

## LOW SPECIFICITY OF THE THIRD GENERATION ELISA FOR HCV DETECTION IN VOLUNTARY BLOOD DONORS IN INDIA

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### ABSTRACT

#### Objective

Third generation anti-HCV ELISA is currently recommended for the diagnosis of HCV infection. We determined its specificity in voluntary blood donors (VBDs) and patients with chronic liver disease (CLD) in relation to confirmatory line immunoassay (LIA) and reverse transcription polymerase chain reaction (RT-PCR).

#### Material and Methods:

1926 serum samples of VBDs and 16 HCV related CLD patients were screened by ELISA. An optical density/cut-off ratio (OCR) of >1 was taken as positive for anti-HCV antibodies. Samples were confirmed by LIA and HCV-RNA detection by RT-PCR. Interpretation of LIA was done as: indeterminate, reactive or non-reactive. Every 50th VBD sample, negative for anti-HCV by LIA was subjected to LIA and RT-PCR to rule out false negativity of ELISA.

#### Results:

Anti-HCV was positive in 34 (1.76%) VBDs and all the CLD patients. Only one (2.9%) VBD was reactive by LIA and 6 (17.6%) were HCV-RNA positive. Serum samples from VBDs with OCR >3 were significantly more often ( $p < 0.05$ ) PCR positive than those with an OCR of <3. In the CLD patients, specimens even with OCR between 1-3 were reactive by PCR. All ELISA negative samples were non-reactive by LIA and PCR.

#### Conclusions:

(i) There is a high false positivity of the third generation

ELISA for the diagnosis of HCV infection in VBDs, (ii) Higher OCR should be used for improving the specificity of ELISA in VBDs, (iii) VBDs with an OCR of >3 should be subjected to HCV-RNA determination.

### INTRODUCTION

Serodiagnosis of Hepatitis C Virus (HCV) infection started about a decade back by employing enzyme linked immunosorbent assay (ELISA) [1]. There are two situations where anti-HCV detection is important; blood banks, where it is routinely used to reduce the risk of post-transfusion hepatitis C and, in clinical practice, to correlate and confirm the clinical suspicion of HCV-related chronic liver disease. First generation assays were less sensitive and specific. To overcome their drawbacks, confirmatory recombinant immunoblot assays (RIBA) were developed [2,3]. With further improvement in the sensitivity and specificity, second and third generation ELISA and immunoblot assays became available [4-7].

The presence of anti-HCV antibodies using these immunological tests does not give any idea about the viraemic status of a patient or a blood donor. To overcome these limitations, RIBA tests are often employed which indicate that most RIBA positive donors have persistent HCV infection [6]. However, the indeterminate results need to be ascertained by doing HCV-RNA test.

Prevalence of anti-HCV antibodies in the blood donor population in India is about 1.7% [8]. The frequency of anti-HCV false positivity by ELISA and indeterminate pattern in supplemental tests is not known. This could create a difficult situation in a low prevalence healthy population [1,6,9]. We initiated a large prospective study with the aim of determining the specificity of the third generation enzyme immunoassay in detecting anti-HCV antibodies in blood donor population and to compare this with patients with chronic liver disease due to hepatitis C.

### MATERIAL AND METHODS:

Nineteen hundred and twenty six consecutive healthy voluntary blood donors (VBDs) attending the blood bank of G. B. Pant Hospital, New Delhi, India, were included in the study. Blood sample collection, storage of serum samples and detection of HCV infection was a simultaneous process. At first, the serum specimen was subjected to anti-HCV detection using the third-generation, ELISA (United Biomedical, NY, USA). The test system detects antibodies directed to core, NS3, NS4 and NS5 regions of the HCV genome using synthetic peptides. The assay was carried out according to the manufacturer's instructions.

Figure 1: Population used

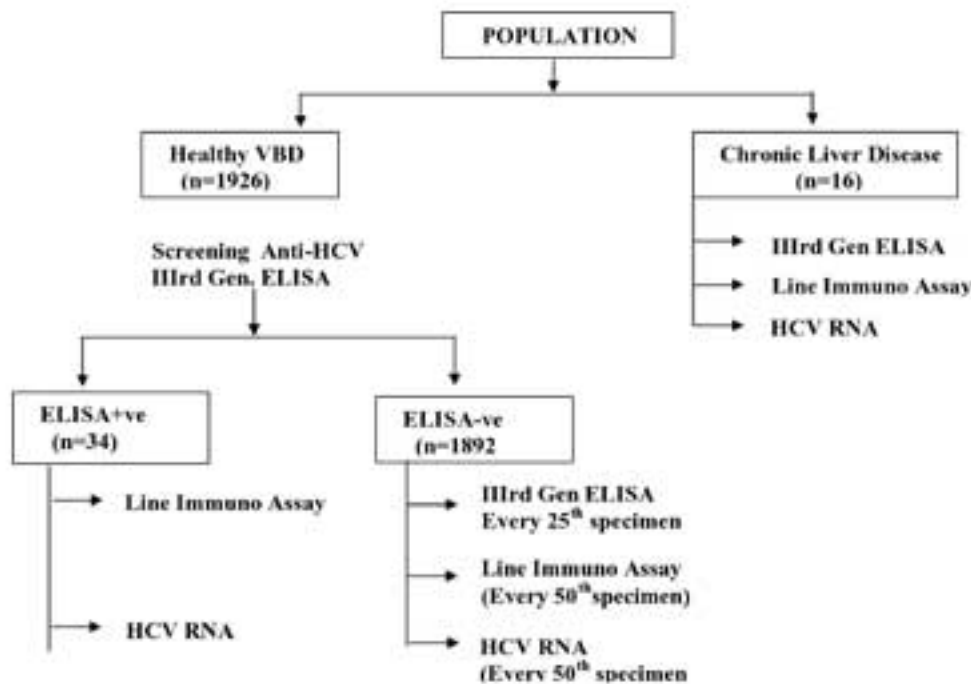


Figure 1 - Study design

In each specimen, optical density (OD) to cut-off ratio (OCR) was calculated. Samples with an OCR <1 were considered to be positive and those with OCR <1 were marked as negative for anti-HCV antibodies. Each positive sample was re-tested to confirm the positivity using ELISA. On the basis of the OCR, the samples were divided into 3 groups:

- (a) OCR between 1 – 3,
- (b) OCR between >3 – 6, and
- (c) OCR > 6.

To check for false negativity of anti-HCV ELISA, every 25th serum sample was re-analyzed by ELISA. Serum transaminase levels were determined for all the 1926 specimens simultaneously.

A confirmatory third generation Line Immunoassay (LiaTek, Organon Teknika, The Netherlands), and HCV RNA detection by RT reverse transcription polymerase chain reaction (RT-PCR) were performed on all ELISA positive specimens. The line immunoassay system uses synthetic peptides, corresponding to HCV envelope (E2 / NS2) in addition to core, NS3, NS4 and NS5 antigens. The assay and the interpretation of the results were carried out according to the manufacturer's instructions. To cross-check the negative ELISA result and to assess the false negativity of the line immunoassay, every 50th ELISA negative specimen was reanalyzed by a repeat-line immunoassay test.

HCV-RNA detection was also carried out using the RT-PCR in all ELISA positive and every 50th ELISA negative specimen. RNA was isolated by the guanidium isothiocyanate method [10] and the amplification was done using oligonucleotide primer sequence from the conserved region of the HCV genome.

A comparative group of sixteen biopsy-proven HCV-related patients with chronic liver disease were also included in the study. Serum of these subjects were analyzed by all the three assays i.e. ELISA, line immunoassay and HCV-RNA by RT-PCR.

## RESULTS

The results of different assays, namely ELISA, line immunoassay and RT-PCR in VBD's and chronic liver disease patients are shown below.

### ELISA

#### Voluntary Blood Donors:

Of the 1926 VBDs screened, 34 (1.76%) subjects revealed an OCR of >1.00, that is they were positive for anti-HCV antibodies. On the basis of OCR, the EIA positive specimens were divided into 3 groups: those having an OCR between 1-3, between 4-6, and above 6. Twenty six of the positive specimens had an OCR in the range of 1-3, seven (21%) revealed a ratio between 4 to 6 and only one

**Table - I****Comparison of methods of HCV detection in VBDs and CLD patients**

OCR	EIA		LIA		HCV RNA	
	VBD	CLD	VBD	CLD	VBD	CLD
1-3	26	5	3(I)	3(I)2(R)	-	5
>3-6	7	6	5(I)	2(I)4(R)	5*	6
>6	1	5	1(R)	5(R)	1	5
<b>Total</b>	<b>34</b>	<b>16</b>	<b>9</b>	<b>16</b>	<b>6</b>	<b>16</b>

I=Indeterminate, R=Reactive, OCR=O.D./Cut-off ratio \*p<0.05 between OCR 1-3 vs.

>3-6

sample had an optical density 10 folds compared to the cut-off value (Table 1). Every 25th EIA negative sample was found to be non-reactive on repeat analysis using enzyme immunoassay.

#### Chronic Liver Disease Patients:

Samples from all sixteen patients with chronic liver disease were reactive by enzyme immunoassay, 5 (31%), 6 (38%), and 5 (31%) patients were found to have an OCR between 1 to 3, 4 to 6 and above 6 respectively. Compared with the VBD group, a significantly higher number of patients with chronic liver disease had an OCR of >3.

#### Line Immuno Assay

##### Voluntary Blood Donors:

LIA had a very poor correlation with ELISA in the subjects with OCR between 1 and 3. Twenty-three of the 26 (86%) ELISA positive specimens were non-reactive by line immunoassay and three specimens showed indeterminate results. In the group of subjects with an OCR between 4 and 6, LIA again showed indeterminate results in six of the seven (86%) specimens. In the third group, there was only one sample which had an OCR ~10 by ELISA. This showed a positive LIA reaction. Every 50th ELISA-negative sample was also found negative by LIA.

#### Chronic Liver Disease Patients:

The LIA results in these specimens were quite different than that seen in VBDs. Even in the subjects with an OCR between 1-3, two of the 5 EIA positive samples were reactive by LIA and the remaining 3 were found to be indeterminate. In samples with an OCR between 4 to 6, LIA showed reactive results in four and indeterminate in two. The sample having an OCR > 6 was found to reactive by LIA.

#### HCV-RNA by Polymerase Chain Reaction

##### Voluntary Blood Donors:

HCV-RNA detection was undertaken in all the 34 anti HCV positive VBDS. In the 26 ELISA positive specimens where the OCR was between 1 and 3, none of the samples was found positive for HCV RNA. In the groups with an OCR between 4 to 6 and > 6, five (84%) and one (100%) subject respectively was found to be positive. Thus overall, only six of the 34 (6%) anti-HCV positive samples were found to be HCV-RNA positive.

#### Chronic Liver Disease Patients:

In the group of chronic liver disease patients, 11 of the 16 (69%) blood samples showed an OCR above 3. However, irrespective of the OCR, all the 16 anti-HCV positive patients with chronic liver disease were found to be HCV-RNA positive (Table 1).

## DISCUSSION

The results of our study indicate a poor correlation between the results of the enzyme immunoassay, the confirmatory line immunoassay and the HCV-RNA detection by RT-PCR in the voluntary blood donor population in India. Only six of the 34 (6%) donors who were found to be anti-HCV-positive were actually detected to have HCV-RNA in their blood. Of these however, only one sample was found to be positive by the confirmatory line immunoassay. This discrepancy was particularly evident when the OCR was between 1 and 3; 88% of the ELISA positive specimens were found to be negative by line immunoassay and the remaining 12% were indeterminate. None of the samples was reactive. Similarly, at this none of the OCR of 1-3, donors were HCV-RNA positive. However, if a hyper value of OCR (>3) was used, the false positivity of ELISA has been well documented in the healthy population where the prevalence of HCV infection is low [1,6,9]. Our results of high false positivity of anti-HCV by 3rd generation ELISA are in conformity with Cordons et al, [11] who have also recommended the use of the polymerase chain reaction for improving the specificity of HCV detection. Few other authors have also concluded that further refinement of antibody screening and confirmatory assays and standardization of molecular testing are necessary to optimize testing and fully characterize the diagnosis of HCV infection [12]. These observations clearly indicate that the addition of a new antigen in the third generation ELISA kit might have improved the sensitivity of the assay over the second-generation kits, but it has not added to the specificity of detection, especially in serum samples positive for anti-HCV and having a low OCR.

Our results also bring to attention the major limitations of the confirmatory immunoblot assays in the VBD population. These tests were not found helpful as even in 8 of the 9 samples with an OCR of >3, the results of LIA were indeterminate. In almost every subject who is LIA indeterminate, HCV RNA testing was required. Hence, there is little rationale for using immunoblot assay in routine blood bank screening. This finding reaffirms the observation of Krarup et al. [13].

On the other hand, the standard third generation EIA was quite sensitive and specific in the patients with chronic liver disease. All the sixteen blood samples that were anti-HCV positive, were also found to be HCV-RNA positive. RIBA was reactive in only 11 of these patients and indeterminate in 5. It therefore appears quite clear that the third generation anti-HCV testing is quite sensitive and specific for chronic liver disease patients and there is no added advantage of doing immuno blot assays.

In summary, the third generation ELISA is quite sensitive and specific for the diagnosis of HCV infection in patients with chronic liver disease. In the voluntary blood donor population however, the test is relatively less specific, specially, at the lower OCR. We recommend that an assay with a higher OCR should be used for VBDs to reduce the false positivity. Blood donors with high OCR should be investigated further by doing RT-PCR testing for HCV-RNA.

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