatic/sub-clinical infection
* Viability and stability in stored blood or plasma
* The hallmark is the persistence of infection

HBV is present in the blood, blood products, and body fluids such as vaginal secretions and in low concentration in the saliva of active carriers.[5] The average incubation period of the virus is 90 days from the time of exposure to onset of symptoms but may vary from 6 weeks to 6 months. Different methods are used for the diagnosis of hepatitis including Rapid card test, ELISA Enzyme Immunoassay (EIA), and Polymerase Chain Reaction (PCR).[6] ELISA is an enzymatic immunoassay technique of the "sandwich" type for the detection of HBV in human serum or plasma. The test uses monoclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg. HBsAg rapid card test is a rapid screening test for the qualitative detection of HBsAg in whole blood, serum, or plasma specimens. The test utilizes a combination of monoclonal and polyclonal antibodies selectively to detect elevated levels of HBsAg in whole blood serum or plasma.[6] ELISA method is expensive and Rapid diagnostic kits are less expensive.

This study is important to determine, the seroprevalence of hepatitis B infection in blood donors at blood centers by using ELISA and RAPID card test techniques.

## Materials and Methods

The study was carried out at the standalone Blood Center for Blood Transfusion (NBTC), which oversees all components of the blood donation chain from collection, screening for transfusion-transmitted diseases ((TTIs), and distribution of labile blood products. A retrospective analysis of

blood donor data from January 2021 to December 2023 was carried out. All donors were healthy voluntary non-remunerated donors (VNRD). The data was collected from our software. An informed consent was taken from all blood donors included in the study before sample collection they were carefully selected for blood donation by trained personnel after a complete physical examination and satisfactorily answering the donor's questionnaire. Clinically healthy individuals between 18 and 65 years of age with a body weight of above 50 kg and hemoglobin ≥12.5 g/dl with no significant medical or surgical history were qualified for the donation process.

**Sample Processing**

Each blood sample was initially tested for HBsAg using the ELISA by Bio-Rad Evolis Manufacture by (BIORAD Monolisa HBsAg Ultra) Method. The specificity of BIORAD Monolisa HBsAg Ultra is 99.02 %. Initial reactive sample tested by rapid card test **[**HEPACARD - DIAGNOSTIC ENTERPRISES**]** method. The specificity of Rapid HEPACARD is 95%
Each Initial reactive donor's Blood components were discarded and labeled as Bio Hazard. All the initial reactive donor samples were tested further for HBsAg by Rapid Card test.

ELISA Method

BIORAD Monolisa HBsAg is based on microwells coated with monoclonal anti-HBsAg antibodies. The conjugate is a polyclonal anti-HBsAg antibody labeled with horseradish peroxidase. Samples and controls are incubated in the wells and HBsAg if present binds to monoclonal anti-HBsAg antibody on the microwell. In a subsequent step, conjugate is added which in turn binds to any specific antigen already bound to the antibody on the well. Unbound conjugate is washed away and a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound conjugate develop a blue color which is converted to a yellow to orange color when the reaction is stopped with sulphuric acid. After incubation the reactions are stopped with sulphuric acid and color is read spectrophotometrically. The intensity of color produced in the wells is directly proportional to the concentration of HBsAg in the sample.[6]

Rapid Card test Method

HEPACARD is a one-step immunoassay based on the antigen capture or sandwich principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with through an absorbent pad where it mixes with HBsAg, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen antibody-colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (test line) 'T' complex is trapped forming an antibody antigen-antibody colloidal gold complex. This forms a pink band indicating the sample is

reactive for HBsAg. To serve as a procedural control, an additional line of antimouse antibody (control line) 'C', has been immobilized at a distance from the test line on the strip. If the test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate. [6]

All the tests were performed according to the manufacturer's instructions with adequate controls. Data regarding the HBsAg positivity and negativity of the respective blood donors were taken from records available in the standalone Blood Centre's software.

**Inclusion criteria**

Blood donors aged 18-65 years, both male and female, without a history of Hepatitis B or any chronic liver disease.

**Exclusion criteria**

Individuals with a known history of HBV infection, pregnant women, or those with contraindications for blood donation.

**Results**

A total number of 90754 blood donors were screened over a period of 3 years from January 2021 to December 2023. Out of 90754 donors, 85959 (94.71%) were male donors and 4795 (5.28%) were female donors [Table 1]. Out of all these units, 526 units (0.57%) were discarded as TTI Reactive. Out of these 526 reactive units, 276 units were HbsAg reactive(52.47%)[Table 2]. Out of these 276, 271 (98.18) were males and 5 (1.81) were females[Table 3]

| Year | Total No Of Donors Tested By ELISA Method | Male Donors | % of Male Donors | Female Donors | % of Female Donors |
|---|---|---|---|---|---|
| 2021 | 25253 | 24251 | 96.03% | 1002 | 3.96% |
| 2022 | 31921 | 30085 | 94.24% | 1836 | 5.75% |
| 2023 | 33580 | 31623 | 94.17% | 1957 | 5.82% |
| Total | 90754 | 85959 | 94.81% | 4795 | 5.17% |

Table 1**:** Year-wise percentage of male donors and female donors.

| Year | Total No of Donors | No of Male HBsAg Reactive Units by ELISA & RAPID Method | No of Female HBsAg Reactive Units by ELISA | Total No of HBsAg Reactive Units by ELISA | % of HBsAg Reactive Units |
|---|---|---|---|---|---|

| | | | & RAPID Method | & RAPID Method | |
|---|---|---|---|---|---|
| 2021 | 25253 | 80 | 2 | 82 | 0.32% |
| 2022 | 31921 | 98 | 0 | 98 | 0.30% |
| 2023 | 33580 | 93 | 3 | 96 | 0.28% |
| Total | 90754 | 271 | 5 | 276 | 0.30% |

Table 2 shows year-wise from 2021-2023 about the trends in the total number of HBsAg reactive units.

| Year | Total No of Donors | Total No of Bags Discarded | % of Bag Discarded | Total No of HBsAg Reactive units | % of HBsAg Reactive Units |
|---|---|---|---|---|---|
| 2021 | 25253 | 174 | 0.68% | 82 | 0.32% |
| 2022 | 31921 | 181 | 0.56% | 98 | 0.30% |
| 2023 | 33580 | 171 | 0.50% | 96 | 0.28% |
| Total | 90754 | 526 | 0.58% | 276 | 0.30% |

Table 3 represented year-wise from 2021-2023 about the trends in the total number of Blood units discarded due to HBsAg Reactivit.

**Discussion**

The discovery of the HBsAg was a breakthrough in decreasing the incidence of post-transfusion hepatitis. Following infection by the hepatitis B virus (HBV), the first serological marker to appear in the blood is the HBV DNA, followed by HBsAg, the DNA polymerase, and the hepatitis B 'e' antigen (HBeAg). Thereafter, the antibodies to the hepatitis B core antigen (anti-HBc), hepatitis B 'e' antigen and the HBsAg can be detected. Screening of donated blood by ELISA for HBsAg is the common method for detecting hepatitis B infection. Screening of blood for the detection of this viral marker, however, does not rule out the risk of transmission of hepatitis B, because, during the host serological response 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 the

'window period'. It represents a carrier state of the disease. With the fairly high incidence of HBsAg in India, there is a definite risk of inadvertently transfusing HBV-infected blood. It is therefore strongly felt that a marker must be utilized for the screening of blood in the Indian population to detect the presence of hepatitis B during the window period. The safety of blood products is one of the major problems concerned with transfusion medicine. At present, HBsAg detection is the only diagnostic screening test for HBV infection identification in the blood transfusion centers of India. According to India's Drugs and Cosmetics Act (1943), each blood unit has to be tested for hepatitis B infection.[7] Hepatitis B virus infection can be estimated by detection of HBsAg in sera. Hence, the present study was done to detect the prevalence of HBV in our local area by screening the blood of blood donors attending our center.

Among the 90754 screened samples of blood donors, 276 of them (0.30%) (271 were Male & 5 Female) were found positive for HBsAg. In the present study, the ELISA (BIORAD) Method was used for the detection of HBsAg as the first kit, and the Rapid Method (HEPACARD) was used as the Second kit.

In the present study, the overall Seroprevalence of HBsAg was observed to be 0.30%. According to the study, this part of Gujarat qualifies as a low prevalence area (less than 2%). The data providing a picture of the hepatitis B infection burden in Ahmedabad, Gujarat has come from HBsAg Seroprevalence studies (Table 2). The present study shows low Seroprevalence of hepatitis B infection in Ahmedabad, Gujarat.

Table 4: Prevalence of HBsAg positive donors in different cities of India [8-12]

| **Place** | **Prevalence** |
|---|---|
| New Delhi | 2.23%, 2.76% |
| Kerala | 3.1% |
| Madurai | 4% |
| Tamil Nadu | 1.37% (-Voluntary) & 2.96% (Replacement) |
| Dehradun | 0.99% |
| Kolkata | 1.66% |
| Kanpur | 2.25% |
| Bangalore | 1.86% |
| Kashmir | 0.35% |
| Present Study | 0.30% |

The risk of transmission of HBV can further be reduced by screening blood donations for anti-HBC, as it is the only marker of HBV during the window period. Active HBV vaccination is another approach to reduce the rate of transmission of HBV. Public awareness, educational, and motivational programs, mass immunization programs ensuring 100% voluntary blood donation, implementation of strict pre-donation counseling, and donor selection criteria will be effective in decreasing the hepatitis B infection rate.

**Conclusion**

The seroprevalence of HBs Ag among blood donors in the local population at a standalone blood center is 0.3%. Both ELISA and Rapid test Kits are effective

tools for screening HBsAg in blood donors. The Rapid Test Kit, while less sensitive than ELISA, can be used as a screening tool in areas where resources are limited, followed by confirmatory testing using ELISA. Regular screening of blood donors for HBV is essential to prevent the transmission of the virus through blood transfusions.

## Recommendations

1. Routine screening of blood donors for HBsAg should be continued and expanded to include more comprehensive tests for hepatitis B.
2. The use of Rapid Test Kits as a preliminary screening tool should be promoted in remote or under-resourced regions.
3. Public health campaigns should be conducted to raise awareness about the importance of blood donor safety and hepatitis B prevention.

## Future Directions

Further studies could explore the correlation between HBsAg seroprevalence and risk factors such as age, gender, and socioeconomic status. Additionally, investigations into the cost-effectiveness and operational feasibility of implanting Rapid Test Kits in various healthcare settings would be valuable.


## Source of Funding

None.


## Conflict of Interest

None.

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