

NON-SPECIFIC AND FALSE-NEGATIVE REACTIONS IN DETECTION OF HBsAg

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Summary. — Variable occurrence of non-specific HBsAg reactions in different materials is described as revealed by tests of different sensitivity. The importance of reliable tests for specific determination of HBsAg is pointed out, especially that of the highly sensitive passive reverse haemagglutination reaction (PRHA). Results comparing confirmatory tests based on animal or human anti-HBs immunoglobulin are presented.

Key words: HBsAg detection; diagnosis; non-specific reaction

Introduction

During the last few years a considerable progress was made in specific determination of the etiological agent of viral hepatitis B (WHO Report 1977). A number of the third generation tests were introduced for the determination of virus structural components (Zuckerman, 1978; Vandervelde, 1978; Burrell *et al.*; 1978; Stanček *et al.*, 1980). In many countries PRHA screening tests are widely used for HBsAg detection in different clinical materials (Ikram and Prince, 1981). Though sensitive, the tests have some disadvantages which have to be born in mind in practical application.

In the present communication we deal with the presence of non-specific reactions and their possible exclusion by means of specific human or animal anti-HBs globulins.

Materials and Methods

Passive reversed haemagglutination (PRHA), enzyme linked immunoassay (ELISA) and radioimmunoanalysis (RIA) tests. Screening and confirmatory tests were carried out according to the instructions of manufacturers. The following kits were used: Hepatest, Wellcome; Cellognost HBsAg, Behring Inst.; Auscell, Abbot Labs.; Sevatest HBsAg, Imuna; Enzygnost HBsAg, Behring Inst.; Ausria II, Abbot Labs. Preparation and application of swine anti-HBs immunoglobulin in testing the specificity of HBsAg is described in Results and Discussion.

Results and Discussion

Occurrence of non-specific reactions in HBsAg detection by third generation methods

With the exception of the RIA test, where HBsAg detection is only seldomly accompanied with non-specific reactions, all other sensitive testing systems

Table 1. Non-specific reactions in HBsAg third generation tests

Results	Detection system				
	Hepatest	Cellognost	Sevatest	Enzygnost	AusRia II
Positive in screenig	15* (18.7 %)*	20 (25.0 %)	17 (21.2 %)	17 (21.2 %)	24 (30.0 %)
Positive in confirmatory test	13 (16.2 %)	15 (18.7 %)	14 (17.5 %)	16 (20.0 %)	24 (30.0 %)
Number of non-specific reactions	2 (2.5 %)	5 (6.2 %)	3 (3.7 %)	1 (1.2 %)	0 (< 1 %)

* — per cent out of total 80 sera checked

Table 2. Relationship between the sensitivity and specificity of different PRHA systems in HBsAg detection

PRHA test	Positive reaction in:		Non-specific reaction
	screening test	confirmatory test	
Auscell	49 (60.5 %)*	39 (48.1 %)*	10 (20.4 %)**
Hepatest	48 (59.2 %)	39 (48.1 %)	9 (18.7 %)
Sevatest I	30 (37.0 %)	30 (37.0 %)	0
Sevatest II	48 (59.2 %)	39 (48.1 %)	9 (18.7 %)

* — per cent out of total 81 tested samples

** — per cent out of positives in screening tests

react to different proportions to non-specific reactive factors present in clinical materials. As shown in Table 1, non-specific haemagglutinins often participate in PRHA reactions, making their results problematic unless proper confirmatory tests are used. In detection of HBsAg by ELISA non-specific reactions occur less frequently and adequate confirmatory tests are necessary in the case of weak positive reactions. As further shown in Table 1 and Table 2, certain differences can be seen even among PRHA tests of different provenience, reflecting the different origin and composition of reactive components in the individual products. The rate of non-specific reactions as revealed by the tests based on neutralization of HBsAg by means of specific antibody, varied from less than 1 % in RIA to 20 % (or more) in the most

Table 3. Non-specific reactions in sera of different origin as detected by PRHA test

Origin of serum	Positive reaction in:		Non-specific reaction	Total
	screening test	confirmatory test		
Patients with viral hepatitis B	300 (90.9 %)*	280 (84.8 %)*	20 (6.7 %)**	330
Patients with undifferentiated viral hepatitis	340 (41.9 %)	270 (33.3 %)	70 (20.6 %)	810
Patients with undifferentiated hepatopathy	120 (14.9 %)	91 (11.3 %)	29 (24.2 %)	805
Umbilical cord blood sera	21 (2.6 %)	1 (0.12 %)	20 (95.2 %)	825

* — per cent out of total serum samples tested

** — per cent out of total positives in screening test

Table 4. Comparison of animal and human anti-HBs globulins in PRHA confirmatory tests

Type of reaction	Antiserum used in confirmatory test:	
	swine anti-HBs	human anti-HBs
Non-specific HBsAg reactions (not reactive with anti-HBs)	58* (28.3 %)	58* (28.3 %)
Specific HBsAg reactions (reactive with anti-HBs)	147 (71.7 %)	147 (71.7 %)

* — identical serum samples tested in both groups

sensitive of PRHA. As demonstrated by Sevatest I (less sensitive preparation) and Sevatest II (more sensitive preparation) there is an indirect relationship between the sensitivity of the tests and their specificity.

Striking differences can be seen among the number of non-specific reactions when sera of different groups of patients are checked for HBsAg presence by PRHA. Table 3 shows the ratio of HBsAg false-positives to HBsAg true-positives could be as high as 20 : 1. On the other hand, the ratio of non-specific to HBsAg specific reactions decreased with the increasing number of sera coming from patients suffering of viral hepatitis B.

Confirmatory test based on application of animal anti-HBs immunoglobulin

The most commercially available confirmatory tests so far use human anti-HBs globulin or human sera containing antibody against HBsAg. An exception is the confirmatory test developed by Ortho Diagnostic System, USA (Raphadex) which uses chimpanzee anti-HBs globulin. Modification of our

Table 5. Pro-zone phenomenon in PRHA reactions

Serum dilution	Hepatest	Sevatest HBsAg	Auscell
1 : 8	±	—	—
1 : 16	+	±	—
1 : 32	+	+	±
1 : 64	+	+	+
1 : 128	+	+	+
1 : 256	+	+	+
1 : 512	+	+	+
1 : 1024	+	+	+
1 : 2048	—	±	+
1 : 4096	—	—	—
Titre of PRHA inhibitor	8	16	32
Titre of HBsAg	1024	1024— —2048	2048

own confirmatory test is based on anti-HBs swine immunoglobulin. Both — the satisfactorily purified HBsAg used for immunization, as well as the refractionated, purified antiHBs globulin — were treated with powdered human plasma before use. By this procedure, the formation and presence of antibody against non-specific haemagglutinins, which would cause false-neutralization effects in the confirmatory tests, is diminished to minimum. This can be further improved by a dilution of anti-HBs globulin to a desirable level before use in the confirmatory test. Table 4 shows the results of parallel checking of swine and human anti-HBs globulin in PRHA. Several PRHA (Hepatest, Cellognost, Sevatest) or ELISA (Enzygnost) test systems were checked in this manner with similar results. All tested sera, positive either in a specific or non-specific manner, reacted equally regardless to the origin of antiserum. This proved the applicability of properly prepared animal anti-HBs globulin in determination of HBsAg specificity.

False-negative reactions occurring during screening of HBsAg by PRHA

Besides non-specific positive reactions also false-negative results may be obtained in screening of HBsAg by PRHA systems. "Pro-zone phenomenon" could sometimes be seen with sera from different patients. As demonstrated in Table 5, the particular serum containing an inhibitor of haemagglutination would appear as HBsAg negative in screening procedures commonly using undiluted or low diluted (1 : 4; 1 : 8) serum samples. The "anti-haemagglutinin factor" could be present up to the dilution 1:64 and its level depends on the PRHA system. To avoid such underisable reactions means either to use other than PRHA screening system or to check several dilutions of serum samples for HBsAg activity in PRHA reaction.

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