

Comparative Evaluation of Commercial Enzyme Immunoassay Kits for Detection of Hepatitis B Seromarkers

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The commercial hepatitis B enzyme immunoassay kits of Abbott Laboratories and Organon Teknika were compared for their sensitivity, specificity, and reproducibility in detecting the hepatitis B seromarkers hepatitis B surface and e antigens and antibodies to hepatitis B core, e, and surface antigens. With the exception of the Organon kit for antibodies to hepatitis B surface antigen, the specificity and reproducibility were about the same for both products, but the level of sensitivity was generally lower for the Organon kits; this, however, may not be critical in routine clinical application. The Organon kits have a longer shelf life and are cheaper.

Over the past several years, a wide variety of methods have been developed and used to detect the hepatitis B seromarkers (2). Among the most sensitive third-generation tests, the enzyme immunoassay (EIA) (1, 5) is the preferred method of most diagnostic laboratories (4). Although a number of manufacturers have been marketing hepatitis B EIA diagnostic kits for some time now, the products of Abbott Laboratories, North Chicago, Ill., appear to have found wide acceptance in clinical laboratories. We had an opportunity to evaluate the hepatitis B Microelisa system (Hepanostika) manufactured by Organon Teknika, Turnhout, Belgium, which incorporates a microtiter strip-plate, solid-phase method, in comparison with the bead-based, solid-phase hepatitis B EIA system of Abbott Laboratories. The EIA kits evaluated included tests for the following seromarkers: hepatitis B surface antigen (HBsAg) and e antigen (HBeAg) and antibodies to hepatitis B core antigen (anti-HBc), e antigen (anti-HBe), and surface antigen (anti-HBs).

The Hepanostika HBsAg, HBeAg, anti-HBc, anti-HBe, and anti-HBs Microelisa diagnostic kits were obtained from Organon Canada Ltd., West Hill, Ontario, Canada. The Hepanostika HBsAg and HBeAg tests are based on a sandwich principle with polystyrene microtiter strips coated with monoclonal anti-HBs and human anti-HBe, respectively. The anti-HBc and anti-HBe tests are based on a sandwich inhibition principle, i.e., blocking of a fixed amount of hepatitis B core antigen or HBeAg by corresponding antibodies present in test serum samples. The anti-HBs test is based on neutralization of a fixed amount of HBsAg by anti-HBs present in the test sample during a preincubation step, which is followed by the standard HBsAg test procedure. The corresponding Abbott EIA kits included for comparison were Auszyme II (HBsAg), Abbott-HBe (HBeAg and anti-HBe), Corzyme (anti-HBc), and Ausab (anti-HBs). Auszyme II, HBeAg, and Ausab are based on a sandwich principle with polystyrene beads coated with corresponding antibody or antigen. Corzyme and anti-HBe tests incorporate a competitive inhibition principle similar to that of the Hepanostika anti-HBc and anti-HBe tests. Both the Organon and Abbott systems use horseradish peroxidase conjugates and offer manual, semiautomated, and automated features to various extents. An HBsAg confirmatory test

procedure is available in both systems; this, however, was not included for the evaluation.

Test samples included a reference panel of 19 sera with known concentrations of purified ad and ay HBsAg subtypes (Abbott Laboratories), a proficiency panel of 10 sera of known reactivity as determined by both radioimmunoassay and EIA techniques (Laboratory Centre for Disease Control, Ottawa, Ontario, Canada), and a test panel of serum samples randomly picked from two batches of routine clinical specimens, one of which comprised sera that were positive for hepatitis B seromarkers and the other of which comprised sera that were negative. Throughout the evaluation, all specimens were identified by code only and blinded to the analyst. All test procedures were done according to manufacturer's instructions. The procedures with longer incubations were chosen for assays that had alternative shorter incubation steps. Both the Hepanostika and Abbott products were independently evaluated by two technologists, and most samples were tested in duplicate or triplicate. Samples yielding discrepant results were retested for confirmation by radioimmunoassay and EIA techniques at the Viral Hepatitis Section, Laboratory Centre for Disease Control, Ottawa.

The detection levels of HBsAg test kits as determined by the sensitivity reference panel were 1.40 ng/ml (ad) and 1.09 ng/ml (ay) for Hepanostika and 0.31 ng/ml (ad) and 0.30 ng/ml (ay) for Abbott Auszyme II. The Hepanostika results also indicated a HBsAg detection sensitivity one-quarter to one-half that of Auszyme II when HBsAg-positive serum samples were tested in serial dilutions. With the HBsAg proficiency panel, which consisted of eight positive and two negative samples having borderline reactivity close to the cutoff, the Hepanostika kit yielded inconsistent results with false-positives and false-negatives with an overall agreement ranging from 40 to 80%, whereas Auszyme II yielded reproducible results with 100% agreement. A total of 74 randomly picked clinical samples from the batches of HBsAg-positive and -negative sera were included for further evaluation of the kits; all samples tested accurately by both the Hepanostika and Auszyme II kits with 100% agreement (Table 1).

The sensitivity of the Hepanostika anti-HBc test kit was found to be one-quarter to one-half that of the Abbott Corzyme kit in detecting anti-HBc as determined by serially diluted anti-HBc-positive serum samples. Among 39 random samples tested from the batches of anti-HBc-positive and -negative routine sera, 5 yielded discordant but reproducible

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TABLE 1. Comparative analysis of Abbott and Organon EIA kits for detection of hepatitis B seromarkers in routine clinical specimens

Characteristic	HBsAg (74) ^a		Anti-HBc (39)		Anti-HBs (44)		HBeAg (33)		Anti-HBe (44)	
	Abbott	Organon	Abbott	Organon	Abbott	Organon	Abbott	Organon	Abbott	Organon
Sensitivity (%) ^b	100	100	100	90	100	100	100	81	100	100
Specificity (%) ^b	100	100	95	89	100	73	100	100	100	100
Reproducibility (%)	100	100	100	100	100	95	100	100	100	100
No. of tests per kit	100/500	192/576	100/500	192	100	96	100	192	100	192
No. of controls per assay or strip ^c	5	2	5	2	5	2	5	4	5	4
Approx minimum time required to complete test (h)	2/18	2/4	3/13	19	5/19	19	23	5	23	21
Shelf life of kit (mo)	3	12	3	9	3	9	3	12	3	12
Cost per test (\$) ^d	3.14	2.30	4.62	3.22	3.35	3.08	6.15	4.14	6.15	4.14

^a Figures in parentheses are the numbers of serum samples tested.

^b Confidence interval (95%) for values of <100% varied from $\pm 30%$ ($n = 11$) to $\pm 15%$ ($n = 19$).

^c Organon controls per strip (12 wells) for HBsAg, anti-HBc, and anti-HBs. Additional controls were required for additional strips, with a minimum of four controls per eight strips.

^d List price, including controls, in Canadian dollars as of June 1986.

results with the respective systems. Retesting of these samples by radioimmunoassay confirmed one Hepanostika result and four Corzyme results, with an overall agreement of 90 and 97%, respectively, for the kits. The Hepanostika anti-HBs test indicated a level of sensitivity two- to fourfold higher than that of the Abbott Ausab test in detecting anti-HBs in serially diluted anti-HBs-positive serum samples. However, for 44 randomly picked samples tested from the batches of anti-HBs-positive and -negative routine sera, the overall agreement was 93% for Hepanostika and 100% for Ausab. The HBeAg kits were evaluated with 33 sera from routine submissions. The Hepanostika kit yielded an overall agreement of 91%, whereas the Abbott-HBe reached 100%. A total of 44 routine sera were tested for anti-HBe, with 100% agreement for both kits. The results of tests done with the routine clinical specimens are shown in Table 1, along with a cost comparison.

With the exception of the anti-HBs test, the Hepanostika kits were found to be generally less sensitive than the Abbott kits, and this was recognizable only when low-positive and weakly reactive samples were tested. The differences in sensitivity could be attributed in part to the difference in the solid phase used in these systems, i.e., microtiter wells versus beads. The 0.25-in. (ca. 0.64-cm) beads used in the Abbott kits provide a larger surface area, perhaps with more uniform coating of antigen or antibody than the Hepanostika microtiter wells. However, as indicated by our results, the reduced sensitivity of Hepanostika may not be critical in routine clinical application because of the high levels of circulating antigens and antibodies generally present in the serum of individuals with exposure to the hepatitis B virus. Moreover, increased sensitivity is likely to result in nonspecific reactions. In fact, a recent report indicated that low-level anti-HBs and anti-HBc reactions are often nonspecific and should be interpreted with caution (3). In this connection, it is also unlikely that the increased sensitivity of the Hepanostika anti-HBs test correlates with protective antibody level. Nevertheless, the importance of a highly sensitive HBsAg test in particular cannot be overemphasized, especially for screening blood donors, in the reference laboratory setting, and for proficiency testing.

On the technical side, Organon offers an automated washer and reader as standard accessories, whereas Abbott normally provides a manually operated washer and reader, with an automated washer-dispenser and reader available as

options either free of charge, depending on the test volume, or at an additional cost. Manual dispensing of reagents is laborious and time-consuming by both methods. For this reason, we consider even the purchase of the automated Abbott dispenser, which comes with an automated washer unit, worthwhile if the Abbott kits are used. The Hepanostika strip-plate system containing 12 wells on each strip offers flexibility to the microtiter method, but the maximum flexibility no doubt is provided by the Abbott bead system. On the economical side, significant differences were noted in the price and the shelf life of the kits (Table 1). From this standpoint, it appears more desirable to use the Hepanostika kits than the Abbott kits. In deciding to use the former, the nature of application and the sensitivity levels of the tests should be taken into account.

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