

Detection of Australia Antigen (HBAg) in Blood Donors and Hepatoma Patients in Mozambique

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SUMMARY

The evaluation of three different methods (counter immuno-electrophoresis, latex agglutination and radio-immunoassay) for Australia antigen detection is discussed with regard to their sensitivity in the analysis of sera from blood donors and primary liver cancer patients.

The counter immuno-electrophoretic method is the least sensitive, particularly in the analysis of sera from hepatoma patients. The latex agglutination test seems to have the same reliability in both groups, but gave a higher percentage of positive results compared with the radio-immunoassay technique.

The value of an improved latex agglutination test for Australia antigen detection in field work in southern Mozambique, is stressed.

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The transfusion of blood containing hepatitis B antigen (HBAg) is associated with the development of acute,¹ chronic² or anicteric³ hepatitis. Evidence has been accumulated in recent years that HBAg is highly prevalent in primary liver carcinoma (PLC)⁴ and other diseases.^{5,6} For these reasons, many blood banks are introducing the detection of HBAg as a compulsory screening test in blood donors. A simple, quick, inexpensive and reliable method, which would require a minimum of equipment and qualified personnel, is most desirable, particularly in countries where high frequencies of HBAg prevail, especially when the demand for blood units exceeds the supply.

Most of the serological methods available for HBAg detection are not suitable for use as a routine test in blood banks, either because they require very sophisticated techniques or because of prohibitive costs. Of the current serological methods, the latex agglutination test (LAT) should be most adequate, and different laboratories are trying to use it for HBAg detection.^{7,8} We had the opportunity of trying one of these tests, developed by Pfizer Laboratories and to compare it with the counter immuno-electrophoresis (CIEP) and radio-immunoassay (RIA) techniques in the detection of HBAg in blood donors and PLC patients in Mozambique.

MATERIALS AND METHODS

Sixty-four serum samples were collected from 35 blood donors and 29 PLC patients. After the addition of a drop of 5% sodium azide solution, they were kept frozen at -20°C until tested.

The CIEP technique was performed according to Pesendorfer *et al.*,⁹ with minor modifications. The antiserum used in this test was obtained from a White patient with haemolytic anaemia of unknown aetiology, who had received more than 60 transfusions. This antiserum gave an identification reaction with reference antiserum kindly supplied by the Institute for Cancer Research of Philadelphia.

Pfizer's HAA Detection Latex Reagent (Lot No. L 12/3), still on trial evaluation and supplied by the manufacturers, was used according to their instructions. The reactions were considered positive when agglutination of latex-sensitised particles became visible within 3 minutes, without any visual aids. In every run negative and positive controls were included, and all doubtful results repeated.

The RIA was performed with the Abbott's Ausria¹⁰ kit of reagents. The cutoff is defined as the product of the mean of the CPM values of negative controls, included in every run, times a 2,1 factor.

Blood samples were obtained from the blood bank of Hospital Miguel Bombarda (Lourenco Marques), and from patients with confirmed diagnoses of PLC.

RESULTS AND DISCUSSION

The results of the samples analysed by the 3 methods are summarised in Table I. The percentage of HBAg positives detected by either of the 3 methods is much higher in the liver cancer group than among the blood donors, which confirms the results found in the literature.⁴

Of 64 samples examined by the 3 methods, the results were coincident in 41 cases, 8 being positive and 33 negative. The 8 coincident cases averaged high CPM values in the RIA method.

However, some of the sera with very high counts in RIA did not react in CIEP.

Considering RIA as one of the most reliable methods now available for detection of HBAg,¹⁰ the ratios CIEP/RIA and LAT/RIA show that CIEP systematically detected fewer positive samples than RIA, while the opposite was observed with LAT. In other words, while CIEP is prone to give a high percentage of false negative results, the LAT technique presently used is biased towards false positive results.

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TABLE I. RESULTS OBTAINED IN 64 SERA SAMPLES ANALYSED FOR HBAG BY 3 DIFFERENT METHODS

Group	No. of samples examined	No. of positive results			Ratio CIEP/RIA	Ratio LAT/RIA
		CIEP	LAT	RIA		
Blood donors	35	4	7	5	1/1,25	1/0,75
PLC patients	29	4	21	18	1/4,50	1/0,86
Both groups	64	8	28	23	1/2,87	1/0,82

The ratio LAT/RIA is more uniform in the 2 groups examined, than CIEP/RIA. It is possible that HBAG detection by CIEP depends, in comparison with LAT and RIA methods, on more stringent conditions such as the source of antiserum and its antibody titre and subtypes, the HBAG subtypes and concentration in the samples to be tested, the technical details involved in the electrophoretical procedure,¹¹ and other unknown factors. The CIEP technique is less suitable for a blood bank service, where there is a need for rapid clearance of the blood units collected.

On the other hand, the LAT method presently tried seemed to give a more constant ratio towards the RIA in both groups. If its margin of error is biased exclusively towards false positives, it could be used safely as a quick preliminary screening test, which would allow the immediate clearance of all negative blood units, while the positive ones could be rechecked by more reliable techniques, such as the RIA. The possibility of false negative results by LAT cannot, however, be excluded with confidence at present.

From the discrepancy found in the ratio CIEP/RIA in the 2 groups examined, it seems that CIEP detects more HBAG positives among the blood donors than in PLC patients, in spite of HBAG titres being higher in PLC patients.

It is known that either RIA or LAT techniques are more sensitive in detecting the *ad* than the *ay* subtypes of HBAG. While the incidence of *ad* and *ay* subtypes of HBAG is more or less the same in acute hepatitis patients, *ad* is much more common in chronic hepatitis and also in HBAG blood donor carriers.¹² Furthermore, it is now being realised that HBAG subtypes tend to follow a different pattern of geographical distribution.¹³ The preliminary results of the analysis carried out in the HBAG positive samples included in the present work did not support a significant difference of their incidence in either of the groups studied.

The discrepancy observed could be due to the fact that prozone occurred more frequently in the PLC patients' sera. However, other mechanisms cannot be excluded. It is known that some factors, like the rheumatoid factor (RF) and complement, may mask the Australia antigen in certain methods.

The higher percentage of positive cases found by LAT in relation to RIA, is commonly attributed to false positive reactions.¹⁰ Some precautions such as, for instance, clotting the test blood samples in the presence of thrombin, the addition of mercapto-ethanol to sera with high RF titres, vigorous shaking of the LAT reagent bottle, elimination of bacteriological contamination of the samples, etc., all

of these, could or have been found to reduce the number of false positive results in the LAT method. These precautions were not taken in the present study.

The 23 cases where the results were not coincident by the 3 methods employed, are summarised in Table II.

TABLE II. TWENTY-THREE SAMPLES WHERE THE RESULTS WERE NOT COINCIDENT BY THE 3 METHODS FOR HBAG DETECTION

RIA*	LAT	CIEP
-(1,3)	+	-
-(1,4)	+	-
-(1,4)	+	-
-(1,5)	+	-
-(1,6)	+	-
-(1,6)	+	-
-(1,9)	+	-
±(2,1)	-	-
+(2,2)	-	-
+(3,7)	-	-
+(7,4)	+	-
+(11,5)	+	-
+(14,7)	+	-
+(15,7)	+	-
+(16,9)	+	-
+(17,1)	+	-
+(19,6)	+	-
+(22,5)	+	-
+(24,2)	+	-
+(25,5)	+	-
+(26,6)	+	-
+(35,7)	+	-
+(37,8)	+	-

* Numbers in brackets are the ratio between the actual CPM values of the blood specimens and the mean CPM of negative control samples. The value of 2,1 defines the threshold of positivity.

All the samples gave systematically negative results in CIEP, thus confirming its lower sensitivity in relation to the other two. Seven samples reacted positively with LAT and negatively with RIA, while 3 serum specimens positive with RIA were negative with LAT. The remaining cases were coincident by LAT and RIA techniques.

The 3 samples RIA positive and LAT negative raise the question of false negatives in the last method. The fact that their CPM values were near the cutoff, i.e. at the threshold of positivity, does not permit a definite conclusion. The electron microscopical examination of these sera could have provided the answer to this question.

CONCLUSION

Summing up, it can be said that the CIEP method, as employed in the present study, is not suitable for routine screening of HBAG among blood donors, and it seems even less sensitive with regard to PLC patients' sera.

The LAT technique, if improved in its specificity, could be a more valuable test for blood bank routine screening of blood donors. Furthermore, if any role by HBAG in the pathogenesis of PLC is confirmed, it would be most desirable to have a quick method for its detection, not only in blood banks but also for field work, particularly in southern Mozambique, where the world's highest incidence of PLC is found²⁷ in a well-defined spatial distribution pattern.²⁸

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