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New Principle for the Simultaneous Detection of Total and Immunoglobulin M Antibodies Applied to the Measurement of Antibody to Hepatitis B Core Antigen

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A new test principle for the simultaneous detection of total approximate titers and immunoglobulin M antibodies has been developed and applied to the detection of antibody to hepatitis B core antigen. The method is based on the combination of a competition radioimmunoassay, for the determination of total antibody titer, with an indirect enzyme-linked immunosorbent assay for the determination of single class antibodies. The interference of the rheumatoid factor was avoided by including heat-aggregated immunoglobulin G in the dilution buffer. The specificity, sensitivity, and clinical application of the test are discussed. The results presented suggest that the simultaneous detection of total and immunoglobulin M antibody to hepatitis B core antigen might be helpful in the differentiation between previous and recent or ongoing hepatitis B infection, as well as in the differential diagnosis of acute hepatitis, in monitoring viral activity in chronic infections, and in helping to differentiate acute from chronic infections. The test principle appears applicable in the accurate diagnosis of other infectious diseases by a single test on only one serum sample.

The demonstration of increasing titers of total antibodies, immunoglobulin M (IgM) class antibody, or both is conventionally accepted for the laboratory diagnosis of most viral infections.

In hepatitis B virus (HBV) infections, antibody to hepatitis B core antigen (anti-HBc) is a recognized marker of actual or past infection. In fact, in acute infections, anti-HBc of the IgM class appears early, reaches a peak in the first month of the disease, and then gradually shifts to the IgG class, which can be detected in slowly decreasing titers for several years, probably lifelong if sensitive techniques are employed (14). Chronically infected individuals usually have very high titers of total anti-HBc, and the titer seems to be correlated with the viral replication and the activity of the disease (6, 10). In such subjects, antibodies of the IgM class are often absent or present in very low titers (9). Determination of anti-HBc has been extensively employed in epidemiological studies (13) and is demonstrated to be a more sensitive indicator than antibody to hepatitis B surface antigen (anti-HBs) of past HBV infection (5).

The determination of anti-HBc titer, the IgM class, or both seems to be essential for an accurate diagnosis in many clinical situations. In fact, a misdiagnosis of acute type B hepatitis might be made in chronic HBs antigen (HBsAg) carriers with hepatitis caused by many noninfectious agents and at least three other infectious agents (hepatitis A virus [HAV], δ, and non-A, non-B agents). It is also well known that cases exist of acute hepatitis B which are HBsAg negative by the available test methods, and in some of these cases, anti-HBs can be detected early and simulates a preexisting immunity to HBV. Furthermore, a patient with chronic active hepatitis can have an acute exacerbation of this disease and can initially be said to have an acute HBV hepatitis. In addition, high-titered or IgM anti-HBc-positive donors have been implicated in cases of posttransfusional hepatitis B (11), and screening of blood donors for anti-HBc has been proposed (12).

The commercially available competitive radioimmunoassay (CORAB; Abbott Laboratories, North Chicago, Ill.) is extremely specific and sensitive for the detection of total anti-HBc, but it cannot distinguish among antibodies of different immunoglobulin classes. Therefore, many methods to individuate an IgM response have been proposed. They are generally expensive and time consuming because they require a preliminary step to separate the different immunoglobulins by rate zonal ultracentrifugation (3), absorption of IgG by staphylococcal protein A (2), or IgM cleavage by 2-mercaptoethanol (2-ME) (15).

The recently developed reverse immunoassay, which uses an anti-human IgM as capture antibody (8), is very sensitive, but it requires a purified core antigen preparation and can only recognize the presence of IgM antibody; therefore, an additional test is required to demonstrate the presence of other immunoglobulin classes or total antibodies.

In this work, we describe a modification of the CORAB assay for the determination, in a single test, of both total approximate titer and IgM anti-HBc by a very simple, reproducible, and inexpensive two-step method.

MATERIALS AND METHODS

Serum specimens. A total of 192 sera from 99 subjects were studied. Sera from patients and controls were divided into 13 groups. Group 1 included 89 sequential sera from 13 patients with typical acute hepatitis B; group 2 included 22 sequential sera from 5 patients with hepatitis B who were HBsAg negative at the clinical onset. Groups 3, 4, and 5 included 18 sera taken at the clinical onset from 5 patients with hepatitis A, 10 with posttransfusional non-A, non-B hepatitis, and 3 with infectious mononucleosis, respectively. Groups 6, 7, 8, and 9 included 38 sera from chronic HBsAg carriers: 9 healthy, 10 with chronic persistent hepatitis, 7 with chronic active hepatitis, and 12 with inactive liver cirrhosis, respectively. Groups 10 and 11 included three sera from patients

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with acute exacerbation of a chronic type B active hepatitis without δ infection and two sera from chronic carriers with acute δ superinfection, respectively. Ten additional sera from healthy donors (group 12), and 10 sera positive at high titer for the rheumatoid factor (group 13) were also tested.

Groups 1 to 11 were patients admitted to the Institute of Infectious Diseases, University of Bari, Italy, between October 1979 and October 1982 as in- or outpatients. Group 12 included clinic and laboratory workers, and group 13 included sera kindly tested and provided by G. Lapadula (Rheumatology Division of the I Medical Clinic, University of Bari, Italy).

Finally, 60 additional sera from chronic HBsAg carriers with unspecified liver diseases were part of an anti-HBc panel of the Institute of Infectious Diseases and were used only to plot the standard curve for total anti-HBc.

All serum specimens were stored at −20°C until tested.

Diagnostic criteria. A patient was regarded as having acute hepatitis B if HBsAg was present in acute-phase sera but was absent in the convalescent phase of the illness (group 1), or in the cases negative for HBsAg (group 2), if a seroconversion or a significant increase in the anti-HBs titer was demonstrated between the acute and the convalescent phase of the disease.

A diagnosis of hepatitis A was made if HAV was detected in fecal specimens collected early in the illness, if anti-HAV of the IgM class was detectable in acute-phase sera, or both.

Patients with posttransfusional hepatitis without serological evidence of infection with HAV, HBV, Epstein-Barr virus, or cytomegalovirus were considered to have non-A, non-B hepatitis.

Infectious mononucleosis was diagnosed by the positivity of the Paul-Bunnel reaction.

All HBV chronic carriers were biopsy classified by the method of De Groote et al. (4) and were selected for the study only if they were followed for at least 24 months.

The diagnosis of δ-agent superinfection in chronic carriers was made by the demonstration of a seroconversion for anti-δ antibody.

RIELISA. The basic principle of the radioimmune enzyme-linked immunosorbent assay (RIELISA) is the combination of a competitive immunoassay for the measurement of total antibodies with an indirect immunoassay for the evaluation of the immunoglobulin class of the antibodies.

Competitive immunoassay and indirect immunoassay are two methods which have been extensively used to quantify specific antibodies. In both methods, antigen is absorbed on a solid phase, usually a plastic surface, but in the former specific antibodies compete with a fixed amount of labeled antibody, whereas in the latter antibodies eventually present in the test serum are allowed to bind to the immobilized antigen before being recognized through a labeled anti-immunoglobulin.

The competitive immunoassay cannot distinguish which class of immunoglobulin competes with the labeled antibody, but it is very specific and sensitive to determine the presence and the titer of total antibodies. On the contrary, the indirect immunoassay can recognize a single class of immunoglobulin, and therefore it is less efficient for the quantification of total antibody titers.

When positive sera are tested in a competitive immunoassay, the prevalent antibody bound to the antigen on the solid phase will be from the test serum, and therefore it is possible to anticipate an additional step that, using a labeled antiserum to a single human immunoglobulin, can recognize a specific class of antibody attached to the antigen on the solid phase. The only crucial point is that the label of the antigen-specific antibody must be different and not interfere with that of the immunoglobulin-specific class antibody.

Total anti-HBc measurement. The CORAB test is a competition radioimmunoassay in which specific antibody in the test serum competes with a fixed amount of ^{125}I-labeled anti-HBc to bind to the antigen fixed on a polystyrene bead. Therefore, the level of anti-HBc in the test serum is inversely related to the radioactivity on the bead. We expressed the results as a percentage of inhibition (INH) of the radioactive binding calculated according to the formula: percent INH = $[(\bar{N} - S)/(\bar{N} - \bar{P})] \times 100$, where $\bar{N}$ and $\bar{P}$ were, respectively, the mean counts per minute of the negative and positive controls, and S was the counts per minute of the sample.

As in other immunoassays, the CORAB test dose-response curve has an S shape, therefore only the linear tract of the curve may be utilized to quantify the results. In preliminary experiments, we were able to demonstrate that when testing sera at a dilution of 1:500 the correlation between the percent INH and the endpoint titer fell in the linear tract of the dose-response curve in almost all anti-HBc-positive sera tested. Then, using the commercial kit CORAB, we assessed the correlation between the percent INH at a dilution of 1:500 and the endpoint titer in a panel of HBsAg and anti-HBc-positive sera. Sixty low-, medium-, and high-titered sera from chronic carriers with unspecified liver diseases were tested for the endpoint titer, which was considered as the last dilution giving 50% or higher INH. The results were used to produce a standard curve.

Both sera for the standard curve and test sera were diluted in 0.01 M phosphate-buffered saline (pH 7.4) containing 0.05% (vol/vol) Tween 20 and 0.1% (wt/vol) bovine serum albumin (PBS-T-A). Both merthiolate (as preservative) and anti-HBc-negative heat-aggregated IgG (63°C for 15 min to avoid rheumatoid factor interference [8]) were added to give final concentrations of 1:10,000 and 1 mg/ml, respectively. Each dilution of sera for the standard curve or the 1:500 dilution of test sera were tested by the CORAB procedure, with the only modification being that washings were made with PBS-T-A.

IgM anti-HBc determination. The presence of IgM anti-HBc was determined in test sera by an indirect ELISA step added to the competition radioimmune assay step described above.

After reading bound radioactivity for total antibody measurement, the polystyrene beads were again transferred to the respective wells, and 200 μl of peroxidase-conjugated goat anti-human IgM (Sigma Chemical Co., St. Louis, Mo.) diluted 1:1,000 in PBS-T-A was added to each well. After 2 h of incubation, each well was washed with 10 ml of PBS-T-A, the beads were transferred to ELISA tubes (Abbott), and 300 μl of *ortho*-phenylendiamine (0.4 mg/ml) in citrate buffer (pH 5) containing 0.006% peroxide was added to each tube. The enzyme-substrate reaction was carried out in the dark for 30 min and was stopped by adding 1 ml of 1 N HCl. Optical density (OD) was measured in a Quantum I (Abbott) spectrophotometer equipped with a 492- to 600-nm filter.

The results of the indirect step of RIELISA were expressed as the $S/\bar{N}$ ratio, where S was the OD of the sample and $\bar{N}$ was the mean OD of six IgM anti-HBc-negative control sera (three preinfection and three taken more than 2 years after a recovered acute hepatitis B) tested at the same dilution and in the same way for total anti-HBc as test sera.

A background was always present, and therefore in each run test serum and labeled anti-HBc were substituted by 200 μl of PBS-T-A to treat two polystyrene beads. The resulting

TABLE 1. Total and IgM anti-HBc levels in sera from different groups of subjects

Group	Serum	Total anti-HBc[a]	IgM anti-HBc[b]
1	Acute hepatitis B HBsAg positive[c]	65 ± 13 (100)[d]	5.7 ± 1.8 (100)[d]
2	Acute hepatitis B HBsAg negative[c]	60 ± 7 (100)	5.6 ± 2.0 (100)
3	Acute hepatitis A[c]	9 ± 6 (0)	1.5 ± 0.4 (0)
4	Acute hepatitis non-A, non-B[c]	13 ± 8 (10)	1.2 ± 0.5 (0)
5	Infectious mononucleosis[c]	4 ± 8 (0)	1.3 ± 0.4 (0)
6	Healthy HBsAg carriers	89 ± 8 (100)	1.2 ± 0.7 (11)
7	Persistent hepatitis	93 ± 3 (100)	1.6 ± 0.7 (20)
8	Active hepatitis	92 ± 4 (100)	2.4 ± 1.8 (57)
9	Liver cirrhosis	64 ± 30 (100)	2.3 ± 1.5 (42)
10	Exacerbation of active hepatitis[c]	96 ± 1 (100)	2.4 ± 2.0 (67)
11	δ-Agent infection in chronic carriers[c]	61 ± 11 (100)	0.3 ± 0.1 (0)
12	Normal controls	37 ± 24 (50)	0.7 ± 0.5 (0)
13	Rheumatoid factor positive controls	56 ± 18 (70)	1.3 ± 0.6 (0)

[a] Values represent the mean and standard deviation of individual percent INH of radioactivity binding.

[b] Values represent the mean and standard deviation of the $S/\bar{N}$ ratio of the OD at 492 nm.

[c] Sera at the clinical onset.

[d] Values in parentheses represent the percentage of positives.

background was subtracted from the OD measured in test sera and controls before the calculation of the $S/\bar{N}$ ratio.

Since the standard deviation of the negative controls was always extremely low, an $S/\bar{N}$ ratio of ≥2.1 was arbitrarily used to signify the lowest level at which anti-HBc IgM was considered present.

Specificity tests. To test specificity, acute and late-convalescent sera from four acute hepatitis B (group 1) were treated with 2-ME (0.2 M final concentration) and tested for total and IgM anti-HBc; the results were compared with the untreated sera tested in the same run. In addition, a blocking step was inserted, before adding peroxidase-conjugated anti-human IgM, to incubate the beads for 1 h at 37°C with rabbit anti-human IgM (Dako, Copenhagen, Denmark) and alternatively with anti-human IgG or IgA (Behring, Scoppito, Italy) diluted 1:100 in PBS-T-A. Finally, the same sera were tested, using CORAB beads pretreated overnight with an IgG fraction purified from an anti-HBc high-titered serum by DEAE-cellulose chromatography.

Serological tests other than anti-HBc. HBsAg, anti-HBs, HBeAg, anti-HBe, and total and IgM anti-HAV were detected by commercially available methods (Abbott). HAV in feces were detected by radioimmunoassay as described previously (1). The Paul-Bunnel reaction and the complement fixation test were performed by using standard techniques. Radioimmunoassay for anti-δ antibody (16) was kindly provided by M. Rizzetto (Turin, Italy).

RESULTS

The results of both total and IgM anti-HBc as determined by RIELISA in all of the 13 groups of subjects are summarized in Table 1.

Standard curve for total anti-HBc measurement. The standard curve, using sera from the 60 HBsAg chronic carriers (Fig. 1), demonstrates that the correlation between the percent INH at a dilution of 1:500 and the endpoint titer was linear in the endpoint range from 1:250 to 1:8,000.

By using this method, it was possible to fractionate the anti-HBc-positive subjects into three categories according to anti-HBc titer: low (endpoint titer of 1:500 or less, which corresponds to a percent INH at 1:500 of 32 to 58%); medium (endpoint of 1:1,000 to 1:8,000 and percent INH at 1:500 of 59 to 88%); and high (endpoint of 1:16,000 or more and percent INH at 1:500 of 89% or more) (Fig. 1).

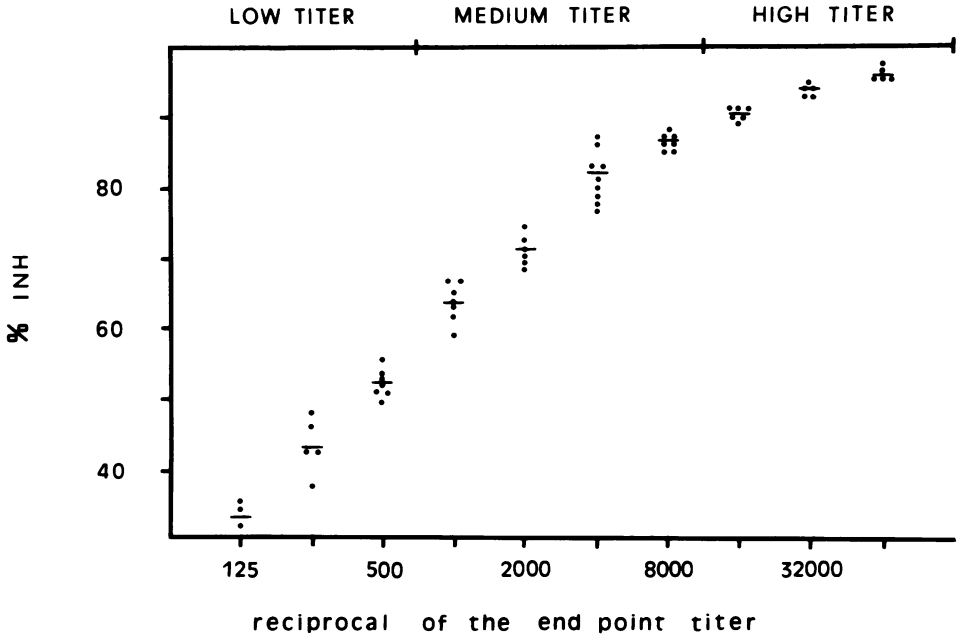

FIG. 1. Calculation of total anti-HBc approximate titer by correlation between the percent INH of radioactivity binding at 1:500 dilution and the endpoint titer in sera from 60 HBsAg chronic carriers. Individual values (●) and mean (horizontal bars) for each endpoint are shown.

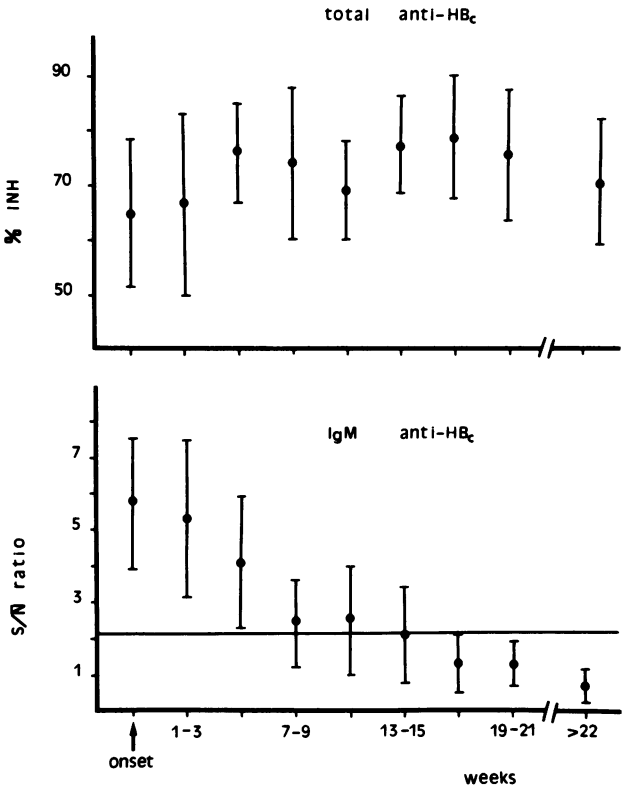

FIG. 2. Pattern of total and IgM anti-HBc levels in sequential sera collected from the 13 patients with HBsAg-positive acute hepatitis B. The mean and the standard deviation of the individual percent INH of radioactivity binding (total anti-HBc) and of the individual $S/\bar{N}$ ratio of the OD at 492 nm (IgM anti-HBc) are given for each group of sera collected at 3-week intervals from the clinical onset of the hepatitis.

The results were highly reproducible, also using different CORAB lots, with an intraassay and interassay variation, expressed as percent INH, of 1.9 and 2.2%, respectively. This variation never determined the dropping of a case from one titer category to another.

Testing three or six sera with known low, medium, and high titer in each run was enough to verify the interassay variation and made unnecessary the calculation of the standard curve each time.

Total anti-HBc titers in acute and chronic HBV infections. In acute HBV infections the anti-HBc titers were low or medium in all cases, and this lasted for the entire course of the disease. The behavior of total anti-HBc in the course of acute hepatitis B is shown in Fig. 2. HBsAg-negative acute hepatitis B usually had a lower total anti-HBc titer than HBsAg positive (Tables 1 and 2).

Interestingly, the level of the total anti-HBc response seemed to be correlated with individual factors; in fact, patients with low anti-HBc titer usually continued to have a low anti-HBc response during the entire course of the disease (data not shown).

In chronic HBV infections, low anti-HBc titers were frequent only in liver cirrhosis and were rare in healthy carriers or in chronic persistent and active hepatitis (Table 2). Although there was not a characteristic titer for a given pathology, the frequence of high anti-HBc titers increased with the activity of the disease, up to 100% in the exacerbation of chronic active hepatitis (Table 2).

TABLE 2. Distribution of total anti-HBc titers in acute and chronic HBV infections as determined in the competitive step of RIELISA

Serum	Total anti-HBc titers (%)			Total no. of cases
	Low	Medium	High	
Acute hepatitis HBsAg positive	3 (23)	10 (77)	0 (0)	13
Acute hepatitis HBsAg negative	2 (40)	3 (60)	0 (0)	5
Healthy carriers	0 (0)	5 (55)	4 (45)	9
Persistent hepatitis	0 (0)	3 (30)	7 (70)	10
Active hepatitis	0 (0)	1 (14)	6 (86)	7
Liver cirrhosis	6 (50)	1 (8)	5 (42)	12
Exacerbation of active	0 (0)	0 (0)	3 (100)	3
δ-Agent superinfection hepatitis	1 (50)	1 (50)	0 (0)	2

IgM anti-HBc determination. (i) Background. Treating CORAB beads with peroxidase-conjugated anti-human IgM, a background reactivity, constant for each lot, was always registered. The pretreatment of the beads with unlabeled anti-human IgM, but not anti-human IgG or IgA, eliminated almost completely the background, indicating the presence of IgM on the beads (Table 3). The human derivation of the hepatitis B core antigen used to coat the beads and the intended use of the CORAB kit not directed to IgM determination made this finding not surprising. According to this view, different lots of beads gave different backgrounds. Pretreatment of the beads with 0.2 M 2-ME lowered the background 20 to 30% and lowered, but did not eliminate, lot-to-lot differences.

Beads pretreated with unlabeled anti-human IgM gave higher background reactivities when used to test human sera, probably because of the binding of nonspecific IgM contained in the test serum which allowed subsequent binding of conjugated anti-IgM.

The mean background of ten different lots of CORAB beads was 0.157 (OD), and the simplest way to eliminate lot-to-lot differences was to subtract the background as described above.

No interference was demonstrated with different lots of iodinated anti-HBc.

(ii) Interference of the rheumatoid factor. In preliminary experiments with only PBS-T-A as sample diluent, false-positive IgM results were obtained in 6 of 10 rheumatoid factor-positive sera. These false-positive results were abolished by including anti-HBc-negative heat-aggreated IgG in the diluent.

(iii) Specificity tests. Acute-phase sera (positive controls) and late-convalescent sera (negative controls) from four cases of acute resolved hepatitis B (group 1) were tested for

TABLE 3. Backgrounds obtained by different types of treatment of CORAB beads (not exposed to human serum) from 10 different lots

Treatment	Background (mean ± SD)
None	0.157 ± 0.037
Anti-human IgG	0.161 ± 0.031
Anti-human IgA	0.158 ± 0.033
Anti-human IgM	0.056 ± 0.010
2-ME	0.115 ± 0.023

specificity. The IgM anti-HBc reactivity was completely abolished by treating sera with 2-ME or by presaturating the solid phase by overnight preincubation with IgG anti-HBc (Table 4). Furthermore, the introduction of a blocking step with anti-human IgG or IgA did not affect the results, whereas with anti-human IgM, the IgM anti-HBc reactivity was again completely abolished.

IgM anti-HBc in acute hepatitis. IgM anti-HBc was detectable in acute-phase sera from all acute hepatitis B, both HBsAg positive or negative, whereas they were absent in acute hepatitis A, non-A, non-B, or infectious mononucleosis (Table 1).

The IgM response was detectable for 2 to 4 months without differences between HBsAg-positive or -negative acute hepatitis B. In all cases, IgM anti-HBc was detectable after the clearance of the HBsAg.

The levels of the IgM anti-HBc, as indicated by the $S/\bar{N}$ ratio, were usually higher in acute than in chronic infections (Table 1).

IgM anti-HBc in chronic HBV infections. IgM anti-HBc was detectable in 30% of all chronic HBV infections. The lower number of positive cases was in healthy carriers (group 6); the higher number was in the exacerbation of chronic active hepatitis (group 10) (Table 1). The number of IgM anti-HBc-positive subjects for each group increased according to the activity of the disease but was not correlated with the liver damage, as indicated by a lower prevalence in liver cirrhosis than in chronic active hepatitis. In the two cases of δ-agent superinfection, IgM anti-HBc was not detectable.

The levels of IgM anti-HBc in chronic infections were lower than at the onset of acute heptatitis B, but the difference was not statistically significant ($P > 0.05$).

According to the observations of Roggendorf et al. (17), IgM anti-HBc was more frequently associated with high total anti-HBc titers. Only in the liver cirrhosis group were IgM-positive cases associated with low total antibody titers (Table 5).

DISCUSSION

The difficulty in obtaining a purified core antigen preparation and the high cost of the commercially available tests have prevented widespread application to the current clinical practice of the total and IgM anti-HBc determinations, despite the central role of such determinations for monitoring chronic infections as well as for an accurate diagnosis of acute cases.

The method proposed here has the major advantage of detecting in a single test both total approximate titer and IgM-specific antibody.

TABLE 4. Results of IgM anti-HBc specificity tests in acute and late-convalescence sera from four acute hepatitis B (group 1)

Treatment	OD at 492 nm[a] (mean ± SD)	
	Acute sera	Convalescent sera
Untreated	0.438 ± 0.063	0.162 ± 0.014
2-ME treated	0.116 ± 0.065	0.098 ± 0.009
Preincubation with IgG anti-HBc	0.175 ± 0.008	0.156 ± 0.007
Blocking with anti-human		
IgG	0.419 ± 0.050	0.185 ± 0.003
IgA	0.392 ± 0.032	0.159 ± 0.003
IgM	0.067 ± 0.048	0.020 ± 0.002

[a] Background (0.143) not subtracted.

TABLE 5. Correlation between IgM and total anti-HBc titers in chronic HBV infections

Serum	No. of IgM anti-HBc positive	Total anti-HBc titers		
		Low	Medium	High
Healthy carriers	1		1	
Persistent hepatitis	2			2
Active hepatitis	4			4
Liver cirrhosis	5	2	1	2
Exacerbation of active hepatitis	2			2
δ-Agent superinfection	0			

Total anti-HBc endpoint titers, either determined in the competitive step of RIELISA or by the standard CORAB procedure, are usually very high in HBV-infected subjects and have a marked experimental error. Therefore, exact titer determinations would require an average of many measurements, which is not practical for routine clinical work. Furthermore, an exact endpoint titer determination does not seem to be essential for clinical considerations (7).

In the present study, the patients were efficiently classified into three principal categories according to the approximately total anti-HBc titer: low, medium, or high. Although the determination of total anti-HBc performed with the 1:500 dilution alone does not correspond to an exact endpoint titer, the relatively low intraassay-interassay variation gave a sufficient approximation to avoid interchange between the three principal titer categories.

When sera from acute hepatitis were tested, total anti-HBc titers were always low or medium, whereas the prevalence of high titers rose from 45% in healthy carriers, up to 100% in the exacerbation of chronic active hepatitis group, and fell again to 42% in inactive liver cirrhosis. This demonstrates that the amount of anti-HBc response was correlated with the activity of the disease but not with the gravity of the hepatic damage.

IgM anti-HBc, as determined in the indirect step of RIELISA, was highly specific, and rheumatoid factor interference was obviated by including heat-aggregated IgG in the dilution buffer.

IgM anti-HBc was detectable in all acute-phase sera from hepatitis B, but not in the late convalescence nor in acute hepatitis A, non-A, non-B, or infectious mononucleosis. In addition, they represent the principal diagnostic marker in HBsAg-negative acute hepatitis B. Serial determinations in acute hepatitis demonstrate that peak values were reached in the first month of the disease, and the time course of the detectability was 2 to 4 months from the clinical onset and lasted after the HBsAg clearance in all cases.

IgM anti-HBc was also detected in 30% of all chronically HBV-infected subjects, and as with total anti-HBc, the prevalence of positivity increased according to the activity of the disease.

IgM anti-HBc levels, expressed as $S/\bar{N}$ ratios, were higher in acute than in chronic infections, but this difference did not reach statistical significance.

RIELISA seems to be less sensitive in determining IgM anti-HBc as compared with the recently developed reverse immunoassay, as demonstrated by the shorter detectability in acute hepatitis (4 months versus up to 6 years) (18) and by the lower prevalence of positive cases in chronic infections (30% versus more than 80%) (9). Because only the higher IgM anti-HBc concentration can be detected by RIELISA, the lower sensitivity may represent an advantage helping in

the rapid differentiation between acute and chronic HBV infections. Certainly, the positivity of IgM anti-HBc, also in some chronically infected subjects, affects its diagnostic potential, but the contemporary determination of total anti-HBc titer allows discrimination in most clinical situations. Clinical features in acute hepatitis are usually easily distinguishable from healthy carriers, chronic persistent hepatitis, or liver cirrhosis, whereas some diagnostic problems may exist in the differentiation of an acute hepatitis from an exacerbation of a chronic active hepatitis. In the latter, total anti-HBc titers are usually very high, in contrast with acute hepatitis in which they are low or medium.

δ-Agent superinfection in chronic carriers seems to inhibit HBV replication, and therefore probably the IgM anti-HBc response would be lowered. In the two cases studied here, the absence of an IgM anti-HBc response allows us to exclude an acute hepatitis B, and the unincreasing titer of the total anti-HBc helps to differentiate it from an exacerbation of a chronic active hepatitis independently from the detection of anti-δ antibody.

In summary, the method proposed here provides a rapid and reliable diagnosis of current or recent HBV infections and can be applied to blood donor screening as well as to the differential diagnosis of acute hepatitis; furthermore, it is useful in monitoring viral activity in chronic infections and helps to differentiate acute from chronic infections.

In the near future, the application of this principle to other systems may become extremely useful for rapidly diagnosing most viral infections.

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