

RESEARCH NOTE

Comparison of Two Enzyme Immunoassays for the Detection of Antibody to Hepatitis B Virus Core Antigen

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Antibodies against hepatitis B core antigen (anti-HBc) are found in combination with the hepatitis B surface antigen (HBsAg) in individuals who were infected and persist as Hepatitis B virus (HBV) carriers. The concomitance of anti-HBc plus the antibody against hepatitis B surface antigen (anti-HBs) means that the infected person cleared the HBsAg and became naturally immunized against HBV (W Szmunes et al. 1976 *Am J Epidemiol* 104: 256-262, E Feret et al. 1987 *Am J Epidemiol* 125: 140-149, ASF Lok et al. 1988 *Hepatology* 8: 766-770, BC Zanalda et al. 1990 *Bol Oficina Sanit Panam* 108: 16-25). Anti-HBc could also be found as the sole serum marker of HBV infection (anti-HBc alone). This last serologic pattern is seen in: (i) the "window" phase of a recent acquired-HBV infection, before the onset of anti-HBs reactivity (ii) in HBV carriers with undetectable HBsAg levels; (iii) in people recovered

from previous HBV infection, but having subsequently lost detectable anti-HBs; (iv) false positive test results (JI Dienstag & DM Ryan 1982 *Am J Epidemiol* 115: 26-39, Feret *loc. cit.*, Lok *loc. cit.*, Zanalda *loc. cit.*). Indeed, Parkinson et al. (1990 *J Med Virol* 30: 253-257) showed weak specificity of anti-HBc tested by enzyme immunoassay (EIA) in comparison with anti-HBc screened by radio immunoassay (RIA). However, these authors found high concordance between these two methods when the EIA positive result was expressed as 70% or more of the total antibody-linking inhibition. In spite of all this knowledge controversies about the interpretation of the anti-HBc alone still remain.

Aiming to identify HBV markers prevalence in the population of N. Sra. do Livramento, a small municipality in the State of Mato Grosso, Central Brazil, an epidemiologic study was conducted between October 1993 and June 1994 (unpublished data). Sera from 740 individuals, a representative sample of the population, were collected, frozen at -20°C and sent to National Reference Center for Viral Hepatitis, Instituto Oswaldo Cruz (IOC), Rio de Janeiro, Brazil. HBsAg and anti-HBs were detected by EIA, using reactivities produced by Bio-Manguinhos, Rio de Janeiro, Brazil (EIA/BM^â). Anti-HBc was detected by EIA, using kits from IOC (EIA/IOC). Anti-HBc kit is produced using recombinant protein, in solid phase and specific polyclonal IgG labelled to peroxidase as conjugate.

Among the 740 participants of the population survey, 169 (22.8%) had at least one of the three HBV markers. A surprisingly high number, 83 (49.2%) out of 169, of anti-HBc alone was found, very different from those seen in other countries (Szmunes *loc. cit.*, Dienstag & Ryan *loc. cit.*, Feret *loc. cit.*, Lok *loc. cit.*). As this could have been caused by false-positive anti-HBc reactions, we decided to re-test sera using EIA reactivities produced by other manufacturer: anti-HBc (Corzyme^â), HBsAg (Auszyme^â) and anti-HBs (Ausab^â) (Abbott, North Chicago, USA). This confirmatory part of the study was performed in the blood bank of Federal University of Mato Grosso.

Tests for anti-HBc were repeated in sera that showed the following results in the first screening: (i) 83 positive sera for anti-HBc alone; (ii) 58 sera positive for both anti-HBc and anti-HBs; (iii) 59 sera randomly selected among 571 negative samples for all HBV markers. Considering EIA (Corzyme^â) as gold-standard, sensitivity and specificity of the EIA/IOC were calculated and the agreement of the results obtained with these two kits were evaluated by the Youden's index J (P Armitage 1971 *Statistical Methods in Medical Research* p. 434-435, Blacwell Sc. Publ., London).

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Seventy one (85.5%) sera remained anti-HBc positive when re-tested by EIA-Corzyme[®]. Of the 58 positive sera for both anti-HBc and anti-HBs, 57 (98.3%) had the anti-HBc positivity confirmed. All 59 sera negative for all HBV markers remained negative for Corzyme[®]. Agreement between the two EIA kits was observed in 187 (93.5%) from 200 re-tested samples (Youlden's index J = 0.82; IC 95% = 0.73-0.91). The calculated sensitivity and specificity for EIA/IOC in anti-HBc detection were 100% (128/128) and 82% (59/72), respectively. The false positive rate of this method was 9% (13/141) (Table).

TABLE

Results of enzyme immunoassays for antibody against hepatitis B core antigen detection in 200 sera selected from a representative sample of the population of N. Sra. do Livramento, State of Mato Grosso, Brazil, tested by Instituto Oswaldo Cruz (IOC) and by Corzyme[®]/Abbott

	Corzyme [®] /Abbott		Total
	Positive	Negative	
IOC Positive	128 ^a	13 ^b	141
IOC Negative	0	59	59
Total	128	72	200

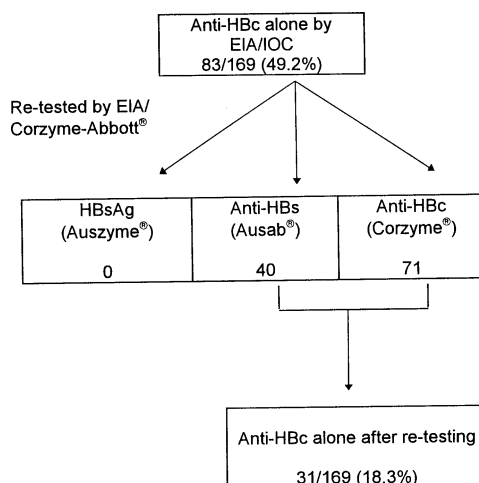
Sensitivity: 128/128 = 100%; Specificity: 59/72 = 82%; Concordance = 187/200 = 93.5%; Youden's index J = 0.82 (IC 95% = 0.73-0.91); a : 71 sera positives for anti-HBc alone + 57 sera positive for both anti-HBc and anti-HBs; b : 12 sera positives for anti-HBc alone + 1 serum positive for anti-HBs and anti-HBc.

All 83 positive sera for anti-HBc alone in EIA/IOC had the negativity of HBsAg confirmed by Auszyme[®]. However, 40 (48.2%) of these samples reacted to anti-HBs when tested by EIA/Ausab[®] (Fig.). Therefore, after re-testing the samples by the three HBV markers, the prevalence of anti-HBc alone in the population study decreased from 49.2% (83/169) to 18.3% (31/169). No significant change in the overall prevalence of HBV infection was observed after repeating the tests (22.8% - 169/740 to 21.2% - 157/740; $\chi^2=0.3$; p=0.5).

In the initial population survey positive tests for anti-HBc alone (EIA/IOC) occurred in half of the subjects positive for HBV markers. Similar results have been shown in other Brazilian surveys (ADC Passos et al. 1993 *Rev Saú Públ* 27: 30-35, MSP Azevedo et al. 1994 *Rev Soc Bras Med Trop* 27: 157-162, HCFV Vasconcelos et al. 1994 *Mem Inst Oswaldo Cruz* 89: 503-507). After re-testing our sera by Abbott kits, the prevalence of anti-HBc alone was very similar to that found in other surveys worldwide (Szmuness *loc. cit.*, Dienstag & Ryan *loc. cit.*, Lok *loc. cit.*). Thus, the most prob-

able explanation for the high prevalence of anti-HBc alone found by us and previously reported in other Brazilian studies is the low sensitivity from the anti-HBs detection rather than a high proportion of false positive anti-HBc results, particularly if EIA/BM[®] product is used. Probably, most individuals with an anti-HBc alone result by EIA/IOC is really a previously HBV infected that cleared HBsAg, but with undetectable anti-HBs level by EIA/BM[®] kits. The poor sensitivity of this method may produce a high amount of false negative results and an erroneous high proportion of anti-HBc alone subjects. HBsAg purified from plasma of asymptomatic blood donors is used in solid phase of EIA for anti-HBs detection and for enzyme-conjugated preparation. The great volume of plasma with high level of HBsAg required for the purification procedure precludes the production of a higher sensitive anti-HBs kit. More encouraging results could be obtained using recombinant HBsAg proteins.

In the present report we found high sensitivity and good specificity for the EIA/IOC in detection of anti-HBc. Probably, the lower specificity here observed is not important if one consider the usefulness of this method to screen blood donors. The high concordance found in results of anti-HBc tested by the two EIA kits and the absence of change in its overall prevalence after re-testing sera warrant that EIA/IOC is a suitable method to detect HBV contact in epidemiological surveys. In fact, to this goal is quite reasonable and less expensive not to use anti-HBs since the anti-HBc is more sensitive and has a better detection of previous HBV contact, except when dealing with anti-HBV vaccine response.



Hepatitis B virus (HBV) markers detected by enzyme immunoassays from Abbott[®] in 83 sera positive for anti-HBc as the sole HBV marker by enzyme immunoassay from Instituto Oswaldo Cruz (IOC).