

## Performance Characteristics of Microparticle Enzyme and Chemiluminescence Immunoassays for Measurement of Anti-HBc Immunoglobulin M in Sera of Patients with HBeAg-Negative Chronic Hepatitis B Virus Infection<sup>∇</sup>

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**The IMx, AxSym, and Architect immunoglobulin M anti-HBc assay systems for detecting hepatitis B virus e antigen-negative chronic hepatitis B virus infection were compared. Despite good intra- and interassay coefficients of variation, significantly different values and low correlation (overestimation by AxSym and underestimation by Architect) were observed. Association and cutoff values for distinguishing patients with viral replication should be established for all methods.**

In patients with hepatitis B virus e antigen (HBeAg)-negative chronic hepatitis B virus (HBV) infection, absolute levels and changing titers of immunoglobulin M antibodies against the HBV core protein (anti-HBc IgM) are considered markers of viral replication (11, 13, 17). The measurement of anti-HBc IgM is an easily performed and inexpensive alternative to frequent serum HBV DNA measurements for monitoring untreated patients, as well as patients treated with alpha interferon or antiviral agents (1, 5, 11–13, 15–18, 20).

Most experience in the detection of anti-HBc IgM in patients with chronic hepatitis B (CHB) is based on microparticle enzyme immunoassays (MEIA) using the IMx analyzer. Most anti-HBc IgM values for patients with HBeAg-negative chronic HBV infection are below the cutoff limit for a diagnosis of acute hepatitis B, either in the negative or in the “gray” zone. When determined by a classic enzyme-linked immunosorbent assay, they range from 20 to 600 U/ml (10), and when tested with a MEIA (the IMx system), the results range from index values of 0.2 to 2.0, approximately 10 to 300 U/ml (1, 7).

In most laboratories, the IMx analyzer has been replaced by the AxSym analyzer (MEIA), and newer analyzers with chemiluminescent assays (CMIA) are gradually being instituted (4, 6, 21). We compared the anti-HBc IgM measurements by the three analyzers for patients with HBeAg-negative chronic HBV infection.

The IMx (MEIA), AxSym (MEIA), and Architect (CMIA) analyzers (Abbott Laboratories, Abbott Park, IL) were used for anti-HBc IgM quantification. The results of the MEIA are expressed as index values (ratio of sample to calibrator) (9),

and the results of the CMIA are in relative light units divided by the cutoff value determined in the preceding calibration (21).

For the evaluation of the inter- and intra-assay variation (coefficient of variation [CV]), we used multiple aliquots of sera from three patients with HBeAg-negative CHB (patients P1 to P3). Each serum sample was tested five times in the same assay and five times over five days with each of the three analyzers. Anti-HBc IgM measurements for 53 consecutive patients with HBeAg-negative chronic HBV infection were compared. The influence of rheumatoid factor (RF) measured by nephelometry (Image immunochemistry systems; Beckman Coulter, Inc., Fullerton, CA) was assessed.

For data entry, frequencies, and comparisons, SPSS version 14.0 (SPSS, Inc., Chicago, IL) was used. The *t* test and the Mann-Whitney test were applied for parametric and nonparametric data comparisons, respectively. For paired comparisons, the Friedman test or the Wilcoxon signed-rank test was used, as appropriate. A two-tailed *P* value of <0.05 was considered statistically significant. The inter- and intra-assay variations were expressed as the mean  $\pm$  1 standard deviation, and the %CV was calculated. All other measurements were reported as medians (interquartile range) and compared by nonparametric procedures.

The intra-assay %CV was 1.7 to 11.7% (mean, 5.2%) for IMx, 0.8 to 3.6% (mean, 2.4%) for AxSym, and 2.4 to 4.2% (mean, 3.5%) for Architect. The interassay %CV was 7.2 to 8.8% (mean, 8.1%) for IMx, 3.6 to 8.6% (mean, 5.3%) for AxSym, and 3.9 to 9.7% (mean, 7.6%) for Architect. The mean anti-HBc IgM values in the replicate measurements were significantly different (*P* < 0.001). With IMx as the reference, AxSym overestimated by 2.8%, and Architect underestimated by 34.2% (Table 1).

The median anti-HBc IgM values for the 53 patients were 0.224 (0.107 to 0.574) by IMx, 0.25 (0.07 to 0.7) by AxSym, and 0.135 (0.04 to 0.69) by Architect, the differences being highly

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TABLE 1. Anti-HBc IgM assay characteristics of the IMx, AxSYM (MEIA), and Architect (CMIA) analyzers<sup>a</sup>

Assay and patient	Interassay results		Intra-assay results	
	Mean assay index score ( $n^b = 5$ )	% CV	Mean assay index score ( $n^b = 5$ )	% CV
IMx				
P1	0.629	7.2	0.616	1.7
P2	0.592	8.9	0.596	11.7
P3	1.039	8.3	1.011	2.3
AxSYM				
P1	0.590	8.6	0.562	3.6
P2	0.627	3.6	0.612	2.7
P3	1.149	8.1	1.116	0.8
Architect				
P1	0.473	9.7	0.474	2.4
P2	0.386	3.9	0.392	4.2
P3	0.628	9.2	0.618	3.9

<sup>a</sup> The inter- and intra-assay variations in results for serum IgM anti-HBc measurements are shown.

<sup>b</sup>  $n$ , number of determinations.

significant ( $P < 0.001$ ). Compared to IMx, AxSym overestimated the anti-HBc IgM by 21%, and Architect underestimated it by 26%. Among the 20 patients with IMx values below the 0.2 cutoff for quantification, 2 (10%) and 5 (25%) had values higher than 0.2 by AxSym and Architect, respectively. Of the 33 patients with anti-HBc IgM values of  $>0.2$  by IMx, 16 (52%) and 3 (10%) had values of  $<0.2$  by Architect and AxSym, respectively. The correlation coefficient ( $r$ ) between IMx and AxSym was 0.78, and that between IMx and Architect was 0.71 (Fig. 1).

Among 28 samples with anti-HBc IgM values of  $>0.2$  by IMx, 14 were positive for RF at  $>20$  IU/ml (range, 20 to 566

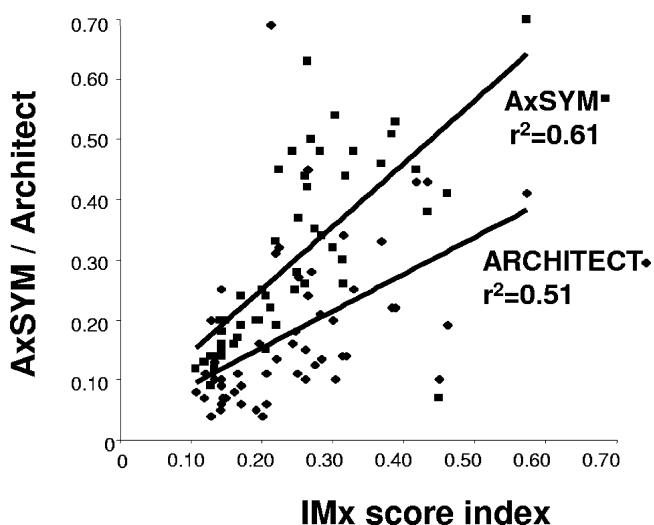


FIG. 1. Anti-HBc IgM measurements for 53 HBsAg-positive, HBeAg-negative patients by AxSym and Architect in reference to measurements by the IMx analyzer. There is low positive correlation between the assays, and the paired differences are significantly different.  $r$ , correlation coefficient.

IU/ml; mean, 65.65 IU/ml). No positive correlation was observed between RF and anti-HBc IgM measurements.

Although commercially available anti-HBc IgM assays have been developed and marketed for the diagnosis of acute HBV infection (9, 19), anti-HBc IgM titers are used for the differentiation between “inactive HBsAg carriers” and patients with HBeAg-negative CHB. In this study, we showed that two MEIA methods and one CMIA method for the quantification of serum anti-HBc IgM have good performance characteristics with low variation and no positive interference with RF but that individual results deviate significantly. In addition, measurements by CMIA appear clinically irrelevant in distinguishing patients with HBeAg-negative CHB when a cutoff of 0.2 set by MEIA is used (7).

Current guidelines do not justify a liver biopsy when the alanine aminotransferase (ALT) level is normal and the HBV DNA level is  $<20 \times 10^3$  copies/ml, and it is common practice to monitor patients with HBeAg-negative chronic HBV infection by serum ALT and anti-HBc IgM measurements and at longer intervals by HBV DNA determinations (2, 23). Raw values provided by commercial anti-HBc IgM methods have been widely used for monitoring such patients (5, 8, 11, 13, 14, 16, 17, 20, 24). The observed differences between the three analyzers advocate the use of not only the same method but also the same analyzer in the follow-up of these patients.

The intermittent elevation of serum ALT (ALT flares) preceded by an increase in HBV DNA is common in patients with HBeAg-negative CHB. ALT flares are followed by an elevation in anti-HBc IgM lasting for several months of biochemical and viral quiescence. In this phase, the only marker of a previous flare is an elevation in anti-HBc IgM (3, 20), usually a moderate score index of  $>0.2$  and rarely a score index of  $>1.2$  by MEIA (diagnostic of acute hepatitis B) (22). Based on our results, the 0.2 cutoff index for viral activity does not apply to methods other than MEIA, and 52% of patients with an anti-HBc IgM index of  $>0.2$  by IMx would be missed by Architect.

CMIA analyzers are most likely going to replace MEIA, especially in large settings, since they are sensitive, fast, and reliable and can combine immunology with biochemical tests. The underestimation of the level of anti-HBc IgM by CMIA was anticipated, since the cutoff for acute infection is set lower and the gray zone is set wider than for the MEIA. A 1.4 conversion factor could decrease the discordance between results above and below the 0.2 limit, but the values would still be significantly different. Moreover, in contrast to MEIA, no association between Architect anti-HBc IgM and HBV DNA (20 patients; data not shown) or ALT measurements was observed, which is in agreement with results of previous studies (21).

We propose that for patients with HBeAg-negative chronic HBV infection, anti-HBc IgM should be quantified by MEIA until an association with viral activity and cutoff values for active disease are established with CMIA.

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