

Comparison of DiaSorin and Bio-Rad Test Kits for the Detection of Hepatitis B Virus Total Core and Surface Antibodies on the Bio-Rad Evolis

Stephen J. Cavalieri, PhD, Sharon Hrabovsky, and Trichel Jorgensen

Key Words: HBV; Virology; Serology; MONOLISA; Immunology; Hepatitis; Antibodies; Evolis; BioRad; DiaSorin

DOI: 10.1309/AJCPHY8P2LOIKFBE

Abstract

The new MONOLISA Bio-Rad kits were compared to DiaSorin kits for the detection of hepatitis B virus (HBV) total core (HBcTAb) and surface (HBsAb) antibodies on the Bio-Rad Evolis immunoanalyzer. The resolved sensitivities, specificities, positive and negative predictive values, and overall accuracy were 91%, 100%, 100%, 96%, and 97%, respectively, for HBcTAb and 99%, 95%, 96%, 99%, and 97%, respectively, for HBsAb. Whereas accuracy and reagent cost were comparable between the kits, Bio-Rad kits required less specimen volume and less instrument processing time to results than the DiaSorin kits.

Serologic analysis remains an important component in the diagnosis and staging of hepatitis B virus (HBV) infections.¹ The HBV total core antibody (HBcTAb) rises approximately 2 to 3 months after exposure to HBV and is a marker of natural infection. HBV surface antibody (HBsAb) is the last serologic marker to appear after HBV infection, becoming detectable approximately 5 to 6 months after exposure. Its presence heralds recovery and is the only marker to appear after vaccination.

The Evolis is an open, automated microplate processor (Bio-Rad Laboratories, Hercules, CA) capable of performing a variety of serologic tests using an enzyme immunoassay format. The ETI-AB-COREK PLUS and ETI-AB-AUK PLUS kits (DiaSorin, Saluggia, Italy) detect HBcTAb and HBsAb respectively, by an enzyme immunoassay method and can both be performed on the Evolis. The ETI-AB-COREK PLUS is a competitive assay, whereas the ETI-AB-AUK PLUS is a noncompetitive assay. Recently Bio-Rad developed 2 new kits for the detection of HBcTAb (MONOLISA Anti-HBc Assay) and HBsAb (MONOLISA Anti-HBs Assay). Both are noncompetitive assays. The purpose of this study was to compare the DiaSorin test kits with the new Bio-Rad test kits for the detection of HBcTAb and HBsAb using the Evolis instrument.

Materials and Methods

Consecutive serum samples from patients with suspected viral hepatitis and having routine orders for HBcTAb and/or HBsAb and consecutive serum samples from patients having routine orders for HBsAb to determine immune status were

tested using the DiaSorin kits on the Evolis as described subsequently at the Creighton University Medical Center clinical laboratory (Omaha, NE). Serum specimens were then frozen at -20°C for testing with the Bio-Rad kits on the Evolis at a later date. Testing using the DiaSorin and Bio-Rad kits was performed according to the manufacturers' instructions. Briefly, microwells sufficient for all samples, calibrators, and control samples were placed in the Evolis. The instrument performed all subsequent steps. Serum samples were added to microwells coated with antigen. Excess sample was removed by washing. Enzyme conjugate (peroxidase) was added to the wells and incubated. Excess conjugate was removed by washing. Substrate was then added and incubated. A stop solution (sulfuric acid) was then added to halt the reaction. Absorbance levels (615-630 nm) in the microwells were then measured by the instrument spectrophotometer. Results were compared with the calibrators using a test- and kit-dependent formula to ascertain whether the test result was reactive, equivocal, or nonreactive.

Data were initially analyzed assuming that the DiaSorin result was the correct one. Sensitivity, specificity, positive and negative predictive values, and overall accuracy were then calculated. Tests resulting in discrepancies were, when possible, repeated in an attempt at resolution. Other data such as optical densities of the samples and the results of other HBV serologic tests were also examined in an attempt to resolve discrepant samples.

Results

Initial and resolved accuracy data for the Bio-Rad MONOLISA Anti-HBc Assay for the detection of HBcTAB are shown in **Table 1**. Initially, the sensitivity, specificity, positive and negative predictive values, and overall accuracy on 147 serum samples were 90.9%, 99.0%, 97.6%, 96.2%, and 96.6%, respectively. Repeated analysis of the 5 discrepancies (1 false-positive and 4 false-negatives) resulted in the resolution of the 1 false-positive only, increasing the specificity, positive predictive value, negative predictive value, and overall accuracy to 100%, 100%, 96.3%, and 97.3%, respectively.

Initial and resolved accuracy data for the Bio-Rad MONOLISA Anti-HBs Assay for the detection of HBsAb are shown in **Table 2**. Initially, the sensitivity, specificity, positive and negative predictive values, and overall accuracy on 172 serum samples were 97.8%, 92.7%, 93.6%, 97.4%, and 95.3%, respectively. Repeated analysis of the 8 discrepancies (6 false-positives and 2 false-negatives) resulted in the resolution of 2 false-positives and 1 false-negative, increasing the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy to 98.9%, 95.0%, 95.8%, 98.7%, and 97.1%, respectively.

Data on all discrepant samples for HBcTAB and HBsAb are shown in **Table 3**. For the 4 unresolved Bio-Rad false-negative HBcTAB samples, all were HBsAb+, and 3 had relatively low optical densities well below the positive cutoff.

Table 1
Comparison of Bio-Rad MONOLISA Anti-HBc With DiaSorin ETI-AB-COREK PLUS for the Detection of Hepatitis B Virus Total Core Antibody

	Initial			Resolved*		
	DiaSorin+	DiaSorin-	Total	DiaSorin+	DiaSorin-	Total
Bio-Rad+	40	1	41	40	0	40
Bio-Rad-	4	102	106	4	103	107
Total	44	103	147	44	103	147

* One false-positive Bio-Rad result became negative on repeated analysis.

Table 2
Comparison of Bio-Rad MONOLISA Anti-HBs With DiaSorin ETI-AB-AUK PLUS for the Detection of Hepatitis B Virus Surface Antibody

	Initial			Resolved*		
	DiaSorin+	DiaSorin-	Total	DiaSorin+	DiaSorin-	Total
Bio-Rad+	88	6	94	91	4	95
Bio-Rad-	2	76	78	1	76	77
Total	90	82	172	92	80	172

* For 2 Bio-Rad+, DiaSorin- results, the DiaSorin results became positive on repeated analysis. For 1 Bio-Rad-, DiaSorin+ result, the Bio-Rad result became positive on repeated analysis.

Table 3
Discrepancy Analysis

Discrepancy (Bio-Rad/DiaSorin)	Initial Category	Repeated Analysis (Bio-Rad/DiaSorin)	Other Data	Resolved Category
HBcTAb-/HBcTAb+	FN	HBcTAb-/NA	OD, 0.239; HBsAb+	FN
HBcTAb-/HBcTAb+	FN	HBcTAb-/NA	OD, 0.064; HBsAb+	FN
HBcTAb-/HBcTAb+	FN	HBcTAb-/NA	OD, 0.053; HBsAb+	FN
HBcTAb-/HBcTAb+	FN	HBcTAb-/NA	OD, 0.119; HBsAb+	FN
HBcTAb+/HBcTAb-	FP	HBcTAb-/HBcTAb-	OD, 0.407	TN
HBsAb-/HBsAb+	FN	HBsAb+/HBsAb+	OD, 0.079	TP
HBsAb-/HBsAb+	FN	HBsAb-/HBsAb+	OD, 0.048	FN
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb+	OD, 0.423	TP
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb+	OD, 0.331	TP
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb-	OD, 0.140	FP
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb eqv	OD, 0.123	FP
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb-	OD, 0.191	FP
HBsAb+/HBsAb-	FP	HBsAb+/HBsAb eqv	OD, 0.140	FP

eqv, equivocal; FN, false-negative; FP, false-positive; HBcTAb, hepatitis B virus total core antibody; HBsAb, hepatitis B virus surface antibody; NA, no data available; OD, initial Bio-Rad optical density value (positive cutoffs for HBcTAb were typically 0.28-0.29; positive cutoffs for HBsAb were typically 0.10-0.13); TN, true-negative; TP, true-positive.

For the 1 unresolved false-negative HBsAb sample, the optical density was well below the positive cutoff. For the 4 unresolved Bio-Rad false-positive HBsAb samples, all had relatively low optical densities slightly above the positive cutoff.

Discussion

To our knowledge, this is the first published comparative evaluation of the new Bio-Rad MONOLISA Anti-HBc and MONOLISA Anti-HBs assays for the detection of HBcTAb and HBsAb on the Evolis instrument. Indeed, there is a paucity of recent comparative data in the published literature on the various available serologic kit assays for the detection of HBcTAb and HBsAb. A 2008 study examined the MONOLISA Anti-HBc compared with the PRISM HBcore (Abbott Laboratories, Chicago, IL), Murex Anti-HBc total (Murex Diagnostics, Chicago, IL), and bioMérieux and Dade Behring Enzygnost (Dade Behring, Marburg, Germany). Specificity was highest with the MONOLISA kit, with sensitivity comparable among the assays.² In a 2006 study, 9 kits for the detection of HBcTAb were compared, but none of the 9 were the kits used in the present study.³

In the present study, there were 5 discrepant samples for the HBcTAb comparison and 8 discrepant samples for the HBsAb comparison (Table 3). Repeated testing resolved 1 Bio-Rad false-positive HBcTAb sample, one Bio-Rad false-negative HBsAb sample, and 2 Bio-Rad false-positive HBsAb samples. For the 4 Bio-Rad false-negative HBcTAb samples, optical densities were well below the positive cutoff for 3, and all were HBsAb+. Although HBsAb positivity in these patients may be due to vaccination, these data suggest that these were actual Bio-Rad false-negative results. For the

4 remaining Bio-Rad false-positive HBsAb samples, 2 had equivocal results when the DiaSorin test was repeated, and all had Bio-Rad optical densities slightly above the positive cutoff. These data suggest that these false-positive samples clustered near the positive cutoff value, with 2 possibly, but not unequivocally, being true-positives.

There appear to be modest technical advantages to the Bio-Rad kits as shown in **Table 4**. DiaSorin kits require 3.5 hours of total instrument processing time (time from sample application to results) vs 2.5 hours for the Bio-Rad kits. In addition, specimen volume requirements are 50 µL for the DiaSorin HBcTAb vs 20 µL for the Bio-Rad and 100 µL for the DiaSorin HBsAb vs 75 µL for the Bio-Rad. Reagent costs for both kits are comparable.

The Bio-Rad MONOLISA Anti-HBc and MONOLISA Anti-HBs assays are comparable in accuracy to the DiaSorin ETI-AB-COREK PLUS and ETI-AB-AUK PLUS assays for the detection of HBsAb and HBcTAb when performed on

Table 4
Comparison of DiaSorin and Bio-Rad HBcTAb and HBsAb Kits for Technical Factors

Parameter	HBcTAb		HBsAb	
	DiaSorin	Bio-Rad	DiaSorin	Bio-Rad
Processing time (h)*	3.5	2.5	3.5	2.5
Specimen volume (µL)	50	20	100	75
Reagent cost (\$) [†]	912/4.75	912/4.75	864/4.50	864/4.50
Accuracy	Comparable		Comparable	

HBcTAb, hepatitis B virus total core antibody; HBsAb, hepatitis B virus surface antibody.

* Time from placing the specimen on the Bio-Rad Evolis for analysis to results.

[†] Cost per kit/cost per test. Each kit contained two 96-well plates. At the time of this study, the DiaSorin and Bio-Rad kits were purchased through Bio-Rad.

the Evolis immunoanalyzer. Although the reagent costs and accuracy are comparable, technical advantages of the Bio-Rad assays are lower specimen volume requirements and shorter instrument processing time.

From the Department of Pathology, Creighton University Medical Center, Omaha, NE.

Address reprint requests to Dr Cavaliere: Department of Pathology, Creighton University Medical Center, 601 N 30th St, Omaha, NE 68131.

Acknowledgments: We gratefully acknowledge Bio-Rad Laboratories for supplying free reagent kits for the purpose of this evaluation.

References

1. Horvat RT, Tegtmeier GE. Hepatitis B and D viruses. In: Murray PE, Baron EJ, Jorgensen JH, et al, eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: ASM Press; 2007:1641-1659.
2. Hourfar MK, Walch LA, Geusendam G, et al. Sensitivity and specificity of anti-HBc screening assays: which assay is best for blood donor screening [published online ahead of print July 30, 2008]? *Int J Lab Hematol*. doi: 10.1111/j.1751-553X.2008.0192.x.
3. Schmidt M, Nübling CM, Scheiblaue H, et al. Anti-HBc screening of blood donors: a comparison of nine anti-HBc tests. *Vox Sang*. 2006;91:237-243.