



## Current Screening Strategy Poses Risk of Spreading of Hepatitis C Virus Infection

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### Significance:

Compulsory screening of blood and blood items before transfusion, legitimate disinfection of dental and surgical instruments, and suitable transfer of contaminated stuff and dispensable syringes are among the issues that should be addressed by using of combo testing or antigen testing that can reduce the transmission of viral infections through transfusions. These procedures can significantly reduce the false positive reports of HCV during the window period, which is reported to be as long as 17 days by using fourth generation of ELISA.

### ABSTRACT

**Background:** Hepatitis C virus is one of the significant causes of morbidity and mortality in the World. Surprisingly, despite national screening campaigns, new cases of HCV are still on the rise.

**Methods:** A total of 5914 healthy blood donors was included in this study after informed consent. Anti-HCV ELISA was used to check presence of antigen in participant's plasma using Monlisa HCV Antigen-Antibody Ultra kit. Final confirmation was done by using real time PCR considered as a gold standard.

**Results:** 0.5% of anti-HCV ELISA negative samples showed presence of antigen in plasma, when checked through core Ag detection method.

**Conclusion:** Our result suggested that HCV core antigen detection and/or combo testing are far safer screening methods for the detection of HCV and the use of these methods can avoid/reduce further spread of this deadly disease.

### Introduction

Hepatitis is a common disease occurring worldwide and is a significant cause of illnesses and deaths (1). According to the World Health Organization, approximately 3% of the world population are infected with hepatitis C virus (HCV) with an increase of 3-4 million cases every year. Round about 65-85% of hepatitis C patients develop chronic hepatitis C further leading to hepatocellular carcinoma and death (2, 3). Awareness in the public regarding the transmission and prevention of HCV infection, provision of safe blood and blood products and accessibility to cost effective HCV antiviral drugs have considerably reduced the HCV prevalence in the developed countries. However, in many developing countries lack of knowledge about transmission and prevention of HCV, insufficient

facilities to screen blood and blood products, and unavailability/unaffordability to effective HCV therapies are the major factors which are responsible for inevitable rise in HCV infection (4, 5). Pakistan is amongst one of the countries that have very high rate of HCV patients both acute and chronic. There are about 10 million people with HCV in Pakistan. Published literatures showed that the prevalence of HCV infection is approximately 4% in Pakistan (6, 7). Recent studies illustrated that 90% of HCV positive patients were not aware of the infection (8, 9). It has been shown that prevalence of HCV increases with age and the reason can be because of exposure to risk factors.

Earlier in 1992, blood transfusions were considered as one of the major route of transmission of HCV owing to approximately 15-20 % of the total transmitted cases (10, 11). By changing the rule from paying the blood donors to just all volunteer has decreased the post-transfusion hepatitis to 10%, which was further reduced to 1 per million transfusions due to pre-transfusion blood screening for different infectious agents including HCV. The few cases that still occur is due to blood transfusions from newly infected patients that are in the window period i.e. antibodies are still not developed. This window period can be as long as 6-8 weeks (12). Moreover, early diagnosis of HCV among immunocompromised and hemodialysis patients is more difficult, where antibody respond late and take more time to develop.

In order to detect the false negative reporting, especially during the window period, we planned this study to evaluate the efficiency of the screening method (Ab detection based) commonly used in most of the blood banks within the country. We found that 0.5 % cases out of the healthy donors were found positive when analyzed by commercially available methods targeting either Antigen or antigen/antibody both.

### Materials and Methods

Five thousand nine hundred and fourteen donors from the blood banks of different health centers and hospitals of Punjab province of Pakistan were included in the present study. Complete history was taken from each blood donor through pre-designed tested questionnaire, which was approved by institutional ethical committee. Blood donors with the ages ranging between 20-60 years and body weights of more than 50 kg were included in this study. Donors with the history of hepatitis, current

or recent systemic disease, drug abuse, recent surgery or transfusion of blood or blood product within last 12 months were excluded from this study. Written informed consent was acquired from each participants. All the research work carried out was in compliance with Helsinki’s declaration.

**Screening of Blood groups and different infectious agents:** Blood groups of all the subjects were checked by slide and tile method using the readily present agents. Screening of hepatitis B virus (HBV) and immune deficiency virus (HIV) was performed by using ABBOTT PRISM® kits according to the manufacture protocol.

**Screening of the subjects for HCV:** All the subjects were initially screened with commercially available assay for the detection of antibodies against HCV (anti-HCV Ab) i.e., ARCHITECT Anti-HCV. Then, all the plasma was further analyzed by two other commercially available kits targeting either only the core antigen using Abbott Architect HCV Chemiluminescent Microparticle Immunoassay (CMIA) or both antigen and antibody using anti-HCV Monolisa® HCV Antigen-Antibody Ultra (Bio-Rad Laboratories Limited, Marnes La Coquette, France). These entire tests were performed as per the manufacturer’s protocol.

**Real-time PCR for HCV RNA detection:** We used RT-PCR as gold standard for the HCV detection in this study. Briefly, the HCV RNA was extracted via Fovergen kit and HCV RNA was amplified using commercially available AmpliSens HCV PCR kit and CFX Bio-Rad real time PCR according to the manufacturer protocol.

**Statistical Analysis:** All the data are expressed in percentages. The statistical analysis was performed using SPSS version 20 for analyzing percentages of different blood groups.

**Results**

**Demographic analysis of the studied population:** Out of 5914 blood donors, 5840 (98.74%) were male and 74 (1.25%) were female. The age-wise distribution among male and female is shown in (Table 1 and 2).

After checking for the prevalence of HCV among different blood group, we found higher prevalence of HCV subjects with A +ve and B+ve blood groups in both female and male, respectively (Figure 1).

**Screening results of other infectious agents**

All the subjects were analyzed for HBV and HIV, out of these subjects it was not found any positive case for HIV, even then 70 (1.18%) subjects were positive with hepatitis B virus (HbsAg). The Blood group distribution between males and females in HBV infected individuals is shown in Figure 3. Higher prevalence of HBV was

found among the subjects with A+ve blood group (Figure 2).

**Table 1:** Gender wise distribution of Blood Donors According to age group

Male donors distribution		
Age group	No. of donors	Percentage
18-30	4246	71.79%
31-40	1162	19.64%
41-50	234	3.95%
51-60	94	1.58%
Female donors distribution		
18-30	56	75.6%
31-40	16	21.6%
41-50	2	2.7%
51-60	0	0

**Table 2:** Distribution of Anti HCV positive cases in Different Districts of Pakistan

Districts	Prevalence of HCV	Districts	Prevalence of HCV
Khaniwal	14.28%	Pattoki	6.97%
Nankana	11.94%	Sargodha	6.66%
Faisalabad	11.76%	Gujranwala	5.88%
Kasoor	9.94%	Sialkoat	5.26%
Bahawalpur	9.09%	Multan	4.34%
Okara	8.74%	Lahore	3.42%
Shiekhupura	8.41%	Sahiwal	2.53%
Pakpatan	7.89%		

**Screening results of HCV through three different methods:**

All the blood donors included in this study were checked for HCV using three different commercially available kits. Out of 5914 subjects, 292 were found positive for the presence of anti-HCV antibodies in their plasma. Surprisingly, the other two methods, either targeting only HCV core Ag or both HCV core Ag and Ab (combo testing) showed 30 more positive cases in addition to the previously reported 292 positive cases analyzed through anti-HCV detection method. So, total number of 322 (292+30) were assigned as HCV affected individuals and were confirmed by RT-PCR.

**Confirmation of HCV through RT-PCR:** To confirm our results, we used RT-PCR against HCV as gold standard. All the included subjects were finally analyzed through RT-PCR and found that out of 5914 subjects, 322 (292+30) were confirmed for the presence of viral RNA in their plasma.

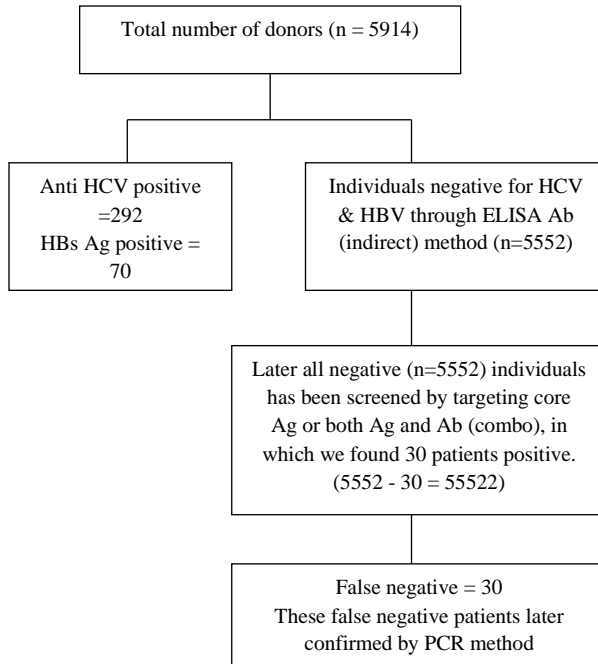


Figure 1. Schematic representation of the study

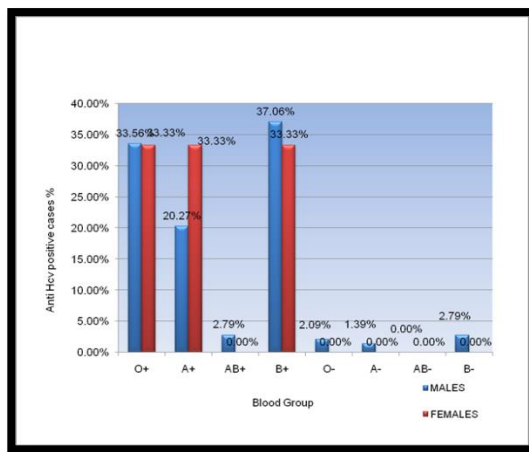


Figure 02. Distribution of Anti HCV Positive case with Blood Groups and Sex

**Incidence of HCV positive cases in different districts of Pakistan:** The distribution of HCV positive cases among different districts was also checked as shown in Figure 4. It was found that prevalence of HCV was 3.42% in Lahore (lowest), 9.94% in Kasur, 8.74% in Okara, 8.41% in Shaikhupura, 5.88% in Gujranwala, 2.53% in Sahiwal, 6.97% in Pattoki, 7.89% in Pakpattan, 11.76% in Faisalabad, 11.94% in Nankana, 6.66% in Sargodha, 5.26% in Sialkot, 14.28% in Khanewal (highest), 9.09% in Bahawalpur, 4.34% in Multan. Prevalence of anti HCV positive cases was highest in Khanewal District.

**Discussion**

We reported that when compared with the universally accepted HCV detection method (RT PCR), the most common method currently in-use (Anti-HCV Ab detection) all across Pakistan and in many other countries of the world as well failed to report all the HCV positive cases. When we compared these results with two other commercially available kits, we found that both the kits were able to detect those false negative samples and showed similar sensitivity and specificity compared to the gold standard (PCR).

There are approximately half million people affected by HCV in the globe and the number is still rising at an astounding rate mostly in the developing countries (12, 13). In Pakistan the number of HCV patients is quickly increasing and it is reported that there are 9 million carriers of HCV (14). In 2008, approximately 91.8 million total blood donations are collected. Blood transfusions increase the risk of getting transfusion-transmissible infections (TTI) such as HCV, HIV, HBV, syphilis and other less common infections such as malaria, toxoplasmosis, and brucellosis etc (15). EIAs are commonly used to detect anti-HCV antibodies for screening of HCV in these donated blood all around the world. New assays that either combines the detection of anti-HCV Ab, and/or antigen be used to lower the transmission rate (16). These methods are of great importance to medical institutes as it can prevent infections, protect doctor-patient relationship, lower the exposure of medical staff to the viral infections more efficiently and also good for early detection and diagnosis of infections (17). Our data showed higher number of male blood donors as compared to females. This was majorly due to physiological gender differences among females as well as other social and economic burdens faced by them. Previous study has also reported that most of voluntary donors were males 96.96% with respect to 3.41% females which is similar to the present study with higher number of young blood donor (18).

We found that the incidence of HCV was 5.4% (322), HBsAg was 1.18% (70) and not a single positive case of anti-HIV was observed in healthy blood donors. Another study conducted in the same province previously reported the incidence rate of HCV has risen significantly during the same time period, even in the presence of better screening methods, treatment and awareness (19). Most frequent incidence of HCV positive cases was seen in Khanewal 14.28%. This may be due to low literacy level, poor hygienic conditions and socioeconomic status in this area. On the contrary, the prevalence in Lahore was the lowest due to better

level of awareness, health care facilities and access to treatment.

We strongly recommend that these new assay/method, may be implemented as soon as possible to decrease the accidental spread of HCV. Compulsory screening of blood and blood items before transfusion, legitimate disinfection of dental and surgical instruments, and suitable transfer of contaminated stuff and dispensable syringes are among the issues that should be addressed by using of combo testing or antigen testing that can reduce the transmission of viral infections through transfusions. These procedures can significantly reduce the false positive reports of HCV during the window period, which is reported to be as long as 17 days by using fourth generation of ELISA.

### Conclusion

Continuous improvement in blood screening, use of sensitive assays and donor requirements led to safer blood transfusions than before. The government drug enforcement agencies may make a policy to use antigen testing or the combo testing rather than only antibody testing (ELISA) to avoid false positive HCV results due late immune response and accidental transmission of HCV among healthy populations.

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Disclosure:** None

**Human/Animal Rights:** No human or animal rights are violated during this study.

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