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Evaluation of Two New Automated Assays for Hepatitis B Virus Surface Antigen (HBsAg) Detection: IMMULITE HBsAg and IMMULITE 2000 HBsAg

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In recent years the diagnostic industry has developed new automated immunoassays for the qualitative detection of hepatitis B virus (HBV) surface antigen (HBsAg) in serum and plasma samples that are performed on analyzers that permit a high-speed throughput, random access, and primary tube sampling. The aim of the present study was the evaluation of two new automated HBsAg screening assays, IMMULITE HBsAg and IMMULITE 2000 HBsAg, from Diagnostic Products Corporation. The new HBsAg assays were compared to well-established tests (Auszyme Monoclonal [overnight incubation, version B], IMx HBsAg, AxSYM HBsAg, and Prism HBsAg [all from Abbott] and Elecsys HBsAg [Roche Diagnostics]). In the evaluation were included seroconversion panels, sera from the acute and chronic phases of infection, dilution series of various HBsAg standards, HBV subtypes and S gene mutants. To challenge the specificity of the new assays, sera from HBsAg-negative blood donors, pregnant women, and dialysis and hospitalized patients and potentially cross-reactive samples were investigated. IMMULITE HBsAg and IMMULITE 2000 HBsAg, although not as sensitive as the Elecsys HBsAg assay, were equivalent to the AxSYM HBsAg assay and showed a higher sensitivity than the Auszyme Monoclonal B and IMx HBsAg systems for detection of acute infection in seroconversion panels. The specificities (100%) of both IMMULITE assays on unselected blood donors and potentially interfering samples were comparable to those of the alternative assays after repeated testing. In conclusion, the new IMMULITE HBsAg and IMMULITE 2000 HBsAg assays show a good sensitivity for HBsAg detection compared to other well-established tests. The specificity on repeatedly tested samples was equivalent to that of the alternative assays. The rapid turnaround time, primary tube sampling, and on-board dilution make it an interesting assay system for clinical laboratory diagnosis.

The envelope protein of hepatitis B virus (HBV), HBV surface antigen (HBsAg), is a transmembrane glycoprotein usually shed in large amounts in the serum of infected individuals, where it is found as spherical particles with a diameter of 22 nm or filaments of similar diameter (29). The *a* determinant of HBsAg, a predicted double-loop structure projecting from the surface of the HBV particle (28), is the major neutralizing epitope. Antibodies to the *a* determinant confer protection in adults to all the common subtypes of HBV. Within the predicted loop regions are also located subtype determinants *d* or *y* and *w* or *r*. A total of nine serotypes have been described (9). These have been related to six genomic groups, groups A to F based, on sequencing of the S gene of isolates from different geographical regions (23, 24).

HBsAg is one of the first serum markers to appear during the course of HBV infection and can be detected 2 to 8 weeks before biochemical evidence of liver dysfunction and the onset of jaundice. HBsAg is cleared within a few months in self-limiting illness. If HBsAg persists for more than 6 months, spontaneous clearance is very unlikely and the infected individual is considered to be a chronic HBV carrier.

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Among the many commercially licensed HBsAg assays offered, enzyme-linked immunosorbent assays are the most commonly used. These assays use either monoclonal or polyclonal anti-HBs bound to a solid phase and a second labeled anti-HBs to detect the captured antigen. Despite the high performance of screening assays, transfusion-associated HBV infection is still reported (13, 14, 18). There are three possibilities to explain false-negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detection limit; i.e., a high proportion of individuals with antibodies against HBV core antigen (anti-HBc) as the only serological marker of infection are low-level chronic carriers of the virus (12, 17). Another explanation is that virus variants yield sequences that are not recognized by the antibodies employed in the assays. In different geographical locations, vaccine-escape mutants are emerging under the selective pressure of active immunization, and there is a danger that they will become dominant strains as vaccination becomes universal (5, 15). Breakthrough infections due to point mutations of the *a* determinant have been described in Europe, Africa, and Asia (4, 6, 11, 25, 26, 30). Vaccine-escape mutants within the *a* determinant of the S gene are not as effectively recognized by conventional diagnostic tests as wild-type particle (7, 16). A further explanation is that there are variants in other parts of the genome that down-regulate the production of HBsAg (3).

In order to reduce the residual risk of transfusion-associated hepatitis B, the sensitivity of HBsAg screening assays is continuously improved. IMMULITE HBsAg and IMMULITE 2000 HBsAg (Diagnostics Products Corporation [DPC], Los Angeles, Calif.) are new fully automated and rapid assays which permit the qualitative detection of HBsAg directly from the patient blood collection tube in a total incubation time of 65 min. In the present study, they were compared with alternative well-established serological assays.

MATERIALS AND METHODS

IMMULITE HBsAg and IMMULITE 2000 HBsAg assays. IMMULITE HBsAg and IMMULITE 2000 HBsAg assays (DPC) are solid-phase, two-step chemiluminescent enzyme immunoassays performed, respectively, on the IMMULITE and IMMULITE 2000 random access immunoassay analyzers. Both assays and analyzers are based on the same test principle and technique, respectively. The IMMULITE 2000 analyzer permits a higher throughput (up to 200 tests per hour) than the IMMULITE analyzer. In contrast to the IMMULITE analyzer, the IMMULITE 2000 analyzer features primary tube sampling and on-board dilution.

The solid phase, a polystyrene bead enclosed within an IMMULITE test unit or IMMULITE 2000 bead pack, is coated with antibody (anti-HBs) directed against the HBsAg. The patient sample and a protein-based buffer are simultaneously introduced into the IMMULITE test unit or the IMMULITE 2000 reaction tube and incubated for approximately 30 min at 37°C with intermittent agitation. During this time HBsAg (either subtype *ad* or *ay*) in the sample binds to the anti-HBs-coated bead. Unbound serum is then removed by a centrifugal wash.

An alkaline phosphatase-labeled anti-HBs is introduced, and the test unit or reaction tube is incubated for another 30-min cycle. The unbound enzyme conjugate is removed by a centrifugal wash. Substrate is then added, and the test unit or reaction tube is incubated for a further 10 or 5 min, respectively.

The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light, allowing multiple readings. The bound complex—and thus also the photon output, as measured by the luminometer—is related to the presence of HBsAg in the sample. A qualitative result is then obtained by comparing the patient result to an established cutoff.

Both systems automatically handle sample and reagent additions, the incubation and separation steps, and measurement of the photon output via the temperature-controlled luminometer. They determine test results for controls and patient samples by comparing the observed signal to a cutoff derived from the adjuster's responses and the bar-coded parameters and generate a printed report that includes any other patient information previously entered via the computer (1).

A single determination uses 100 μl of the patient sample. In the case of the IMMULITE assay, the sample cup should contain at least 250 μl more than the total volume required for all tests to be performed on the sample.

Result calculations are performed automatically by the IMMULITE system. The cutoff is set equal to the average counts per second of the adjuster (from the most recent adjustment) multiplied by curve parameter 1. (See the "Low Adjustor CPS" and "Curve Parameter 1" fields in the IMMULITE kit information screen, which can be accessed from the menu via Data Entry: Kit Entry.)

The result is positive if the sample's counts are above the cutoff and negative if the sample's counts are below the cutoff. A positive result indicates that HBsAg is present and was detected in the patient sample. Specimens found to be initially reactive (IR) for HBsAg should be retested in duplicate to verify that the IR result is repeatable. If one or both of the duplicates of the retested sample are reactive, the patient sample should be tested using the IMMULITE HBsAg confirmatory assay. Only those samples in which the HBsAg is neutralized by the confirmatory test procedure are considered confirmed positive for HBsAg.

If neither of the duplicates of the reassayed sample is reactive, the patient sample should be considered negative for HBsAg.

IMMULITE HBsAg and IMMULITE 2000 HBsAg confirmatory assay. The IMMULITE HBsAg confirmatory kit (DPC) confirms the presence of HBsAg in a patient sample that has tested positive for the antigen. The sample, divided into two aliquots, is combined with either the blocking reagent or the control reagent in the sample cup. The solutions are transferred, along with a protein-based buffer, into the test unit or reaction tube. The solutions incubate for approximately 30 min at 37°C with intermittent agitation. During this time, unbound HBsAg in the patient sample binds to the anti-HBsAg-coated bead. In the sample containing the blocking reagent, most of the HBsAg present in solution will bind to the blocking antibody (goat anti-HBsAg) and will not bind to the coated bead. The control reagent does not contain blocking antibody, and HBsAg present in the sample remains free to bind to the coated bead. Serum components not bound to the coated bead are removed by a centrifugal wash. The sample is confirmed positive if the signal from the neutralized sample is at least 50% less than the signal from the control.

The IMMULITE HBsAg confirmatory kit requires 700 μl of the patient sample (350 μl for the blocking reaction and 350 μl for control reaction).

Results of the assay are valid only if the signal generated by the blocked HBsAg positive control is at least 50% lower than that of the unblocked positive control. If the blocked sample (undiluted, diluted 1-in-500, or diluted 1-in-25,000) generates a signal that is at least 50% lower than that of the corresponding unblocked sample, the sample is confirmed positive for HBsAg. If the signal from the blocked patient sample does not decrease by at least 50%, the previous HBsAg positive result is not confirmed.

Alternative assays. Alternative assays included the Elecsys HBsAg assay (Roche Diagnostics, Penzberg, Germany) and the Auszyme Monoclonal, IMx HBsAg, AxSYM HBsAg, and PRISM HBsAg (all from Abbott, Delkenheim, Germany). For the Auszyme Monoclonal assay, overnight incubation was performed (version B) [referred to as Auszyme Monoclonal (B)] in order to achieve a high sensitivity.

For testing of HBsAg variants and mutants, Murex (Dartford, Germany) HBsAg version 3—a microtiter plate-based sandwich assay using a mixture of monoclonal antibodies which permit the detection of wild-type and mutant HBsAg—and Enzygnost HBsAg 5.0 (Dade-Behring, Marburg, Germany) served as reference assays.

Resolution of discrepant test results was performed by using the VIDAS HBsAg assay (Biomérieux, Marcy-l'Etoile, France). For the present evaluation, the 90-min assay protocol was used.

Anti-HBs, anti-HBc, anti-HBc-immunoglobulin M (IgM), HBV envelope antigen (HBeAg), and anti-HBe determinations were performed using IMx assays (IMx AUSAB, IMx CORE, IMx CORE-M, IMx HBeAg, and IMx anti-HBe assays [all from Abbott]) and AxSYM tests (AxSYM AUSAB, AxSYM CORE, AxSYM CORE-M, AxSYM HBeAg, and AxSYM anti-HBe tests [all from Abbott]) and the Elecsys anti-HBs assay (Roche Diagnostics).

All the tests were performed and interpreted in accordance with the manufacturers' recommendations. The main characteristics of HBsAg assays are shown in Table 1.

Specimens. The following specimens were tested to evaluate the sensitivity.

Serial dilutions. Serial dilutions of the following were used: Paul Ehrlich Institute (PEI) (Langen, Germany) standard for HBsAg subtypes *ad* (HBsAg subtype *ad*, 1,000 PEI units (PEIU)/ml = 2,360 IU/ml) and *ay* (HBsAg subtype *ay*, 1,000 PEIU/ml = 3,210 IU/ml) and HBsAg reference material from the World Health Organization (WHO) (HBsAg subtype *ad*) (1st International Standard EST 1985; Code 80/549, 100 IU/ml).

(ii) Sensitivity panels. Two sensitivity panels were used: the HBsAg sensitivity panel PHA 806 (Boston Biomedica Inc. [BBI], West Bridgewater, Mass.) and the SFTS panel (Société Française de Transfusion Sanguine, Nord-Pas-de-Calais, France). HBsAg sensitivity panel PHA 806 includes 10 subtype *ad* and 10 subtype *ay* sera with decreasing HBsAg concentrations (0.31 to 0.01 PEIU/ml). The SFTS panel consists of nine sera with decreasing concentrations of HBsAg ranging from 2.4 to 0.05 ng/ml.

(iii) Low-titer panel. A low-titer panel (BBI PHA 105) composed of 14 serum or plasma samples with low HBsAg concentration (0.2 to 0.8 IU/ml) and one negative serum was used.

(iv) Serial dilutions of spiked serum. Serial dilutions of a serum, which was spiked with purified heat-inactivated HBsAg (subtype *ad*/*ay*; 32472; Scantibodies Laboratory Inc., Santee, Calif.) up to a concentration of 0.125 mg/ml, were used.

(v) Commercial seroconversion panels. Thirty-eight commercially available seroconversion panels (from BBI; BioClinical Partners, Franklin, Mass.; Serologicals, Livingston, United Kingdom; North American Biologicals Inc. [NABI], Boca Raton, Fla.; and Pyramid Profile Diagnostics, Sherman Oaks, Calif.), consisting of follow-up samples which were collected at weekly or monthly intervals from patients suffering from acute hepatitis B, were used. All the panels were characterized for HBV-specific serological markers (anti-HBs, anti-HBc, anti-HBc-IgM, HBeAg, and anti-HBe).

(vi) HBsAg-positive sera. HBsAg-positive sera from patients at different stages of HBV infection ($n = 410$) were used. All these serum samples were preselected on the basis of the results of HBV-specific markers or clinical data as follows. (i) Four follow-up samples from a patient with acute HBV infection documented by

TABLE 1. Characteristics of automated and microtiter HBsAg screening assays

Screening type and test	Manufacturer	Test principle[a]	Capture antibody	Conjugate	Sample vol (μl)	Incubation time (min)	Cutoff calculation	Interpretation (signal/cutoff)	
								Positive result	Negative result
Automated									
IMMULITE, HBsAg	DPC	Sandwich CEIA	MAb[b] (mouse)	Polyclonal (goat)	100	70	By calibration	≥1	<1
IMMULITE, 2000 HBsAg	DPC	Sandwich CEIA	MAb (mouse)	Polyclonal (goat)	100	70	By calibration	≥1	<1
Elecsys HBsAg	Roche	Sandwich CEIA	MAb (mouse)	MAb (mouse)	50	18	By calibration	≥1	<1
AxSYM HBsAg	Abbott	Sandwich ELFA	MAb (mouse)	MAb (mouse)				≥1	<1
Prism HBsAg	Abbott	Sandwich CEIA	MAb IgM (mouse)	Polyclonal (goat)	400		Mean NCC + 0.2 × mean PCC[c]	≥1	<1
VIDAS HBsAg	Biomérieux	Sandwich ELFA	MAb (mouse)	MAb (mouse)	150	90	By calibration	≥0.1	<0.1
Microtiter									
Enzygnost HBsAg 5.0	Dade-Behring	Sandwich EIA	MAb (mouse)	Polyclonal (goat)	100	105	By calibration	≥1	<1
HBsAg (version 3)	Murex	Sandwich EIA	MAb (mouse)	Polyclonal (goat)	100	105	By calibration	≥1	<1

[a] Abbreviations: CEIA, chemiluminescent enzyme immunoassay; ELFA, enzyme-linked fluorescent assay; EIA, enzyme immunoassay.
[b] MAb, monoclonal antibody.
[c] Abbreviations: NCC, negative control counts; PCC, positive control counts.

HBsAg seroconversion were preselected. (ii) Eight samples from patients with anti-HBc-IgM activity (>200 PEIU/ml) (Serologicals) were preselected. High anti-HBcIgM titers (>100 PEIU/ml) are observed during acute hepatitis B (for a review, see reference 32). (iii) A total of 398 individual and follow-up HBsAg positive samples (confirmed with Abbott AxSYM HBsAg neutralization assay) from patients suffering from chronic hepatitis (HBsAg present for at least 6 months) were preselected. HBeAg/anti-HBe testing was performed for 269 patients. Forty-three individuals were HBeAg positive and anti-HBe negative. Antibody to HBeAg was present in 226 individuals. According to clinical data, 125 patients were suffering from chronic hepatitis B. A total of nine patients were liver transplant recipients; heart and renal transplantation was performed in two and five individuals, respectively. Results of HCV antibody tests were available for 224 patients, 32 of whom were presenting an HCV coinfection. A total of 81 individuals were tested for human immunodeficiency virus antibody; human immunodeficiency virus coinfection was observed in 5 individuals.

(vii) Dilution series of HBsAg-positive serum samples. Dilution series of different HBsAg positive serum samples of different subtypes in HBV and surface antigen mutants in negative serum pools were tested in order to assess the influence of genetic variability on HBsAg assays as follows. (i) The SFTS panel consists of nine HBsAg-positive specimens of different subtypes (*ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4*, *adrq−*, and *adrq+*) with an analyte concentration of 10 ng/ml. The HBsAg subtype of each specimen was serologically determined. (ii) Dilution series of serum samples from patients infected with different subtypes (*adw2*, *ayw4*, *ayw2*, *ayw3*, *adr*, and *adr*/*ayr*) in HBV-negative serum were used. (iii) Crude yeast extracts of two recombinant surface antigen mutants, F134Y/G145R and P142S/G145R/N146D, were used. Mutant F134Y/G145R was obtained from an HBsAg-positive liver transplant recipient treated with anti-HBs antibody. Mutant 142S/G145R/N146D was from a patient suffering from chronic hepatitis B who showed HBsAg–anti-HBs seroconversion while undergoing interferon therapy (5, 21). DNA extraction from serum, amplification, and sequencing were performed as described by Weber et al. (33).

For the evaluation of specificity, selected specimens were comparatively tested with different HBsAg assays (Tables 2 and 3) using (i) specimens from HBsAg-negative German blood donors (IMMULITE HBsAg, $n = 5{,}122$; IMMULITE 2000 HBsAg, $n = 1{,}750$); (ii) routine laboratory diagnostic samples ($n = 48$); (iii) samples from hospitalized patients (IMMULITE HBsAg, $n = 200$; IMMULITE 2000 HBsAg, $n = 193$); and (iv) potentially interfering samples (IMMULITE HBsAg, $n = 112$; IMMULITE 2000 HBsAg, $n = 53$), including rheumatoid

TABLE 2. Overview of results obtained with IMMULITE HBsAg and Elecsys HBsAg assays

Source	No. of samples tested	No. of samples positive by IMMULITE HBsAg assay with Elecsys HBsAg result that was:		No. of samples negative by IMMULITE HBsAg assay with Elecsys HBsAg result that was:		Agreement (%)
		Positive	Negative	Positive	Negative	
Seroconversion panels	363	168	0	19	176	94.8
Acute HBV infection	12	12	0	0	0	100
Chronic HBV infection	398	398	0	0	0	100
Blood donors	1,733	0	0	0	1,733	100.0
Routine samples	48	2	0	0	46	100.0
Hospitalized patients[a]	195	8	0	0	187	100
Potentially cross-reactive sera	112	0	0	0	112	100.0
Total	2,861	588	0	19	2,254	99.3

[a] Eleven samples were not considered for final interpretation because not enough sample material was available for resolution of discrepant results.

TABLE 3. Overview of results obtained with IMMULITE 2000 HBsAg and Elecsys HBsAg assays

Source	No. of samples tested	No. of samples positive by IMMULITE 2000 HBsAg assay with Elecsys HBsAg result that was:		No. of samples negative by IMMULITE 2000 HBsAg assay with Elecsys HBsAg result that was:		Agreement (%)
		Positive	Negative	Positive	Negative	
Seroconversion panels	355	167	0	14	174	96.1
Acute HBV infection	12	12	0	0	0	100
Chronic HBV infection	98	98	0	0	0	100
Blood donors	1,750	0	0	0	1,750	100.0
Routine samples	48	2	0	0	46	100.0
Hospitalized patients[a]	193	8	0	0	185	100
Potentially cross-reactive sera	53	0	0	0	53	100.0
Total	2,509	287	0	14	2,208	99.4

[a] Seven samples were not considered for final interpretation because not enough sample material was available for resolution of discrepant results.

factor-positive sera and samples from various groups of patients (patients suffering from alcoholic liver disease, acute or chronic viral and bacterial infections, or autoimmune diseases) and from pregnant women.

Tables 2 and 3give an overview of the serum samples that were analyzed with the different assays.

Data analysis and resolution of discrepant results. The performance of the IMMULITE HBsAg assay and that of the IMMULITE 2000 HBsAg assay were compared to those of the Elecsys HBsAg, Auszyme Monoclonal, IMx HBsAg, and AxSYM HBsAg assays for the seroconversion panels. The mean number of days earlier by which HBsAg was detected by an assay in comparison to the others was determined for 38 seroconversion panels tested. The statistical significance of the reduction for each test was determined using the Wilcoxon test for matched pairs.

The calculation model for time delays between assays established by the PEI (8) was used. This method considers seroconversion to be theoretically possible the following day after the last negative follow-up sample is obtained. The total number and the average of days of time delay for the 38 panels were calculated in comparison with results from the most-sensitive assay.

Anti-HBc and anti-HBs determinations were performed for the resolution of discrepant results between HBsAg assays. A sample was considered to be a true positive if it was repeatedly reactive (RR) in at least two assays and confirmed by neutralization assay and if anti-HBc was positive (with the exception of seroconversion panels) and anti-HBs was negative. For seroconversion panels and sensitivity panels, repeated reactivity in one single assay and the presence of HBV DNA (if tested) was considered to indicate a true-positive result. Conversely, a test result was interpreted as true negative if it was negative by the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays and in at least one comparative assay and confirmatory testing (if enough sample material was available) and if it showed one of the following serological constellations: anti-HBc and anti-HBs negative (HBV negative), anti-HBc and anti-HBs positive (resolved infection), or anti-HBc negative and anti-HBs positive (HBV vaccination). Discrepant samples were only considered for final interpretation if complete testing, including neutralization assay, was performed.

In the case of discrepancy between results of the HBsAg assays for the anti-HBc positive samples, the HBsAg result was considered to be false positive if anti-HBs was present at a titer of >100 IU/liter.

RESULTS

Sensitivity. The detection limit of the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays was comparable to that of the IMx HBsAg assay for the PEI standard *ay* (0.05 PEIU/ml [Table 4]) and to that of the Elecsys HBsAg assay for the BBI 806 sensitivity panel subtype *ay*. The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays showed a higher sensitivity for subtypes *ad* and *ay* for the BBI sensitivity panel 806 than did the Auszyme Monoclonal assay. The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays showed a lower sensitivity for HBsAg subtype *ad* and *ay* detection in dilution series of HBsAg PEI and WHO standards and BBI HBsAg sensitivity panel PHA 806 subtype *ad* than did the Elecsys HBsAg assay (Table 4). No difference in sensitivity between the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays and the Elecsys HBsAg assay was observed for the BBI HBsAg low-titer performance panel PHA 105. Four samples with low HBsAg concentrations (0.1 to 0.3 IU/ml) tested negative by the IMx HBsAg assay.

TABLE 4. Detection limit of HBsAg assays for PEI, WHO, and SFTS standards and the BBI 806 sensitivity panel

Standard or panel	Units	Detection limit of assay				
		IMMULITE HBsAg	IMMULITE 2000 HBsAg	Elecsys HBsAg	Auszyme (B)	IMx HBsAg
Standards						
PEI *ad*	PEIU[a]/ml	0.075	0.075	0.014	0.005	0.033
PEI *ay*	PEIU/ml	0.05	0.05	0.017	0.005	0.055
WHO subtype *ad* (NIBSC code 80/549)	IU/ml	0.1	0.1	0.0125	0.156	ND[a]
SFTS	pg/ml	0.12	0.25	0.12	ND	ND
Panels						
BBI 806 subtype *ad*	PEIU/ml	0.06	0.03	0.02	0.31	ND
BBI 806 subtype *ay*	PEIU/ml	0.03	0.02	0.02	0.21	ND

[a] ND, not determined.

TABLE 5. Comparison of different HBsAg assays for detection of acute HBV in BBI seroconversion panels

PHM Seroconversion panel	Subtype	Sequence no. of sample (day of blood donation) with first positive result					
		IMMULITE HBsAg	IMMULITE 2000 HBsAg	Elecsys HBsAg	Auszyme Monoclonal	IMx HBsAg	AxSYM HBsAg
903	*ad*	5 (14)	5 (14)	4 (10)	5 (14)	5 (14)	5 (14)
904	*ad*	3 (18)	3 (18)	2 (7)	3 (18)	3 (18)	
906	*ad*	2 (137)	2 (137)	2 (137)	2 (137)	3 (150)	
908	*ad*	6 (20)	6 (20)	6 (20)	7 (33)	7 (33)	6 (20)
909	*ad*	5 (14)	5 (14)	4 (9)	4 (9)	5 (14)	
911	*ad*	21 (79)	21 (79)	20 (77)	21 (79)	21 (79)	21 (79)
912	*ad*	8 (42)	8 (42)	8 (42)	8 (42)	8 (42)	8 (42)
914	*ad*	4 (153)	4 (153)	2 (146)	5 (158)	5 (158)	4 (153)
916	*ay*	9 (61)	9 (61)	9 (61)	10 (64)	10 (64)	10 (64)
918	*ad*	2 (7)	2 (7)	2 (7)	3 (12)	2 (7)	
919	*ad*	6 (19)	6 (19)	5 (12)	6 (19)	6 (19)	6 (19)
922	*ad*	7 (21)	7 (21)	5 (14)	6 (16)	7 (21)	6 (16)
923	*ad*	3 (15)	3 (15)	3 (15)	3 (15)	4 (22)	
926	Indeterminate	5 (15)	4 (13)	4 (13)	4 (15)	5 (15)	5 (15)
927	Indeterminate	2 (4)	3 (7)	2 (4)	3 (7)	3 (7)	2 (4)
929	*ad*	6 (18)	6 (18)	5 (14)	6 (18)	6 (18)	5 (14)
930	*ad*	2 (3)	2 (3)	2 (3)	3 (8)	2 (3)	2 (3)
931	Indeterminate	6 (21)	5 (19)	5 (19)	6 (21)	6 (21)	5 (19)
932	*ad*	10 (61)	10 (61)	9 (50)	10 (61)	10 (61)	10 (61)
933	*ad*	3 (7)	3 (7)	3 (7)	4 (9)	3 (7)	3 (7)

Although a saturation effect was observed with high HBsAg concentrations (125 μg of inactivated HBAg *ad*/*ay* subtype/ml), there was no false-negative result (data not shown).

The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays showed a higher sensitivity than the Auszyme Monoclonal (B) and IMx HBsAg assays for diagnosis of acute HBV infection. Fourteen and sixteen of thirty-eight serconversions were detected one bleed later with the Auszyme HBsAg assay in comparison with the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively (Tables 5 and 6). Only three acute infections were detected earlier with the Auszyme HBsAg assay than with the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays. Six and seven of twenty-four seroconversions were found reactive one to two bleeds earlier with the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively, than with the IMx HBsAg assay (Tables 5 and 6).

With the AxSYM HBSAg assay, one and three of thirty-two seroconversions were detected one bleed later than with the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively (Tables 5 and 6). The AxSYM HBsAg assay detected six and four acute infections one bleed earlier than the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively. Of 38 seroconversion panels tested in parallel with the Elecsys HBsAg assay, 17 and 13 acute HBV infections were found reactive one to two bleeds later with the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays (Tables 5 and 6).

With the calculation model of the PEI, which considers seroconversion to be theoretically possible the following day after the last negative follow-up sample is obtained, the total number of days of time delay in comparison with the most-sensitive assay was 21 and 16 for the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively. The mean time delay for detection of HBsAg in comparison with the most-sensitive assay was 0.55 days (range, 0 to 3 days) and 0.43 days (range, 0 to 3 days) for the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, respectively. The performances of the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays were comparable to that of the AxSYM HBsAg assay (0.38 days; range, 0 to 1 day), significantly better than that of the Auszyme Monoclonal (B) assay (1.42 days; range, 0 to 15 days), and that of the IMx HBsAg assay (1.21 days; range, 0 to 8 days). The Elecsys HBsAg assay showed a statistically significantly better sensitivity (0.03 days) than the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays ($P < 0.01$, Wilcoxon matched paired test).

All the samples from patients suffering from acute or chronic hepatitis B tested positive by the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays and the Elecsys HBsAg assay (Tables 2 and 3).

HBsAg subtypes and mutants. The detection limit of the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays for the different subtypes of the SFTS panel varied between 0.125 and 0.5 ng/ml. The Elecsys HBsAg assay showed independently of HBsAg subtype a detection limit of 0.125 ng/ml (Table 7).

The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays showed a lower sensitivity for detection of HBV subtypes than the Elecsys HBsAg and AxSYM HBsAg assays (Table 8). Conversely, the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays were more sensitive than the Murex HBsAg version 3 and Enzygnost HBsAg 5.0 assays for HBV subtype detection.

The Murex HBsAg version 3 assay showed the highest sensitivity for the detection of recombinant escape mutants, followed by the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays and the Enzygnost HBsAg 5.0 assay (Table 8). The Elecsys HBsAg assay detected only mutant F134Y/G145R in undiluted crude yeast extract. Insertion mutants (human serum) were detected by all the three assays with a variable sensitivity (Table 8).

Specificity. Eleven of 3,389 unselected samples from blood donors tested by the IMMULITE HBsAg and Prism HBsAg

TABLE 6. Comparison of different HBsAg assays for detection of acute HBV in BCP, Serologicals, NABI, and Pyramid seroconversion panels

Seroconversion panel	Sequence no. of sample (day of blood donation) with first positive result					
	IMMULITE HBsAg	IMMULITE 2000	Elecsys HBsAg	Auszyme Monoclonal	IMx HBsAg	AxSYM HBsAg
BCP 64006	8 (35)	8 (35)	7 (29)	8 (35)		8 (35)
BCP 60409	>8 (>29)	8 (29)	7 (27)	>8 (>29)		8 (29)
BCP 63701	7 (28)	7 (28)	6 (26)	>7 (>28)	>7 (>28)	7 (28)
BCP 62347	14 (54)	13 (50)	13 (50)	14 (54)		13 (50)
BCP 62675	6 (19)	6 (19)	6 (19)	7 (21)		6 (19)
BCP 61042	5 (25)	5 (25)	5 (25)	5 (25)	5 (25)	4 (14)
BCP 61066	5 (22)	5 (22)	5 (22)	5 (22)		5 (22)
BCP 63426	4 (12)	4 (12)	4 (12)	9 (33)	4 (12)	4 (12)
BCP 61832	2 (13)	2 (13)	2 (13)	2 (13)		2 (13)
BCP 62433	6 (22)	5 (19)	5 (19)	6 (22)		6 (22)
BCP 62967	11 (45)	11 (45)	11 (45)	11 (45)		11 (45)
BCP 63253	10 (70)	10 (70)	10 (70)	10 (70)		10 (70)
BCP 64121	6 (27)	6 (27)	6 (27)	7 (34)		6 (27)
Serologicals 22663	8 (28)	8 (28)	8 (28)	9 (31)		8 (28)
NABI 405	4 (11)	3 (7)	3 (7)	4 (11)		3 (7)
NABI 406	5 (14)	5 (14)	5 (14)	6 (16)		5 (14)
NABI 409	2 (3)	2 (3)	2 (3)	3 (7)		2 (3)
Pyramid RP 009	1 (0)		1 (0)	2 (2)		
No. of samples testing positive/no. of samples tested (%)[a]	168/363 (46.3)*	167/355 (47.0)*	187/361 (51.8)*	143/363 (39.4)*	73/189 (38.6)*	154/327 (47.1)*
No. of samples testing positive/no. of positive samples (%)	168/187 (89.8)	167/182 (91.8)	187/187 (100)	143/187 (76.4)	73/97 (75.2)	154/168 (91.7)

[a] *, the differences in performance between the Elecsys HBsAg assay and the alternative assays were statistically significant ($P < 0.01$ [Wilcoxon matched paired test]).

assays were IR with the IMMULITE HBsAg assay. All the samples were negative after retesting. With the Prism HBsAg assay, no initial reactivity was observed.

Of 1,733 blood donors tested in parallel with the IMMULITE HBsAg and Elecsys HBsAg assays, 10 (0.58%) anti-HBc negative samples were IR with the IMMULITE HBsAg assay. For one sample there was not enough material available for retesting. Five (0.29%) samples were RR. IMMULITE HBsAg neutralization assay was performed for four RR reactive samples. The neutralization assay was not interpretable for all four samples (a negative value was obtained for the nonneutralized control and no reduction of the signal was observed for the blocked sample). The final interpretation for these 10 serum samples was as follows: 4 samples were HBsAg negative (IMMULITE HBsAg IR), and 6 were not considered for final interpretation because not enough sample material was available for resolution of discrepant results. Of the 1,750 samples from German blood donors tested in parallel with the IMMULITE 2000 HBsAg and Elecsys HBsAg assays, 3 (0.17%) anti-HBc negative samples were IR and RR with the IMMULITE 2000 HBsAg assay. The neutralization assay was performed for two RR samples. The neutralization assay was not interpretable (a negative value was obtained for the nonneutralized control, and no reduction of the signal was observed for the blocked sample). None of the samples were considered for final interpretation, since complete confirmatory testing due to low sample volume could not be performed. The Elecsys HBsAg assay was IR for five (0.29%) anti-HBc negative samples. One (0.06%) sample was RR. There was not enough material available to perform the Elecsys HBsAg neutralization assay.

Among the routine laboratory diagnostic samples ($n = 48$), two anti-HBc-positive samples were concomitantly positive in the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays and the Elecsys HBsAg assay, and these results were confirmed by the Elecsys HBsAg neutralization assay. The Elecsys HBsAg assay gave a repeatedly positive result for an anti-HBc-negative sample. The Elecsys HBsAg neutralization assay was negative.

TABLE 7. Sensitivity of HBsAg assays for detection of HBsAg in subtypes of SFTS panel

SFTS sample no.	Genotype	Subtype	Lowest HBsAg concn (ng/ml) with positive result		
			IMMULITE HBsAg	IMMULITE 2000 HBsAg	Elecsys HBsAg
95	A	*ayw1*	0.25	0.25	0.125
96	D	*ayw2*	0.25	0.25	0.125
97	D	*ayw3*	0.25	0.125	0.125
98	E	*ayw4*	0.25	0.25	0.125
99	C	*ayr*	0.125	0.125	0.125
92	A	*adw2*	0.5	0.5	0.125
93	E	*adw4*	0.5	0.5	0.125
94	C	*adrq+*	0.5	0.5	0.125
100	C	*adrq−*	0.25	0.25	0.125

TABLE 8. Sensitivities of HBsAg assays for detection of different HBV subtypes and mutants

HBV subtype or mutant	Highest reciprocal dilution with a positive result				
	IMMULITE HBsAg or 2000 HBsAg	Elecsys HBsAg	Murex HBsAg (version 3)	Enzygnost HBsAg 5.0	AxSYM HBsAg
Subtypes					
adw2	100,000	400,000	100,000	100,000	400,000
ayw4	440,000	440,000	6,400	6,400	440,000
ayw2	1,000,000	1,000,000	200,000	200,000	1,000,000
ayw3	128,000	128,000	128,000	32,000	128,000
adr	6,000	30,000	6,000	30,000	30,000
adr/ayr	4,000	32,000	4,000	4,000	32,000
Mutants					
F134Y/G145R	10	1[a]	1,000	1[a]	ND[b]
P142S/G145R/N146D	10	—[c]	1,000	1[a]	ND

[a] Undiluted.
[b] ND, not determined.
[c] —, not detected.

Of the 200 samples from hospitalized patients, 8 anti-HBc-positive sera tested positive by the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays, in agreement with the Elecsys HBsAg assay. A total of 11 anti-HBc-negative sera were IR with the IMMULITE HBsAg assay but negative with the Elecsys HBsAg assay. For two samples, there was not enough material available for retesting, eight sera were negative after repeated testing, and sample 206 (anti-HBc negative; anti-HBs, 13 IU/liter) was RR. There was not enough material available for confirmatory testing. Two anti-HBc-positive samples were positive by the IMMULITE HBsAg assay but negative by the Elecsys HBsAg assay. The first serum sample became negative after retesting, while the second sample (anti-HBs, 15 IU/liter) remained positive. There was not enough material available for a neutralization assay. A total of eight anti-HBc negative sera were IR with the IMMULITE 2000 HBsAg assay but were negative with the Elecsys HBsAg assay. For two samples, there was not enough material available for retesting. Three sera were negative on retesting, and one was RR; there was not enough material available for a neutralization assay. Three anti-HBc-positive samples (also IMMULITE HBsAg RR) were positive with the IMMULITE 2000 HBsAg assay but negative with the Elecsys HBsAg assay. For the first sample there was not enough material available for retesting. The two others were repeatedly IMMULITE 2000 HBsAg reactive. The neutralization assay for one of them was not interpretable, while it was positive for the other one. No VIDAS HBsAg result was available. Three serum samples were IR with the Elecsys HBsAg assay but negative with the IMMULITE HBsAg assay. Two sera became negative after repeated testing. There was not enough material available for the third sample to perform repeated testing.

The results obtained by testing potentially cross-reactive serum samples are shown in Table 2. No false-positive result was observed with IMMULITE HBsAg in potentially interfering serum samples. One sample from a patient with primary Epstein-Barr virus infection was initially borderline positive with the Elecsys HBsAg assay. There was not enough material available for retesting.

DISCUSSION

The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays showed a higher sensitivity than the Auszyme Monoclonal (B) and IMx HBsAg assays for the diagnosis of acute HBV infection. The sensitivities of both IMMULITE assays were comparable to those of the AxSYM HBsAg assay. The Elecsys HBsAg assay showed a significantly higher sensitivity for seroconversion panels than did the alternative assays.

The sensitivities of the different HBsAg screening assays for HBV subtypes or genotypes and mutants were variable. The IMMULITE HBsAg and IMMULITE 2000 HBsAg assays seem to be accurate for HBsAg screening in different geographical locations, since low concentrations of different HBV subtypes, ranging between 0.125 and 0.5 pg of HBsAg/ml, were detected.

As shown by the data of the present evaluation, the sensitivity of commercial assays is variable for the different surface mutants (Table 8). Variations in the pre-S region, or mutations in the surface antigen itself and especially in the *a* determinant which is recognized by anti-HBs, may render HBsAg undetectable by HBsAg screening assays (3, 22).

The IMMULITE HBsAg assay showed an overall higher rate of initial reactivity in anti-HBc-negative blood donors and hospitalized patients than did the Elecsys HBsAg assay (0.5 versus 0.1%) and the Prism HBsAg assay (0.2 versus 0.03%), respectively. After retesting, the specificity was 100% and equal to that of the alternative assays. If the percentage of IR samples from blood donors was considered, IMMULITE HBsAg showed a false positivity rate comparable to that of the Auszyme Monoclonal (B) assay (27; I. J. Skurrie and S. M. Garland, Letter, Lancet **i:**299-300, 1988). A high rate (2.4%) of false-positive results was obtained with the IMx HBsAg assay among specimens from blood donors, hospitalized patients, pregnant women, and intravenous drug addicts (31). The IMMULITE 2000 HBsAg assay showed an initial reactivity comparable to that of the Elecsys HBsAg assay.

For the resolution of discrepant results between HBsAg screening assays it is important to consider additional HBV markers. HBsAg in combination with anti-HBc determination

TABLE 9. Calculations of sensitivity, specificity, and predictive values for various assays[a]

Test	Result	No. of samples with indicated HBsAg status		% Sensitivity[b]	% Specificity[b]	PPV (%)[b]	NPV (%)[b]
		Positive	Negative				
IMMULITE HBsAg	Positive	588	0	96.9 (95.2–98.1)	100 (99.9–100)	100 (99.5–100)	99.2 (98.7–99.5)
	Negative	19	2,254				
IMMULITE 2000 HBsAg	Positive	287	0	95.3 (92.3–97.4)	100 (99.9–100)	100 (98.9–100)	99.4 (98.9–99.7)
	Negative	14	2,208				
Elecsys HBsAg	Positive	606	0	99.8 (99.1–100)	100 (99.9–100)	100 (99.5–100)	99.9 (99.8–100)
	Negative	1	2,254				
AxSYM HBsAg	Positive	557	0	96.7 (94.9–98.0)	100 (99.5–100)	100 (95.8–100)	78.7 (68.7–86.6)
	Negative	19	70				

[a] Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b] Values in parentheses are 95% confidence intervals.

shows positive and negative predictive values of 100% (Table 9). Anti-HBc is the most important marker for confirmation of an HBsAg-positive result since the HBsAg neutralization assay may give false-positive results, especially in the presence of low HBsAg concentrations. However, during acute hepatitis B, anti-HBc may not be detected in the first days to weeks in up to 8% of the cases (20). Another explanation for isolated HBsAg-positive results may be the absence of anti-HBc antibody response as a consequence of iatrogenic or acquired immunodeficiency. Occasionally, anti-HBc reactivity is absent in patients with selective immunodeficiency or immunocompromised HBsAg carriers (2, 10, 19, 20, 25, 32–34).

The results of the present study show that there exists some degree of variability between two nearly identical assays performed on analyzers based on the same technology. This was especially evident in the case of seroconversion panels; in 9 of 38 panels tested in parallel, a difference of one bleeding day was observed between the two assays. Differences in performance were also observed for unselected blood donors. Discrepancies between identical assays performed on two separated identical analyzers are also observed with assays from other manufacturers (unpublished observations).

In conclusion, the new IMMULITE HBsAg and IMMULITE 2000 HBsAg assays show a good sensitivity for HBsAg detection, with performances equivalent to those of other immunoassays. Although the Elecsys assay is one of the most-sensitive HBsAg assays available on the international market (31), the IMMULITE HBsAg and IMMULITE 2000 HBsAg assays seem to show a better sensitivity to HBsAg mutants. However, the number of samples with recombinant HBsAg in this study was too small to draw general conclusions. The specificity observed with repeatedly tested samples was equivalent to those observed for the alternative assays. The rapid turnaround time, primary tube sampling, and on-board dilution make it an interesting assay system for clinical laboratory diagnosis.

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