

# Hepatitis B and C Prevalences Among Blood Donors in the South Region of Brazil

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*The prevalence of hepatitis B and C infection has been determined in a seroepidemiological survey among blood donors from the south of Brazil (Florianópolis, State of Santa Catarina). These markers has also been correlated with the levels of alanine aminotransferase (ALT), a surrogate marker to prevent post-transfusion hepatitis. Sera from 5000 donors were randomly collected in the period of April to November 1991. The prevalences of HBsAg, anti-HBs and anti-HBc were respectively 0.78%, 7.02% and 13.98%. The anti-HCV prevalence after confirmation testing with line immunoassay (LIA), was 1.14%. Normal values of ALT ( $\leq 32$  U/ml) were found in 59.78%, values slightly above the mean (ALT between 32-70 U/ml) in 37.74% and high values of ALT ( $\geq 70$  U/ml) in 2.48%. The positivity of anti-HCV antibodies increased with the elevation of ALT levels. This correlation was not observed in relation to HBsAg. There exists a diversity in the recognition of HCV epitopes among HCV positive donors. Via the confirmation test used, we could observe that 94.7% of donors recognize the structural core antigen. Besides that, we observed that 5.26% of the HCV reactive sera recognized only epitopes located in the NS4 and/or NS5 region, indicating the importance of these epitopes for the improvement of assays.*

Key words: blood donors - hepatitis C - hepatitis B - alanine aminotransferase - anti-HCV - anti-HBc

Since the detection of hepatitis B virus (HBV) in the 1960's and hepatitis A virus (HAV) in the 1970's, a considerable proportion of sporadic and post-transfusion hepatitis could not be classified and therefore was referred as non-A, non-B (NANB) hepatitis (Dienstag 1983). In 1989, the main causative agent of post-transfusion NANB hepatitis was characterized, and named hepatitis C virus (HCV) (Choo et al. 1989). This was the start for the development of different anti-HCV antibody assays, and subsequently confirmation tests (Immunoblot and polymerase chain reactions) (Barbara & Contreras 1991).

We present here data regarding the seroprevalence of hepatitis B and C markers among volunteer blood donors from the south of Brazil, in Florianópolis, State of Santa Catarina. A correlation between liver enzyme level - alanine aminotransferase (ALT) - and anti-HBc and anti-HCV antibodies is also established.

## MATERIALS AND METHODS

**Subjects** - From April to November 1991, plasma samples from 5000 volunteer blood donors were selected randomly from blood donations at

the Hematology and Hemotherapy Center (HEMOSC) of Florianópolis, SC. Ninety percent of the donors were males, 97.5% were white and only 2.5% were black, in contrast with other geographic areas of Brazil. The donors aged from 18 to 65 years, but 82.4% aged less than 40 years.

**Serologic tests** - The HBV markers tested by enzyme immunoassay included hepatitis B surface antigen (HBsAg, Auszyme monoclonal - Abbott Lab.), antibody to hepatitis B core antigen (anti-HBc, National Reference Center for Viral Hepatitis/ IOC), and antibody to hepatitis B surface antigen (anti-HBs, Bio-Manguinhos/FIOCRUZ). The anti-HCV assay was performed with the use of a commercial enzyme linked immunosorbent assay (Innotest HCV Ab II, Innogenetics). Reactive assays were confirmed by a commercial available Line Immuno Assay (InnoLIA HCV Ab II, Innogenetics). ALT levels were determined using a colorimetric Reitman and Frankel method (Labtest). A normal ALT level using this assay was less than 32 U/ml. The ALT threshold for exclusion of donation was 70 U/ml, according to specifications of Alter et al. (1981). All tests were performed according to the manufacturer's instructions.

**Statistical Analysis** - Statistical evaluations were performed using the Epiinfo 5.01b program developed by Andrew G Dean and others (Centers

for Disease Control, Atlanta, GA, USA);  $\alpha = 0.05$ .

**RESULTS**

The overall prevalence for the different blood-borne diseases evaluated in 5000 volunteer blood donors is illustrated in Fig. 1.

Concerning HBV markers among this population, HBsAg was detected in 39 individuals (0.78%), anti-HBs in 351 (7.02%) and anti-HBc in 699 (13.9%). From 351 individuals reactive for anti-HBs and 699 for anti-HBc, 11 (3.13%) and 19 (2.72%) were also positive for anti-HCV, respectively. Anti-HCV could not be detected in any of the 39 HBsAg positive patients (Table I). The odds ratio in donors with HBV markers (anti-HBc and/or anti-HBs) was 4.2 times higher for HCV than in donors without HBV markers ( $2.41 < OR < 7.32$ ; confidence limit of 95%, according to Cornfield).

In Fig. 2 we can see the blood donors classified according to the hepatitis B virus infection. Persons without any HBV markers (84.62%) were classified as susceptible for HBV infection. Immune individuals (anti-HBs and/or anti-HBc positive) accounts for 7.02%. Persons positive for HBsAg and anti-HBc were classified as HBsAg carriers (0.78%), 7.58% has anti-HBc as an unique marker for HBV infection.

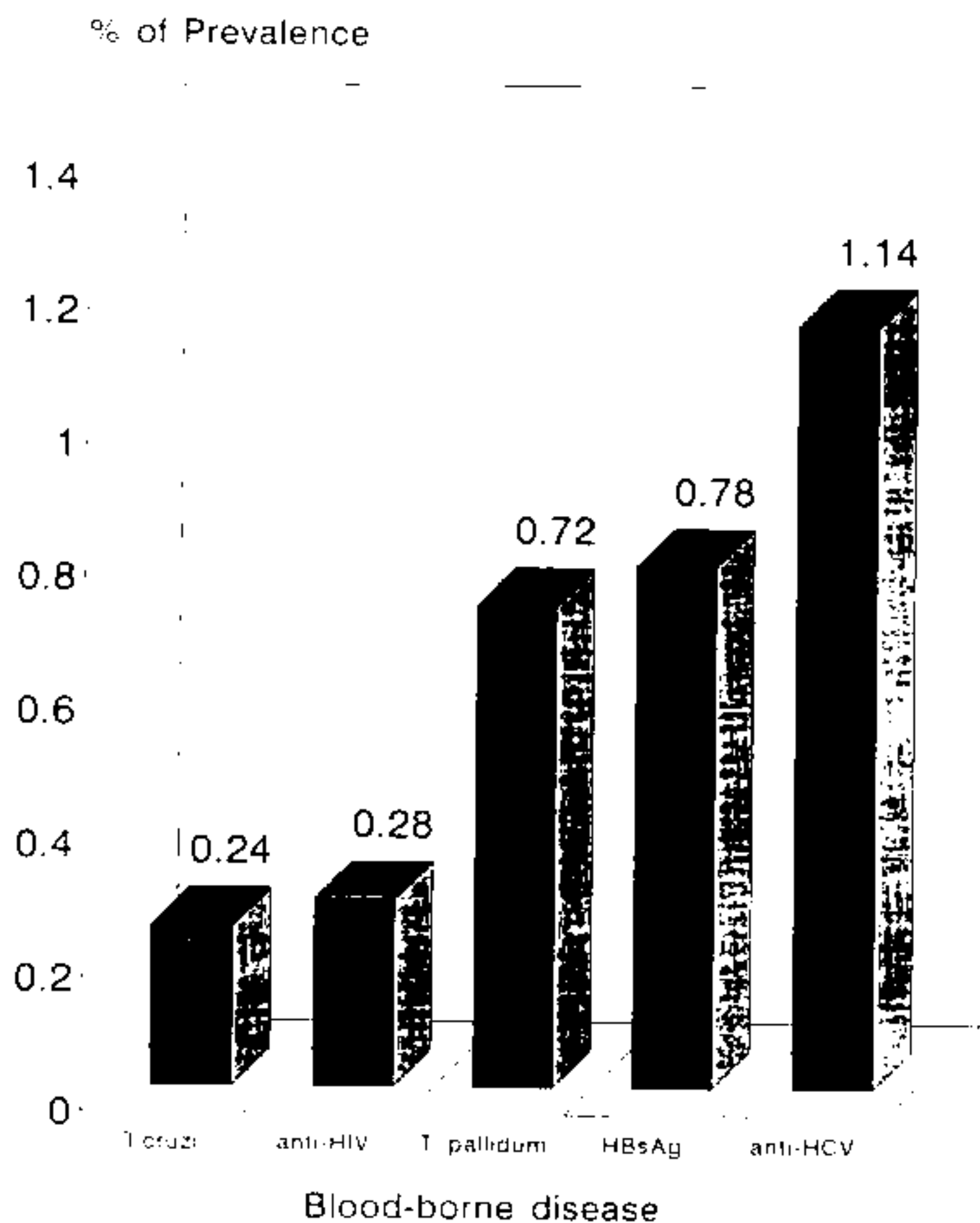


Fig 1: prevalence of different blood-borne disease in blood donors from the South of Brazil. Analysis of 5000 donors.

TABLE I

Prevalence of anti-HCV antibodies in blood donors presenting serological markers for hepatitis B

HBV Marker	Number (%)	anti-HCV+ (%)
HBsAg	39 (0.78)	0 (0.00%)
anti-HBc	699 (13.98)	19 (2.72%)
anti-HBs	351 (7.02)	11 (3.13%)
Absence of HBV marker	4231 (73.22)	27 (0.64%)

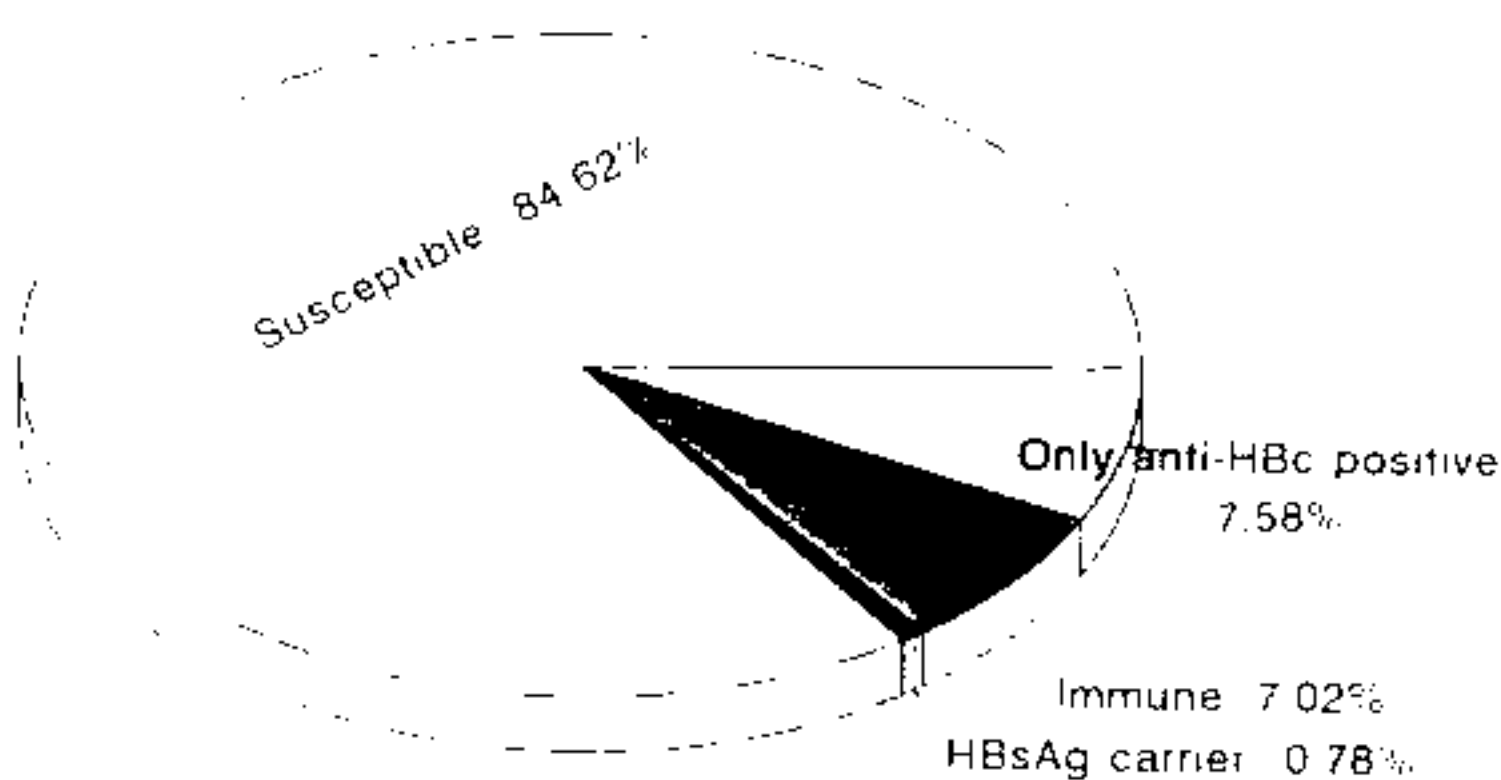


Fig 2: classification of blood donors in relation to HBV infection. Analysis of 5000 donors from the South of Brazil.

Table II shows the ALT value distribution for the population analyzed. Normal ALT values ( $\leq 32$  U/ml) could be seen in 2989 donors (59.78%). Slightly altered ALT values (32-70 U/ml) were observed in 1887 donors (37.74%). Clearly altered ALT levels ( $> 70$  U/ml) were found in 124 donors (2.48%). It could be noted that anti-HCV positivity was linked with the increase of ALT levels. Among donors with slightly altered ALT values the anti-HCV prevalence was 2.2 times as high as in donors with normal ALT levels, and 13 times higher in donors with elevated ALT levels when compared to normal ALT. These results were statistically significant (Chi-square for linear trend= 35.30;  $p < 0.001$ ). HBsAg prevalence is 2.4 times higher in persons with highly altered ALT levels when compared to normal ALT groups. Nevertheless in the case of HBsAg we could not observe the same correlation (Chi-square for linear trend= 1.63;  $p > 0.20$ ). Among donors which are anti-HBc positive, we could find a significant tendency in relation to the ALT values as observed for anti-HCV (Chi-square for linear trend= 81.37;  $p < 0.001$ ).

The immune response against different HCV epitopes are presented in Table III. Forty-six out of 57 HCV positive donors (80.70%) had antibodies against both, core and non-structural epitopes (NS4 and/or NS5) from the HCV genome. Antibodies to core epitopes solely were found in 8 do-

TABLE II

Correlation between ALT values and HCV and HBV infections in blood donors from the South Region of Brazil

ALT level	Number (%)	Hepatitis C		Hepatitis B	
		anti-HCV+(%)	HBsAg+(%)	anti-HBc+(%)	
<32 U/ml	2989 (59.78)	19 (0.64)	20 (0.67)	269 (9.90)	
32-70 U/ml	1887 (37.74)	27 (1.43)	17 (0.90)	383 (20.30)	
>=70 U/ml	124 ( 2.48)	11 (8.87)	2 (1.61)	20 (16.13)	
Total	5000 (100.0)	57 (1.14)	39 (0.78)	672 (13.44)	

TABLE III

Antibody response directed to different epitopes corresponding to HCV genome presented in line immunoassay

Epitopes	Number/Total	%
Core	8/57	14.03
Core + NS4	11/57	19.30
Core + NS5	4/57	7.02
Core + NS4 + NS5	31/57	54.39
NS4 + NS5	3/57	5.26

nors (14.03%). Three donors (5.26%) had exclusive antibodies against epitopes from the non-structural part of the HCV genome (NS4 and/or NS5). This results show the diversity of the immune response in the recognition of HCV epitopes among HCV positive donors.

### DISCUSSION

There are few data concerning the prevalence of Hepatitis C infection in blood donors from Brazil because only very recently screening for anti-HCV became compulsory.

The serological analysis of 5000 volunteers blood donors from the South Region of Brazil, Florianópolis, demonstrated that anti-HCV is the most important marker among blood-borne diseases of this region.

The prevalence of anti-HCV antibodies was 1.14%, and about 1.5 times higher than HBsAg (0.78%) and *Treponema pallidum* (0.72%), 4 times higher than anti-HIV (0.28%) and 5 times higher than anti-*T.cruzi*. A similar distribution was demonstrated by Vanderborcht et al.(1993), among blood donors from Rio de Janeiro where the prevalence of anti-HCV after confirmation testing was 2.7%, 2.4 times higher than in Florianópolis. The same screening and confirmatory tests were used in both studies.

In other parts of the world, the prevalence of anti-HCV is variable. In Africa, Coursaget et al.(1990), found 4.2% of anti-HCV antibodies in

adult population, whereas lower prevalence of 0.9-1.2% was found in European blood donors (Aymard et al. 1990, Esteban et al. 1992). In USA, Hsu et al. (1991) found 0.53% of the donors positive for anti-HCV. Our results from the South region of Brazil, showed to be similar to the prevalence found in Japan (Watanabe et al. 1990). Nevertheless, these results were relative to the screening test of first or earlier 2nd generation tests.

The prevalence of HBsAg asymptomatic carriers is rather low (0.78%) comparing with other geographic areas of the country. In Brazil, prevalence studies of HBsAg in several groups of populations show that there exists a south to north tendency on the increase of HBsAg prevalence (Fay et al. 1985). High prevalences of 5.1% are found in West Amazon region (Bensabath & Boshell 1973).

Simultaneous infection of HBV and HCV characterized by the presence of HBsAg and anti-HCV was not found. However, the prevalence of 5.85% of anti-HCV was observed in donors with anti-HBs and/or anti-HBc, in contrast to 0.64% of anti-HCV antibodies found in donors with absence of any HBV marker. As hepatitis B and C are epidemiologically similar in respect to factors such a parenteral risk (Alter et al. 1989), it seems that there exists a correlation in the mechanism of transmission of both viruses.

This study showed that 15.38% of the donors had had previous contact with HBV. From these, 7.58% had anti-HBc as an unique marker. It is important to assign that the presence of this antibody is not suitable for use as a surrogate marker for HCV infection, since only 2.37% of the anti-HBc reactive donors were positive for anti-HCV, also ALT values in this group was not significant.

Anti-HBc is the only marker that can be present between HBsAg clearance and anti-HBs appearance, or as a residual marker after anti-HBs disappearance (McMahon et al. 1981, Hadler et al. 1984). In order to better understand the importance of anti-HBc marker in relation to transmission of

HBV by blood transfusions, we are investigating the presence of HBV-DNA genomes in those blood donors.

It is known that normal ALT values can differ taking in account some parameters like body weight and sex (Stevens et al. 1990), however HEMOSC adopted the threshold value for blood donors exclusion of 70 U/ml according to Alter et al. (1991).

Considering the analysis in stratified form of ALT distribution and anti-HCV antibodies, we found that anti-HCV is 13 times higher in donors with elevated ALT levels (11 in 124) than in group of normal ALT (19 in 2989). Nevertheless almost 40% of donors had alterations of ALT levels, although only 2.4% of them had levels above 70U/ml, of which 8.8% present anti-HCV antibodies. This means that only 19.3% of the universe of anti-HCV positive donors would be eliminated, if the proposed ALT criteria of exclusion would be applied. Low correlation between ALT and anti-HCV was also observed by Hetland et al. (1990).

As an additional study, we are focusing on the remaining blood bags which presented elevated ALT in the absence of anti-HCV antibodies. It is important to investigate the presence of viral HCV genomes by PCR, since some early studies have indicated that the antibody response to HCV infection in some cases can be delayed in the course of natural infection, often proceeded by alterations of liver enzymes (Sankary et al. 1992, Yoshida et al. 1993). Those results could further establish criteria for more accurate discard of potential infective blood bags.

Finally, 94.74% of the anti-HCV reactive blood donors presented antibodies against epitopes corresponding to the structural core region of the HCV genome. Beside this, 5.3% of the donors reacted with only NS4 and/or NS5 epitopes indicating the importance of those epitopes in order to increase the sensitivity of screening tests. Many tests from different manufacturers are commercially available. Although the antigens are not completely identical, Baath et al. (1992) showed good concordance between the different tests studied and emphasized the importance of the use of appropriate antigens for HCV tests.

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