

# Updated Seroprevalence of Hepatitis B Surface Antigen and Anti-Hepatitis Core Antibody Among Blood Donors in Yemen

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**Purpose:** Hepatitis B virus (HBV) is one of the most common infectious pathogens worldwide. Various studies found a prevalence of HBV infection among blood donors ranging from 2% to 18%. Hence, this study aimed to provide an updated prevalence of HBsAg and anti-HBcAb among blood donors.

**Patients and Methods:** This was a cross-sectional study to investigate the donation records of blood donors in Sana'a, Yemen, over one year (January to December 2019). Eligible blood donors were included in the study. The serum samples of blood donors were tested for HBsAg and anti-HBcAb (IgG & IgM) using the electrochemiluminescence (ECL) and enzyme immunoassay (EIA) techniques.

**Results:** A total of 16,367 blood donors were recruited in this study, of whom 14,300 (87.4%) donated only once during this study (single, non-duplicated blood donors), while 2067 (12.6%) were repeated or duplicated. The overall prevalence of HBsAg and anti-HBcAb was 2.4% and 10.8%, respectively. Among single non-duplicated blood donors, HBsAg and Anti-HBcAb were 2.3% and 10.6% and 3.0% and 12.5% for repeated blood donors, respectively. There were statistically significant differences between HBsAg and Anti-HBcAb in terms of donor type and testing techniques.

**Conclusion:** The seroprevalence of HBsAg and anti-HBcAb among the blood donors was 2.0% and 10.3%, respectively. The ECL technique is more sensitive, has a lower error rate, and shows an advantage over the manual EIA technique. Duplicated blood donors influence the accuracy of the seroprevalence of HBsAg and anti-HBcAb.

**Keywords:** hepatitis infections, serological markers, transfusion transmissible infections, blood transfusion, Yemen

## Introduction

The World Health Organization (WHO) Global Database on Blood Safety estimates over 92 million blood donations annually.<sup>1</sup> However, blood transfusion is not entirely free from the risk of transmission of infectious agents, such as Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Treponema pallidum, and malarial parasites.<sup>1</sup> Due to infectious agents such as HIV, HBV, HCV, and syphilis, about 1.6 million units are discarded.<sup>2</sup> WHO has recommended that pathogens causing transfusion-transmissible infections (TTIs) must be tested on all blood collected from blood donors.<sup>1</sup> HBV has infected over 2 billion people, with 360 million having chronic HBV infections and 3.0 million new infections per year.<sup>3,4</sup> Furthermore, over 1.1 million deaths per year are associated with HBV infections, and is the fifth leading cause of cancer in the world.<sup>5,6</sup>

Several studies reported different rates of HBsAg in blood donors, from 1.2%, 1.4%, and 2.3% in Saudi Arabia,<sup>9</sup> Jordan<sup>7</sup> and Iraq,<sup>8</sup> to 4.3%, 5.1%, and 5.62% in Sudan,<sup>10</sup> Syria,<sup>11</sup> and Egypt,<sup>12</sup> respectively. In Yemen, the prevalence of

HBV infection among blood donors ranges from 2% to 18%, as documented by many studies, where Yemen consequently falls into the intermediate to high endemicity category.<sup>13–15</sup>

Previous studies revealed different results regarding viral hepatitis and HIV prevalence among blood donors.<sup>14,16</sup> It was noted that there was a discrepancy in the results, necessitating more investigation to improve and understand these variations and their impact. Hence, the purpose of this study was to update the prevalence rate of HBsAg and anti-HBc among blood donors and determine the reliability of using chemiluminescence and ELISA techniques as well as identifying the effect of duplicated or repeated blood donations on the results.

## Materials and Methods

### Study Population

The study is a prospective, cross-sectional design from January 2019 to December 2019. Serological testing of donor samples was done in the Virology laboratory. This study was conducted on all blood donors who visited the National Blood Transfusion and Research Centre in Sana'a, Yemen. Donors were divided into two groups: those who donated once during the study and those who donated more than once.

### Study Tools

A designed, self-administered questionnaire was used in collecting the data. Blood donors who could not read and write were interviewed face-to-face by a trained public health specialist to fill out the questionnaire. The questionnaire included demographic information (age, gender, residency, occupation) and blood donation history. The NBTRC has no electronic or digital donor registry records and relies mainly on the blood donor logbooks recorded manually by the staff members. Hence, a critical issue is monitoring donation history for repeated blood donors who are still not eligible to donate due to inadequate donation intervals.

Individuals coming for blood donations were divided into four (4) groups according to occupation: students, professionals, manual workers, and military personnel. Professional workers are individuals who have educational qualifications, such as teachers, engineers, health workers, and employees, among others. Manual workers have low academic qualifications or have their own businesses, such as carpenters, plumbers, drivers, and waiters, among others.

Routine examinations, including blood pressure, hemoglobin level, pulse rate, and other general health checkups, were performed on all the blood donors. Healthy individuals aged 16 to 65 years old and weighing more than 45 kg were considered for blood donations. Both voluntary and replacement blood donors who met the required criteria for blood donation were included in the study.

### Assessment of HBsAg and Anti-HBcAb

During the study, two techniques were employed. The Electrochemiluminescence (ECL) technique was used as the primary method for detecting HBsAg and Anti-HBcAb, and the enzyme immunoassay (EIA) technique (manual) was used as a backup if the ECL technique is not available.

Blood samples (5 mL) were aseptically collected by trained lab technicians using a disposable syringe. The blood sample was allowed to clot in a sterile tube before centrifugation. A total of 11,121 (77.8%) serum samples were separated and analyzed for HBsAg and Anti-HBcAb (IgG and IgM) by the ECL technique using the Immunoassay Cobas e 411 analyzers (Roche ELECSYS® 2010 GmbH; Germany). The remaining 3178 (22.2%) serum samples were analyzed for HBsAg and total anti-HBcAb (IgG, IgM) by the enzyme immunoassay (EIA) technique (Monolisa™ HBsAg ultra, no.72348; Monolisa™ anti-HBc plus, no.72316; Monolisa™; BioRad Diagnostics, 92430 Marnes-la-Coquette, France) according to manufacturer instructions.

The HBsAg is the first line of screening for HBV infections in blood donors prior to donation. Samples that have initially reactive results are tested repeatedly in duplicate. If one or both tests are positive, the blood donor is deferred indefinitely for donation. Confirmatory testing using HBV NAT (Nucleic acid testing) is not routinely done but is available in the Blood Center and special private laboratories at the blood donor's expense.

## Study Ethics

Consent to conduct this study was reviewed and approved by the Faculty of Medicine, Taiz University (IRB-2019-01-012) and the National Blood Transfusion and Research Centre in adherence to the Declaration of Helsinki. Informed consent was obtained from the study participants prior to study commencement. Blood donors' data confidentiality was strictly observed, and all related ethics were fully considered.

## Statistical Analysis

The HBsAg and Anti-HBcAb prevalence were observed and expressed as frequencies and percentages. Socio-demographic variables and other population characteristics were computed and analyzed using SPSS (version 21). The Chi-squared test was employed to determine the statistical difference. The significance level was set at  $P$  value  $<0.05$ .

## Results

Screening for HBsAg and Anti-HBcAb was determined for all blood donors. A total of 16,367 blood donor specimens were collected and subjected to routine investigations and detection of HBsAg and Anti-HBcAb. The overall HBsAg and anti-HBcAb were 2.4% and 10.8%, respectively. Of the total blood donors, 14,300 (87.4%) were single, non-duplicated blood donors, while 2067 (12.6%) were recorded as repeated or duplicated blood donors. Single, non-duplicated blood donors were selected for further data analysis, whereas repeated or duplicated blood donors were excluded to prevent result duplications. The seropositivity of HBsAg and Anti-HBcAb among 14,300 selected (single or non-duplicated) blood donors was 2.3% and 10.7%, compared to 3.1% and 11.7% of HBsAg and Anti-HBcAb among 2067 repeated or duplicated blood donors, respectively. This difference in the rates of HBsAg and Anti-HBcAb was found to be statistically significant ( $P < 0.05$ ) (Table 1). Further analysis revealed that the seropositivity of anti-HBc alone was higher (10.8%) in comparison to the seropositivity of HBsAg/anti-HBc (1.2%) in all blood donors. For non-duplicated blood donors, 10.6% (CI: 1.044–1.329) was reactive to Anti-HBc only but 1.7% (CI: 0.532–1.101) was reactive to both HBsAg and anti-HBc, whereas, 12.5% (CI: 0.955–0.995) for duplicated blood donors were positive for anti-HBc, and only 1.6% (CI: 0.921–2.012) for HBsAg and anti-HBc (Table 2).

Among the single non-duplicated blood donors, the number of males was 14,148 (98.9%), with a mean age of 30.07 years. Of those, 10,369 (72.5%) were replacement donors, and 3933 (27.5%) were voluntary donors (Table 3). Moreover, the seropositivity of HBsAg and anti-HBcAb was higher among males, with 2.3% and 10.6% rates, respectively. Predominantly, blood donors were manual workers (32.7%) and professionals (32.2%). The seropositive rates of HBsAg and Anti-HBcAb among the blood donors ranged from 2.2% to 2.3% and from 10.0% to 11.1% for HBsAg and Anti-HBcAb, respectively. The difference in the rates of HBsAg and Anti-HBcAb, according to occupational categories, was found to be statistically insignificant ( $\chi^2 = 0.3$  and  $P = 0.961$ ).

**Table 1** The Overall Frequency of HBs Ag and Anti-HBc Ab Among Population of the Study

Blood Donors Categories	Total		HBs Ag				95% CI	P	Anti-HBc Ab				95% CI	P
			Reactive		Non-Reactive				Reactive		Non-Reactive			
	N	%	N	%	N	%			N	%	N	%		
Duplicated Blood donors	2067	12.6	63	3.0	2004	97.0	0.737–0.970	0.029 <sup>a</sup>	258	12.5	1809	97.5	0.955–995	0.008 <sup>a</sup>
Single non-duplicated blood donors	14,300	87.4	324	2.3	13,976	97.3	1.032–1.633		1510	10.6	12,790	89.4	1.044–1.329	
All donors	16,367	100.0	387	2.4	15,980	97.6		<0.001 <sup>a</sup>	1768	10.8	14,599	89.2		<0.001 <sup>a</sup>

**Note:** <sup>a</sup>Statistically significant, 95%.

**Abbreviations:** CI, confidence interval;  $P < 0.05$ , significant; N, number; %, percentage.

**Table 2** The Frequency of Anti-HBc Ab and HBsAg/Anti-HBc Among Population of the Study

Blood Donors Categories	Total		Anti-HBc Only				95% CI	HBsAg/Anti-HBc				95% CI
			Reactive		Non-Reactive			Reactive		Non-Reactive		
	N	%	N	%	N	%		N	%	N	%	
Duplicated Blood donors	2067	12.6	258	12.5	1809	97.5	0.955–995	33	1.6	2034	98.4	0.921–2.012
Non-duplicated blood donors	14,300	87.4	1510	10.6	12,790	89.4	1.044–1.329	167	1.7	14,133	98.8	0.532–1.101
All donors	16,367	100.0	1768	10.8	14,599	89.2		200	1.2	16,167	98.8	

**Abbreviations:** N, number; %, percentage; 95% CI, confidence interval.

There was no statistical significance in the difference in the prevalence of HBsAg or Anti-HBcAb among donors according to their residency. The lowest positivity rates of HBsAg (2.1%) and Anti-HBcAb (9.4%) were observed among blood donors from Azal, while the highest seropositive rates were found in Sheba and Algand regions, with a rate of 2.1% (HBsAg) and 12.4% (Anti-HBcAb), respectively.

**Table 3** Seropositive Rates of HBs Ag, and Anti-HBc Ab in Relation to Demographic Characteristics of Blood Donors

Demographic Characteristics		Total N =14,300		HBs Ag				Anti-HBc Ab			
				Reactive 324 (2.3)		Non-Reactive 13,976 (97.7%)		Reactive 1510 (10.6)		Non-Reactive 12,790 (89.4)	
		N	%	N	%	N	%	N	%	N	%
Occupation	Students	2966	20.8	67	2.3	2902	97.7	294	9.9	2672	90.1
	Professional worker	4596	32.1	100	2.2	4496	97.8	504	11.0	4095	89.0
	Military	2058	14.4	48	2.3	2010	97.7	227	11.0	1831	89.0
	Manual workers	4680	32.7	109	2.3	4571	97.7	490	10.5	4190	89.5
	$\chi^2$					0.3				2.3	
	P					0.961				0.518	
Gender	Males	14,148	98.9	321	2.3	13,827	97.7	1497	10.6	12,651	89.3
	Females	152	1.1	3	2.0	149	98.0	13	8.6	139	91.4
	$\chi^2$					0.1				0.7	
	P					0.808				0.418	
Residency	Capital city	8159	57.0	193	2.4	7966	97.6	903	11.1	7256	88.9
	Algand	777	5.4	17	2.2	760	97.8	96	12.4	681	87.6
	Azal	5115	35.8	105	2.1	5010	97.9	481	9.4	4634	90.6
	Sheba	140	1.0	6	4.3	134	95.7	17	12.1	123	87.9
	Tehama	109	0.8	3	2.8	106	97.2	13	11.9	96	88.1
	$\chi^2$					4.1				17.1	
	P					0.388				0.013 <sup>a</sup>	

(Continued)

Table 3 (Continued).

Demographic Characteristics		Total N =14,300		HBs Ag				Anti-HBc Ab			
				Reactive 324 (2.3)		Non-Reactive 13,976 (97.7%)		Reactive 1510 (10.6)		Non-Reactive 12,790 (89.4)	
		N	%	N	%	N	%	N	%	N	%
Age groups	16–25	4462	31.3	91	2.0	4371	98.0	398	8.9	4062	91.1
	26–35	6964	48.7	159	2.3	6805	97.7	671	9.6	6293	90.4
	36–45	2327	16.3	56	2.4	2271	97.6	319	13.7	2008	86.3
	45–55	497	3.5	16	3.2	481	96.8	106	21.3	391	78.7
	More than 55	50	0.3	2	4.0	48	96.0	16	32.0	34	68.0
	$\chi^2$			3.9				127.8			
	<i>P</i>			0.418				<0.001 <sup>a</sup>			
Type of donor	Volunteer	3933	27.5	119	3.0	3814	97.0	456	11.6	3477	88.4
	Replacement	10,367	72.5	205	2.0	10,162	98.0	1054	10.2	9313	89.8
	$\chi^2$			14.2						6.1	
	<i>P</i>			0.000						0.013	
Techniques	ECL	11,121	77.8	224	2.0	10,897	98.0	1144	10.3	9977	89.7
	EIA	3179	22.2	100	3.1	3079	96.9	366	11.5	2813	88.5
	$\chi^2$			14.3				3.9			
	<i>P</i>			<0.001 <sup>a</sup>				0.047 <sup>a</sup>			

Note: <sup>a</sup>Statistically significant.

Abbreviations:  $\chi^2$ , Chi-square; *P* < 0.05, significant; N, number; %, percentage.

Most blood donors were aged 26 to 35 years, accounting for nearly half of the donor population (48.7%). Notably, the seropositivity rate of HBsAg and Anti-HBcAb gradually rises as age increases, with the highest rates of 4.0% and 32.0%, respectively. In contrast to HBsAg, the results of anti-HBcAb among the various age groups were found to be statistically significant.

Furthermore, the difference in the prevalence rates of HBsAg among voluntary (3.0%) and replacement donors (2.0%) was statistically significant ( $\chi^2 = 14.2$  and *P* < 0.001). Similar observations were found for anti-HBcAb among volunteers (11.6%) compared to replacement (10.3%) donors ( $\chi^2 = 6.1$  and *P* = 0.013). As shown in Table 3, we calculated the difference between the results of HBsAg using ECL (2.0%) and EIA (3.1%) techniques, and it was found to be statistically significant ( $\chi^2 = 14.3$  and *P* < 0.001). Similarly, a significant difference exists ( $\chi^2 = 3.9$  and *P* = 0.047) in the results of anti-HBcAb using the 2 techniques (ECL, 10.3%; EIA, 11.5%).

Blood groups were also carried out on 11,069 donors. Of these, 10,076 (91.0%) and 993 (9.0%) were positive and negative for the Rhesus factor, respectively. The rates of O, A, B, and AB blood groups were 55.6%, 32.8%, 8.9%, and 2.7, respectively (Table 3). Positivity of HBsAg was found to be highest in group A (6.6%) blood donors, while anti-HBcAb was found in group B blood donors (26.7%). However, the difference between the results of blood groups and HBs Ag ( $\chi^2 = 0.1$  and *P* = 0.707) and Anti-HBc Ab ( $\chi^2 = 0.8$  and *P* = 0.359) was found to be statistically insignificant (Table 4).

**Table 4** Distributions of Blood Groups and Rh in Relation to HBs Ag and Anti-HBc Ab

Blood Group	Total		Rh Total			HBs Ag				Anti-HBc Ab			
						Positive		Negative		Positive		Negative	
	N	%		N	%	N	%	N	%	N	%	N	%
O	6152	55.6	+	5593	50.5	131	2.3	5462	97.7	659	11.8	4934	88.2
			-	559	5.1	11	2.0	548	97.7	60	10.7	499	89.3
A	3632	32.8	+	3326	30.0	99	3.0	3227	97.0	414	12.4	2912	87.6
			-	306	2.8	11	3.6	295	96.4	33	10.8	273	89.2
B	991	8.9	+	894	8.1	18	2.0	876	98.0	110	12.3	787	87.7
			-	97	0.9	1	1.0	96	99.0	14	14.4	83	85.6
AB	294	2.7	+	263	2.4	5	1.9	258	98.1	33	12.5	230	87.5
			-	31	0.3	0	0.0	31	0.2	3	9.7	28	90.3
Total	11,069	100.0		11,069	100.0	276	2.5	10,793	97.5	1326	12.0	9743	88.0
$\chi^2$						8.8				3.0			
P						0.271				0.884			

**Abbreviations:**  $\chi^2$ , Chi-square;  $P < 0.05$ , significant; N, number; %, percentage; +, positive; -, negative.

## Discussion

The overall prevalence rate (2.4%) of HBsAg among blood donors in this study was analogous to the studies carried out in various locations such as Yemen (2.6%),<sup>17</sup> Ethiopia (2.6%),<sup>18</sup> and India (2.12%).<sup>19</sup> However, the seropositivity rate of the present study was higher than our previous study (1.9%).<sup>16</sup> A low prevalence of HBsAg was reported in Iraq (0.24%),<sup>20</sup> Jordan (0.3%),<sup>21</sup> and Malaysia (0.03%).<sup>22</sup> The present study revealed a lower prevalence rate of HBsAg than those reported rates in Saudi Arabia (3.24%),<sup>23</sup> Yemen (4.1%),<sup>14</sup> Sudan (5.8%),<sup>24</sup> Nigeria (5.8%),<sup>25</sup> Ethiopia (3.0%),<sup>26</sup> Cameroon (11.2%),<sup>27</sup> the Central African Republic (16.7%)<sup>28</sup> and Mauritania (11.8%).<sup>29</sup>

The positivity rate of anti-HBcAb in the present study was 10.9%. Comparable rates were reported in Syria<sup>30</sup> (10.3%) and Tamil, India (10.9%).<sup>31</sup> A higher rate of anti-HBcAb was found in Ibadan (16.9%), Iran,<sup>32</sup> and Abuja (17.7%), Nigeria.<sup>33</sup> Lower rates, however, were reported in studies in Iran<sup>34</sup> (4.9%), Saudi Arabia (5.7%),<sup>35</sup> and Jordan (2.0–4.1%).<sup>21</sup>

Notably, significantly higher seropositivity for HBsAg (3.1%) and Anti-HBcAb (11.7%) among duplicated blood donors compared to single non-duplicated blood donors was observed. This could be explained by duplicating test results for donors who donated more than once. Since digital donor registry records are not available in the center, test results could have been recorded repeatedly. Similarly, repeated blood donors, who were ineligible to donate due to inadequate donation interval, could have contributed to the increased seroprevalence of infection. Monitoring donation history is a critical and currently existing problem. Another possible explanation for this was that most of the duplicated blood donors could be paid blood donors chosen by patients' relatives without care if free for viral hepatitis infections. The prevalence of infection is extremely high among paid donors and making blood donation their source of income.<sup>36</sup> It was reported that HCV prevalence among paid blood donors was as high as 5.7% or more.<sup>6</sup> On the contrary, lower HCV prevalence is seen among voluntary donors and employer-organized blood donations, with rates of between 1.1 to 2.3% and 0.46%, respectively.<sup>37,38</sup> The present study also found that the difference in results between these two groups was statistically significant. To get the acceptable prevalence rate of HBsAg and Anti-HBcAb, we excluded all duplicated cases of blood donors.

This present study further revealed that a significant difference exists between the results of ECL and manual EIA techniques. This could be related to the advantage of fully automatic ECL over manual colorimetric EIA, where the anticipation of contamination is higher. The difference in the results obtained by these techniques could be linked to the

sensitivity of ECL, where another study found that the ECL assay was two to four times more sensitive than the colorimetric EIA, at least for the detection of protein antigens or antibodies.<sup>39</sup> This explained the difference in the prevalence rates of HBsAg (2.0% and 3.1%) and Anti-HBcAb (10.3% and 11.5%) by ECL and EIA in this present study and the previous study, where the rate of HBs was 4.1% and 1.9% by ECL and EIA, respectively.<sup>14,16</sup>

Results of this study showed that the frequency of HBsAg in blood donors was not associated with their occupation. This was similar to the results found in the previous study.<sup>14</sup> Nevertheless, the incidence of HBsAg and anti-HBcAb in the present study, according to their occupational categories, was lower than the reported rate in the first study. This could be attributed to the different techniques used in the present study (ECL) than our previous study<sup>14</sup> (EIA). The present study confirms this validity, where the difference in the prevalence rates of HBsAg (2.0% and 3.1%) and Anti-HBcAb (10.3% and 11.5%) was obtained by ECL, and manual colorimetric EIA was found to be statistically significant.

The prevalence rate (2.3%) of HBsAg in this study with respect to the occupation of blood donors was similar to the findings in Cameroon (2.4%).<sup>27</sup> A higher rate of HBsAg (8.9% to 11.0%) was reported in Sierra Leone.<sup>40</sup> This study also observed a lower (2.3%) prevalence of HBsAg among students than the studies in Ethiopia (4.7%),<sup>41</sup> (3.4%),<sup>42</sup> and Sierra Leone (10.7%).<sup>40</sup> In contrast to this present study, our previous study revealed statistical significance ( $\chi^2 = 1.2$  and  $P < 0.001$ ) in the different rates of anti-HBcAb (ranging from 8.8% to 16.2%) concerning their occupational categories. These differences could be related to the larger sample size and greater precision of the ECL technique used in the present study compared to the smaller sample size and low precision of the manual EIA technique employed during the previous study.<sup>14</sup> Higher HBsAg among students (11.7% and 6.9%) and the military (5.1% and 8.4%) were reported in Saudi Arabia<sup>43</sup> and Gabon.<sup>44</sup>

Male donors showed a seropositivity rate of 2.3% for HBsAg. This is consistent with the study carried out in Basra, Iraq (2.3%), and Sana'a, Yemen (2.7%).<sup>16</sup> Statistically, no difference exists between HBsAg rates in males and females ( $P = 0.808$ ). Similarly, the prevalence was reported with the same findings ( $P = 0.464$ ) with rates of 2.4% and 1.7% in males and females,<sup>45</sup> respectively. Lesser positivity rates of HBsAg were reported in the study in Jordan, with 0.013% and 0.37% in males and females, respectively.<sup>46</sup> Furthermore, a similar rate of HBsAg among females was reported in Kenya (1.3%)<sup>47</sup> while a higher rate was found in the other studies (3.7%)<sup>9</sup> and (17.7%).<sup>48</sup> A lower rate (1.0%) of HBsAg among females than in the present study was reported in Cameroon<sup>27</sup> and Kenya<sup>47</sup> with an account of 1.0% and 1.3%, respectively. However, higher rates of HBsAg positivity in males were documented in other studies (13.1%),<sup>47</sup> (10.5%),<sup>27</sup> and (10.6%).<sup>28</sup>

Notably, the seropositivity of HBsAg and Anti-HBcAb rises as age increases. This was also noted in various studies conducted internationally.<sup>14,35,49</sup> A recent study revealed prevalence rates of HBsAg and Anti-HBc Ab among the different age groups fairly similar to that study conducted in Sana'a city, Yemen.<sup>16</sup>

The rates of anti-HBcAb in relation to the residence of blood donors were statistically significant ( $P = 0.013$ ). In contrast, the positivity rates of HBsAg were not significant. This result is consistent with the study conducted in Sana'a city, Yemen.<sup>14</sup> In the current study, the prevalence of HBsAg and Anti-HBcAb ranged from 2.1% to 4.3% for HBsAg and 9.4% to 12.4% for Anti-HBcAb, which was lower than in the previous study,<sup>14</sup> as it ranged from 3.3% to 10.8% for HBsAg and 12.7% to 35.1% for Anti-HBcAb.

The variation of HBsAg and Anti-HBcAb prevalence rates in the present study compared to our previous study<sup>14</sup> could be related to several reasons. First, the exclusion of duplicated donors in the present study; second, the advantage and quality of ECL employed in the present study over the manual EIA technique, which was used in the previous study; and lastly, the former study classified the residency of donors based on their governorates of residence. Hence, to overcome the problem of the low number of donors representing such governorates, we classify the residency of donors according to the new Republic of Yemen classification system, which is based on the province classification system. All the aforementioned reasons could play a significant role in obtaining reliable results, which can reflect the exact rate of HBsAg among this segment of the Yemeni population.

The positivity rate (3.0%) of HBsAg among the voluntary in the present study was higher than the studies in Saudi Arabia (2.4%)<sup>35</sup> and Ethiopia. (2.5%).<sup>42</sup> There was a lower prevalence (2.0%) among replacement donors in this study than in the studies of Saudi Arabia (4.5%)<sup>35</sup> and Ethiopia (4.1%).<sup>42</sup> The high rates of HBsAg and Anti-HBcAb among voluntary blood donors as compared to replacement donors can be explained as a result of the careful selection of patients' relatives as family replacement donors. Hence, most such donors have prior knowledge, as they are free of

infection and eligible to donate blood, in contrast to volunteers who have no prior knowledge of infections that affect blood donation. Hepatitis B reactive blood donors are informed of their infection status verbally and are advised to seek for more medical consultation. They are deferred to donate indefinitely.

Methods used for screening blood donors in Yemen may differ from one blood bank to another, but mostly use high-accuracy techniques, especially in central blood banks and donation centers, which use ELISA and Chemiluminescence, while others use chromatography, particularly in remote areas. The lack of materials and kits caused an acute shortage of supplies during the war, which prevented the use of the previously mentioned modern methods in detecting hepatitis viruses in blood donors.

The WHO recommended Hepatitis B vaccinations among the Yemeni population, to be part of the national immunization program of Yemen in 1998, especially among neonates who are at a higher risk of vertical transmission.<sup>50</sup> According to the latest WHO/UNICEF Estimates of National Immunization Coverage (WUENIC) for Yemen,<sup>51</sup> which are based on data reported until 5 October 2021, the vaccination coverage of the country is 73% for HepB3 (Hepatitis B vaccine, third dose). Vaccine coverage is defined as the percentage of infants (children under one year of age) who received certain vaccine-doses.<sup>51</sup> Moreover, Hepatitis B vaccinations are carried out by health workers, and are available to citizens.

## Conclusion

The seroprevalence of HBsAg and anti-HBcAb among Yemeni donors was 2.0% and 10.3%, respectively. The ECL technique showed enhanced sensitivity and advantage over the manual EIA technique. Duplicated blood donors influence the accuracy of the seroprevalence of HBs Ag and Anti-HBc Ab. Further study on first-time volunteer donors is required to better understand the prevalence of HBV among healthy adults. Screening of other infections, including HIV, HCV, and the prevalence of other transfusion-related infections is necessary among Yemeni blood donors.

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## Disclosure

The authors report no conflicts of interest in this work.

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