

SIGNIFICANCE OF ISOLATED HEPATITIS B CORE ANTIBODY IN BLOOD DONORS FROM SÃO PAULO

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SUMMARY

The clinical significance of isolated anti-HBc is still a challenge. To elucidate the real importance of this finding in our blood donors, an investigation algorithm was tested. One hundred and twelve isolated anti-HBc seropositive blood donors underwent clinical evaluation and retesting of HBV markers. Those who presented repeatedly reactive isolated anti-HBc, received a single dose of hepatitis B recombinant vaccine to verify anti-HBs early response. A HBV-DNA determination by PCR was done for those who did not test positive to anti-HBs after vaccine. The level of anti-HBc was recorded as a ratio of the sample-to-cut-off values (S:C ratio) in 57 candidates at donation. Comparing true and false-positive anti-HBc results, the different S:C ratios of them were statistically significant and when less than 2, implying in a false-positive result probability over 80%.

A high percent of false-positive results (16.07%) was verified after anti-HBc retesting. HBV immunity was characterized in 49.11%, either by anti-HBs detection in retesting (15.18%), or after a single dose HBV vaccination (33.93%). HBV-DNA was negative in all tested donors. In conclusion, this algorithm was useful to clarify the meaning of isolated anti-HBc in most of our blood donors.

KEYWORDS: Hepatitis B; Anti-HBc; Antibodies; HBV-DNA; HBV vaccine.

INTRODUCTION

Post-transfusion hepatitis B is still a relevant subject. Regardless of all efforts to guarantee safety of blood, hepatitis B residual risk is the highest among transfusional transmitted diseases, accounting for 1 in 63,000 transfused units³⁴.

Antibody to hepatitis B core antigen (anti-HBc) is the most sensitive marker of previous hepatitis B contact. It appears in acute phase of HBV (hepatitis B virus) infection and usually persists after virus clearance¹⁵. Diagnostic problems may arise when anti-HBc is found without HBsAg or anti-HBs seropositivity¹⁹.

Isolated anti-HBc serological profile may be associated with: (a) a chronic carrier state in which HBsAg is not detectable; (b) remote infection with loss of measurable anti-HBs; (c) passive transfer of anti-HBc; (d) nonspecific, cross-reacting antibody; and (e) the period when HBsAg has disappeared and anti-HBs has not yet been detected^{14,32}.

Hepatitis B vaccination of these individuals has been largely used to understand the meaning of isolated anti-HBc^{3,21,24}. Those who are immune will present an anamnestic anti-HBs response, after a single dose of vaccine^{12,20}.

Introduction of HBV-DNA detection by polymerase chain reaction (PCR)¹⁸ has implemented the confirmation of HBV infection in carriers with isolated anti-HBc^{8,17,22,36,39}.

The ability to differentiate HBV chronic infection, immunity or false-positive results in donors with isolated anti-HBc pattern has obvious significance for the prognosis, treatment and well being of such individuals³². Thus, this study intends to establish an investigative algorithm to elucidate the real meaning of isolated anti-HBc on our blood donors.

PATIENTS AND METHODS

Routines for notification and counseling of blood donors: Once a blood donor presents a reactive screening serologic test, a letter is sent inviting him to come back to our institution, for an additional laboratory testing. When the blood donor screening test presents HBsAg negative and total anti-HBc positive, the additional laboratory testing will include HBsAg, anti-HBc and anti-HBs detection. If the additional laboratory testing reveals an isolated anti-HBc pattern, the blood donor will be asked to refer to the General Transfusion Research Group of São Paulo University - Hematology Department, to have this serologic pattern investigated.

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Casuistic: From August 1994 to July 1997, 124 donors with isolated anti-HBc were enrolled in our protocol at the General Transfusion Research Group Clinic. Twelve were excluded because they had not completed the different phases of the protocol. From the 112 individuals involved, 87 (77.7%) were male and 25 (22.3%) were female, the mean age was 37.2 years, ranging from 19 to 59. Donors were followed-up during a minimum period of 30 days, and a maximum of 25 months. On average, the three phases of the protocol were completed in 120 days.

Inclusion criteria:

- a) Blood donors from Fundação Pró-Sangue Hemocentro de São Paulo, who had the following serologic result after blood screening and additional laboratory testing:
HBsAg and anti-HBs negative and total anti-HBc positive.
- b) Screening serologic tests negative for anti-HCV, anti-HIV, anti-HTLV I/II, Chagas Disease and Syphilis.
- c) Informed consent to participate on this protocol.

Exclusion Criteria:

- a) No adherence to the different protocol phases, as it is shown at Figure 1.
- b) Prior Hepatitis B vaccination.
- c) Any reference concerning vaccination side effects.

Protocol development: The protocol was developed in three different phases (Figure 1).

Phase 1

Clinical evaluation: All blood donors underwent a clinical evaluation, being questioned about their age, gender, race, risk factors to hepatitis B (tattoos, acupuncture, sexual promiscuity, sexual or household contact with hepatitis B carriers, receipt of blood transfusion, percutaneous exposure), previous HBV infection symptoms, alcoholic ingestion, previous positive blood screening tests to HBV.

Physical examination was routinely carried out in all donors, searching for suggestive signs of hepatic diseases, including jaundice, spiders angiomas, hepatomegaly, splenomegaly, ascites, palmar erythema, gynecomastia (men) and dilated abdominal wall veins.

Additional laboratory testing: In Phase 1, all donors were retested for: HBsAg, anti-HBc and anti-HBs. After these tests, three different results were obtained:

1. Anti-HBc negative in the new blood sample. Samples were tested twice, using the same commercial kit for blood screening at donation. The blood donation serologic test was considered a false-positive result.
2. HBsAg negative, both anti-HBc and anti-HBs positive: donor was informed to be immune to hepatitis B.
3. Persistent isolated anti-HBc positivity: donors underwent phase 2.

Investigation for anti-HBc IgM was also performed in 66 blood donors at retesting.

Phase 2

Hepatitis B vaccination: Those who were confirmed to be isolated anti-HBc positive were vaccinated with 20 µg hepatitis B recombinant single dose vaccine (Engerix B®). After a period of 30 days, another anti-HBs determination was performed. Those who were anti-HBs positive (titers over 10 mIU/mL), were informed to be immune.

Phase 3

HBV-DNA research: HBV core/precore DNA detection by polymerase chain reaction¹⁸ was performed in all donors who failed to produce anti-HBs in phase 2.

Laboratory methods: HBsAg and total anti-HBc – Micro Enzyme Immunoassay, performed by commercial kit methods (Organon® and Ortho®).

Anti-HBs - Micro Enzyme Immunoassay, performed by commercial kit methods (Organon®, Roche® and Murex®). Anti-HBs tests were considered positive when titers over 10 mIU/mL were detected.

Anti-HBc IgM - Micro Enzyme Immunoassay, performed by commercial kit methods (Wellcome® and Organon®).

Once a blood sample was tested utilizing one of the commercial kits above, all the additional samples of that donor were retested using the same commercial kit.

Sample-to-cut-off ratio (S:C ratio): Only after January 1995 anti-HBc ratio of the sample-to-cut-off values (S:C ratio) was routinely incorporated in the serologic result at our institution. Consequently, this index could only be calculated in 57 donors at donation.

Statistical analyses: The different groups of donors were compared in relation to the mean of anti-HBc S:C ratio at donation, through the analysis of variance for independent samples²⁹.

RESULTS

Donors' clinical data, presented on Table 1, showed that almost 80% blood donors did not present risk factors for HBV infection. Other data, such as previous symptoms of hepatitis, alcohol abuse as well as hepatomegaly, were present in 6.25 to 16% blood donors.

One hundred and twelve blood donors were retested for HBsAg, total anti-HBc and anti-HBs. None was HBsAg positive at retest, 17 (15.18%) were total anti-HBc and anti-HBs positive and 18 (16.07%) were negative for all the hepatitis B markers retested. Seventy-seven blood donors (68.75%) presented repeatedly reactive isolated anti-HBc and received a single dose of hepatitis B recombinant vaccine. Thirty-eight donors (33.93%) presented an anamnestic response and 39 did not test positive to anti-HBs after vaccination. HBV-DNA was performed in all donors of the last group, and was not detectable in any of them. Figure 2 shows the four different groups of donors obtained at the end of the study according to the serologic results shown above.

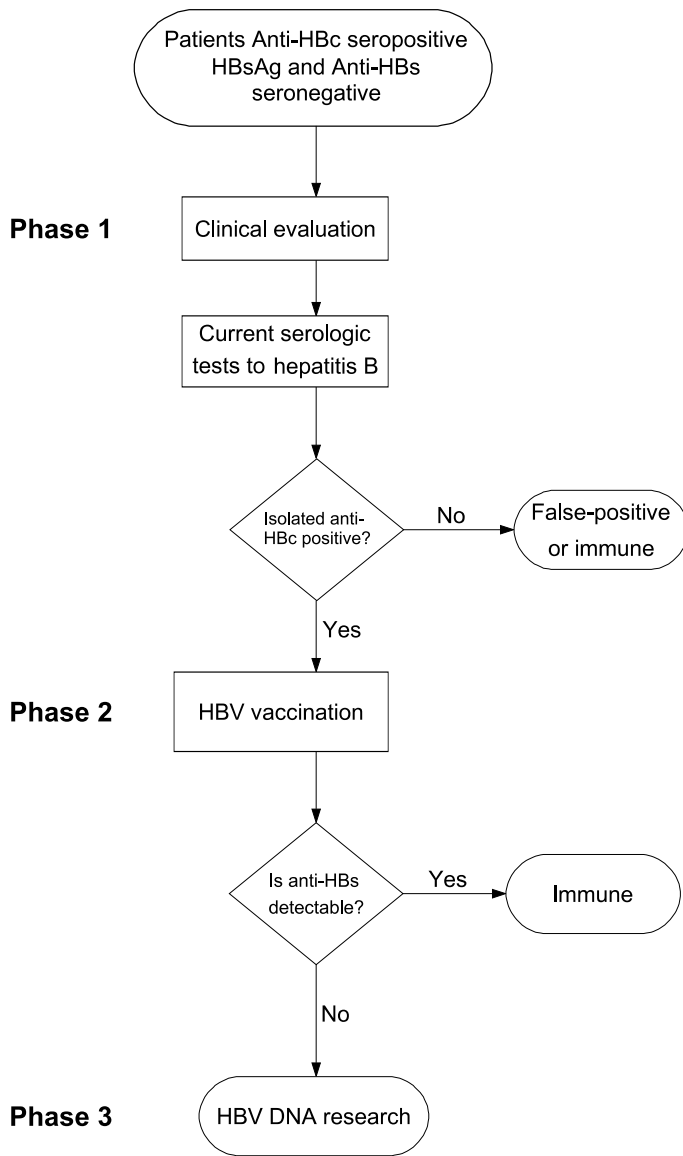


Fig. 1 - Protocol development.

Anti-HBc IgM detection was performed in 66 blood donors at retesting, 12 in group I, 11 in group II, 24 in group III and 24 in group IV. Only 2 blood donors were anti-HBc IgM positive, one in group III (S:C ratio = 4.1) and one in group IV (S:C ratio = 1.4). Blood donor in group III was vaccinated with a single dose HBV vaccine, according to our algorithm. After 30 days, anti-HBc IgM was negative and anti-HBs was detected. Anti-HBc IgM did not confirm to be positive at retesting in group IV blood donor. Considering this donor had an anti-HBc IgM negativity at retesting and the low S:C ratio, the prior anti-HBc IgM positivity was considered a false-positive result.

Descriptive measures and comparison of the results of 57 donors anti-HBc S:C ratio, described on Table 2, show a statistically significant

Table 1
Clinical data in 112 blood donors presenting isolated anti-HBc

	n	%
No risk factors for HBV infection	88	78.6
Risk factors for HBV infection	24	21.42
Household contact with HBV carrier	6	5.35
Sexual promiscuity	4	3.57
Tattoo	4	3.57
Receipt of blood transfusion	3	2.67
HBV sexual partner carrier	2	1.78
Percutaneous exposure+blood transfusion	2	1.78
Acupuncture < 12 months	1	0.89
HBV sexual partner carrier+blood transfusion	1	0.89
Domestic contact with HBV carrier+acupuncture < 12 months	1	0.89
Previous hepatitis symptoms	7	6.25
Alcohol abuse	18	16.07
Previous hepatitis B marker reactivity	1	0.85
Physical examination		
Palpable liver	11	9.82

n = number of donors

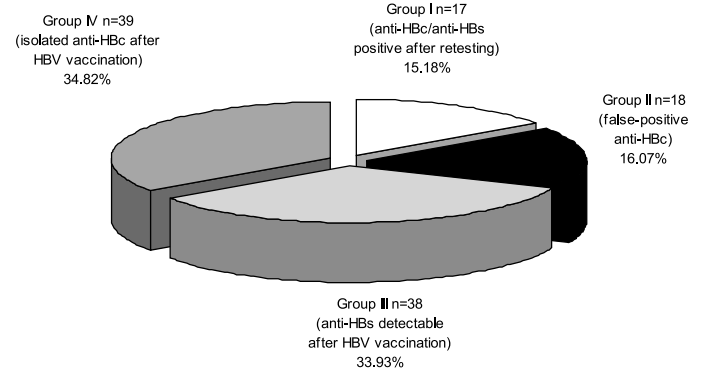


Fig. 2 - Distribution of the patients on different groups at the end of the protocol.

difference of Group II (false-positive anti-HBc) in relation to the others (p = 0.0001).

DISCUSSION

In our institution, 3.49% of the collected blood units are discarded due to anti-HBc reactivity, whereas overall discard for HBsAg is only 0.31%. Although most of the discarded anti-HBc positive blood units are associated with anti-HBs, about 10% of them present isolated anti-HBc, the meaning of which is still unclear².

The application of the proposed algorithm enabled the definition of the significance of anti-HBc in 66.07% of the studied population. In

Table 2
Descriptive measures and comparison of the results among groups – anti-HBc S:C ratio variable at donation

Variable	Group	n	Minimum	Maximum	Median	Mean	S.D.	p-value
S:C ratio	I	7	5.00	8.40	6.00	6.36	1.29	0.0001 ^(*)
	II	12	1.20	5.00	1.30	1.72	1.09	
	III	17	1.70	7.90	5.30	5.68	1.69	
	IV	21	1.20	9.30	5.20	4.70	2.66	

(*) : Analysis of variance; S:C ratio = sample-to-cut-off ratio values; n = number of donors; S.D. = Standard Deviation; p = statistical probability

15.27%, anti-HBs was detected after retesting. The interpretation could be either a spontaneous anti-HBs formation or variations of anti-HBs titers³⁷. Undoubtedly, a young healthy blood donor could be in a hepatitis B window phase at the time of donation^{14,32}. All the 12 blood donors from this group that were tested for anti-HBc IgM were negative concerning this marker. Consequently, variations in anti-HBs titers seem to have occurred more frequently in this studied group.

The negativity of anti-HBc at the retesting indicates a false-positive result at donation, found in 18 out of 112 blood donors. Many authors have observed low specificity for total anti-HBc tests when enzyme immune assays are used^{7,16,19,32,33}. False-positive results can occur as an unspecific immature lymphocyte B activation after any infectious process³⁰. Radioimmunoassay (RIA) is an alternative to improve anti-HBc specificity²⁷, however the reintroduction of RIA in blood bank routine does not seem to be plausible, considering the problems in manipulating radioactive material¹⁹. An interesting feature of this group was the statistically significant low S:C ratio, when compared to the other groups ($p < 0.0001$). Most of the donors (83.33%) in group I had this index lower than 2 at donation, similarly to the results of AOKI *et al.*³, who verified that when total anti-HBc S:C index was lower than 2.2, a false-positive result was very likely. Considering that these donors had a negative anti-HBc at retesting they were advised to reintegrate as blood donors, according to the institution reentry protocol.

In 33.93% of the studied donors, a typical anamnestic response²⁰ was found after a single dose HBV vaccine. This type of response is expected in 2% to 34% of isolated anti-HBc individuals^{3,20,21,24}, providing evidence against chronic infection and enhancing protective immunity¹². Anti-HBc IgM reactivity in one blood donor in this group is not easy to be interpreted. As it does not seem to be a false positive result, it could really be due to an acute episode of hepatitis B, that promptly seroconverted to anti-HBs.

In the proposed algorithm, HBV-DNA was negative in all donors which persisted isolated positive anti-HBc after retesting and HBV vaccination. In fact, 39 blood donors remaining isolated anti-HBc positive were HBV-DNA-negative.

Many authors^{8,10,17,22,23,31,39} have found chronic HBV carrier state in isolated anti-HBc individuals when studying the general population. The algorithm was carried out to identify a sub-population with a higher probability of being HBV infected. Maybe these objectives were not achieved, due to a β error, low prevalence of HBV on this population^{11,35}. Using the same PCR technique in HBsAg negative and anti-HBc positive blood donors from many Blood Centers in São Paulo State, SUCUPIRA³⁸

found 7 (1.34%) HBV-DNA positive in 522 blood samples. On the other hand, CRUZ⁹ found HBV-DNA positivity in 23.3% of São Paulo City blood donors who had presented previously isolated Hepatitis B core antibodies. False-negative or false-positive PCR results must be considered to explain such discrepancy between these studies. The sensitive of PCR assay to detect the presence of HBV depends upon the DNA extraction technique, the sequence of primers of the reactions, and the experimental conditions of the technique¹³. A better standardization of PCR assays is necessary to incorporate this technique routinely in clinical laboratories. This objective is prone to be reach as commercial kits are available²⁸ and the value of adding genomic amplification testing to hepatitis B blood donor screening is under investigation^{1,5,6,26}.

Therefore, we could not define the real meaning of isolated anti-HBc in group IV, considering that HBV-DNA was negative but anti-HBc was positive, and they were informed that they could not be reintegrated as blood donors. They are still coming for medical appointments and the serologic pattern presented before persists.

In order to diminish the loss of blood donated units, one could consider adopting lower cut-off levels for anti-HBc^{17,25,27}, since test signals near the cut-off, are judged to be false-positive. This strategy would be useful mainly in regions with high prevalence of HBV, although more specific anti-HBc screening assays⁴ would be more efficacious. Nucleic acid amplification testing (NAT) of blood donors added to antibody screening test will certainly reduce the residual risk of post-transfusion hepatitis B and will help to elucidate some controversial serologic patterns such as isolated anti-HBc.

RESUMO

Significado do anti-HBc isolado em doadores de sangue de São Paulo

O significado do anti-HBc isolado continua a ser tema relevante para aqueles envolvidos com o atendimento a doadores de sangue soropositivos. Um algoritmo de investigação foi testado com o objetivo de avaliar em nosso meio o real diagnóstico desses doadores. Cento e doze doadores com anti-HBc isolado foram submetidos a avaliação clínico-epidemiológica e testes sorológicos para o VHB. Aqueles confirmadamente anti-HBc isolado, receberam dose única da vacina recombinante contra o VHB, e após 30 dias foi pesquisada a formação do anti-HBs. Naqueles que não formaram anti-HBs após vacina, foi realizada a pesquisa do HBV-DNA por PCR. O índice do “cut-off” sobre a densidade ótica foi determinado em 57 indivíduos por ocasião da doação. Na comparação entre falsos e verdadeiros anti-HBc positivos, o

índice C.O./D.O. mostrou significância estatística. Assim quando este índice foi menor que 2, a possibilidade de resultado falso-positivo foi de 83,33%.

Verificamos ainda elevada porcentagem de resultados falsos-positivos (16,07%) após a simples repetição do anti-HBc. Imunidade ao VHB pôde ser caracterizada em 49,11%, tanto pela detecção do anti-HBs nos testes de repetição (15,18%) quanto pela vacinação em dose única contra a hepatite B (33,93%). O HBV-DNA foi negativo em todos os doadores testados. Concluimos que este algoritmo foi útil para esclarecer o significado do anti-HBc isolado na maioria dos doadores de sangue estudados.

REFERENCES

1. ALLAIN, J.P. – Genomic screening for blood-borne viruses in transfusion settings. **Clin. Lab. Haemat.**, 22: 1-10, 2000.
2. ALMEIDA NETO, C.; MENDONÇA, M.C.; SANT'ANNA, M.V.S. *et al.* - Análise epidemiológica de 222 doadores de sangue com o anti-HBc reativo e o AgHBs não reativo. In: CONGRESSO NACIONAL DO COLÉGIO BRASILEIRO DE HEMATOLOGIA, 15., Águas de Lindóia, 1995. **Anais**, p. 83.
3. AOKI, S.K.; FINEGOLD, D.; KURAMOTO, I.K. *et al.* - Significance of antibody to hepatitis B core antigen in blood donors as determined by their serologic response to hepatitis B vaccine. **Transfusion**, 33: 362-367, 1993.
4. BUSCH, M.P.; DODD, R.Y.; LACKRITZ, E.M. *et al.* - Value and cost-effectiveness of screening blood donors for antibody to hepatitis B core antigen as a way of detecting window-phase human immunodeficiency virus type 1 infections. The HIV Blood Donor Study Group. **Transfusion**, 37: 1003-1011, 1997.
5. BUSCH, M.P.; KLEINMAN, S.H.; JACKSON, B. *et al.* - Committee report. Nucleic acid amplification testing of blood donors for transfusion-transmitted infectious diseases: report of the interorganizational task force on nucleic acid amplification testing of blood donors. **Transfusion**, 40: 143-159, 2000.
6. BUSCH, M.P.; STRAMER, S.L. & KLEINMAN, S.H. - Evolving applications of nucleic acid amplification assays for prevention of transmission by blood components and derivatives. In: GARRATY, G., ed. **Applications of molecular biology to blood transfusion medicine**. Bethesda, American Association of Blood Banks, 1997. p. 123-176.
7. CASPARI, G.; BEYER, H.J.; ELBERT G. *et al.* - Unsatisfactory specificities and sensitivities of six enzyme immunoassays for antibodies to hepatitis B core antigen. **J. clin. Microbiol.**, 27: 2067-2072, 1989.
8. CHUNG, H.T.; LEE, J.S.K. & LOK, A.S.F. - Prevention of posttransfusion hepatitis B and C by screening for antibody to hepatitis C virus and antibody to HBcAg. **Hepatology**, 18: 1045-1049, 1993.
9. CRUZ, C.F.N. - **Estudo de doadores de sangue com a presença do anticorpo anti-HBc sérico como marcador isolado de infecção pelo vírus da hepatite B**. São Paulo, 1997. (Tese de Doutorado - Escola Paulista de Medicina da Universidade Federal de São Paulo).
10. DAVIS, A.R.; PINK, J.M.; LIEN, A.H. & WYLIE, B.R. - Isolated antibody to hepatitis B core antigen in blood donors and immune response to hepatitis B vaccination. [Letter] **Aust. N. Z. J. Med.**, 23: 215, 1993.
11. DOUGLAS, D.D.; TASWELL, H.F.; RAKELA, J. & RABE, D. - Absence of hepatitis B virus DNA detected by polymerase chain reaction in blood donors who are hepatitis B surface antigen negative and antibody to hepatitis B core antigen positive from a United States population with a low prevalence of hepatitis B serologic markers. **Transfusion**, 33: 212-216, 1993.
12. DRAELOS, M.; MORGAN, T.; SCHIFMAN, R.B. & SAMPLINER, R.E. - Significance of isolated antibody to hepatitis B core antigen determined by immune response to hepatitis B vaccination. **J. Amer. med. Ass.**, 258: 1193-1195, 1987.
13. GOMES, S.A.; YOSHIDA, C.F. & NIEL, C. - Detection of hepatitis B virus DNA in hepatitis B surface antigen-negative serum by polymerase chain reaction: evaluation of different primer pairs and conditions. **Acta virol.**, 40: 133-138, 1996.
14. HOLLAND, P.V. - Notification and counseling of blood donors. **Vox Sang.** (Basel), 70: 46-49, 1996.
15. HOOFNAGLE, J.H. & DI BISCEGLIE, A.M. - Serologic diagnosis of acute and chronic viral hepatitis. **Semin. Liver Dis.**, 11: 73-83, 1991.
16. HUGHES, W.; BARR, A.; DOW, B.C.; FOLLETT, E.A. & BARBARA, J.A. - A multicentre assesment of the specificity of ten anti-HBc screening tests. **Transfus. Med.**, 5: 225-230, 1995.
17. IIZUKA, H.; OHMURA, K.; ISHIJIMA, A. *et al.* - Correlation between anti-HBc titers and HBV-DNA in blood units without detectable HBsAg. **Vox Sang.** (Basel), 63: 107-111, 1992.
18. KANEKO, S.; MILLER, R.H.; FEINSTONE, S.M. *et al.* - Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. **Proc. nat. Acad. Sci.** (Wash.), 86: 312-316, 1989.
19. KROES, A.C.M.; QUINT, W.G.V. & HEIJTINK, R.A. - Significance of isolated hepatitis B core antibodies detected by enzyme immunoassay in a high risk population. **J. med. Virol.**, 35: 96-100, 1991.
20. LAI, C.L.; LAU, J.Y.N.; YEOH, E.K.; CHANG, W.K. & LIN, H.J. - Significance of isolated anti-HBc seropositivity by Elisa: implications and the role of radioimmunoassay. **J. med. Virol.**, 36: 180-183, 1992.
21. LOK, A.S.F.; LAI, C.L. & WU, P.C. - Prevalence of isolated antibody to hepatitis B core antigen in an area endemic for hepatitis B virus infection: implications in hepatitis B vaccination programs. **Hepatology**, 8: 766-770, 1988.
22. LUO, K.X.; ZHOU, R.; HE, C.; LINAG, Z.S. & JIANG, S.B. - Hepatitis B virus DNA in sera of virus carriers positive exclusively for antibodies to the hepatitis B core antigen. **J. med. Virol.**, 35: 55-59, 1991.
23. MARUSAWA, H.; UEMOTO, S.; HIJIKATA, M. *et al.* - Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. **Hepatology**, 31: 488-495, 2000.
24. McMAHON, B.J.; PARKINSON, A.J.; HELMINIAK, C. *et al.* - Response to hepatitis B vaccine of persons positive for antibody to hepatitis B core antigen. **Gastroenterology**, 103: 590-594, 1992.
25. MOSLEY, J.W.; STEVENS, R.D.; AACH, R.D. *et al.* - Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. **Transfusion**, 35: 5-12, 1995.
26. MÜLLER-BREITKREUTZ, K.; BAYLIS, S.A. & ALLAIN, J. - Nucleic acid amplification tests for detection of blood-borne viruses. 5th. EPFA/NIBSC Workshop, Amsterdam 1998. **Vox Sang.** (Basel), 76: 194-200, 1999.
27. PARKINSON, A.J.; McMAHON, B.J.; HALL, D.; RITTER, D. & FITZGERALD, M.A. - Comparison of enzyme immunoassay with radioimmunoassay for the detection of antibody to hepatitis B core antigen as the only marker of hepatitis B infection in a population with a high prevalence of hepatitis B. **J. med. Virol.**, 30: 253-257, 1990.
28. PAWLOTSKY, J.M.; BASTIE, A.; HÉZODE, C. *et al.* - Routine detection and quantification of hepatitis B virus DNA in clinical laboratories: performance of three commercial assays. **J. virol. Meth.**, 85: 11-21, 2000.
29. ROSNER, B. - **Fundamentals of Biostatistics**. 2. ed. Massachusetts, PWS Publ., 1986. p. 246-480.

30. SÄLLBERG, M. & MAGNIUS, L.O. - Enzyme immunoassay for anti-hepatitis B core (HBc) immunoglobulin G1 and significance of low-level results in competitive assays for anti-HBc. **J. clin. Microbiol.**, 27: 849-853, 1989.
31. SÁNCHEZ-QUIJANO, A.; JAUREGUI, J.I.; LEAL, M. *et al.* - Hepatitis B virus occult infection in subjects with persistent isolated anti-HBc reactivity. **J. Hepat.**, 17: 288-293, 1993.
32. SCHIFMAN, R.B.; RIVERS, S.L.; SAMPLINER, R.E. & KRAMES, J.E. - Significance of isolated hepatitis B core antibody in blood donors. **Arch. intern. Med.**, 153: 2261-2266, 1993.
33. SCHMIDT, P.J.; LEPARC, G.F. & SAMIA, C.T. - Comparison of assays for anti-HBc in blood donors. **Transfusion**, 28: 389-391, 1988.
34. SCHREIBER, G.B.; BUSCH, M.P.; KLIEINMAN, S.H. & KORELITZ, J.J. - The risk of transfusion-transmitted viral infection. The Retrovirus Epidemiology Donor Study. **New Engl. J. Med.**, 334: 1685-1690, 1996.
35. SCULLY, L.J.; SUNG, H.; PENNIE, R. & GILL, P. - Detection of hepatitis B virus DNA in the serum of Canadian hepatitis B surface antigen negative, anti-HBc positive individuals, using the polymerase chain reaction. **J. med. Virol.**, 44: 293-297, 1994.
36. SHIH, L.N.; SHEU, J.C.; WANG, J.T. *et al.* - Serum hepatitis B virus DNA in healthy HBsAg negative Chinese adults evaluated by polymerase chain reaction. **J. med. Virol.**, 32: 257-260, 1990.
37. SOUTO, F.J.D.; FONTES C.J.F.; OLIVEIRA, J.M.; GASPAR, A.M.C. & LYRA, L.G.C. - Comparison of two enzyme immunoassays for the detection of antibody to hepatitis B virus core antigen **Mem. Inst. Oswaldo Cruz**, 92: 89-90, 1997.
38. SUCUPIRA, M.C.A. - **Prevalência do DNA do HBV em amostras de doadores de sangue com AgHBs negativo e anti-HBc positivo**. São Paulo, 1997. (Dissertação de Mestrado - Instituto de Ciências Biomédicas da Universidade de São Paulo).
39. ZABALETA, M.E.; TORO, F.I.; RUIZ, M.E. *et al.* - Assessment of former and newly developed HBV assays in a third world setting. **J. med. Virol.**, 38: 240-245, 1992.

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