IMMULITE®

HBsAg

Hepatitis B Surface Antigen

DPC

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IMMULITE® HBsAg

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established for testing of newborns.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use

IMMULITE HBsAg is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE automated immunoassay analyzer for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma (EDTA, heparinized, citrate). It is intended for *in vitro* diagnostic use for the laboratory diagnosis of acute and chronic hepatitis B virus infections in conjunction with other serological and clinical information. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates who are at high risk of acquiring HBV during the perinatal period.

Caution: Performance characteristics for the IMMULITE HBsAg were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE HBsAg or another legally-marketed HBsAg assay.

Catalog Number: **LKHB1** (100 tests), **LKHB5** (500 tests)

Test Code: **HBS** Color: **Orange**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of Hepadnaviridiae, a DNA virus, and is found world-wide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevation, and syptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear in serum is hepatitis B surface antigen (HBsAg). Presence of this antigen indicates an ongoing infection with HBV, and is detectable in the acutely ill and in chronic carriers.[1,2,4]

Principle of the Procedure

IMMULITE HBsAg is a solid-phase, two-step chemiluminescent enzyme immunoassay. The solid phase, a polystyrene bead enclosed within a Test Unit, is coated with an antibody directed against the hepatitis B surface antigen (anti-HBs).

The patient specimen is added to the Test Unit containing a coated bead. An alkaline phosphatase-labeled anti-HBs antibody is also added to the Test Unit. After the wash and incubation steps, chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase. The photon output, as measured by the luminometer, is related to the presence of HBsAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated results.[8]

The IMMULITE HBsAg assay may be performed on human serum or plasma (heparinized, sodium citrate, or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens which are not tested within 24 hours should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested.

Multiple freeze-thaw cycles are not recommended.

Volume Required: 100 µL serum, or plasma (heparinized, sodium citrate or EDTA). (Sample cup must contain at least 250 µL more than the total volume required.)

Storage: 2 days at room temperature (15°–28°C).[11]
3 days at 2–8°C.[6,11]
For longer storage: at –20°C.[7]

Warnings and Precautions

For *in vitro* diagnostic use.

Assay performance characteristics have not been established when the IMMULITE HBsAg assay is used in conjunction with other manufactures' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens. Pooled specimens must not be used.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

The HBsAg Adjustor, HBsAg Low Positive and HBsAg Positive Controls contain HBsAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

The components have been tested with FDA-approved methods and were found to contain no infectious agents such as HIV 1, HIV 2 and HCV. These components have also been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories, 1993.*

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water for the wash step and to avoid system contamination.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay. Barcodes encode information about the test, including expiration dates, component lot numbers, adjustment parameters, and cutoff parameters.

HBsAg Test Units (LHB1)
Each barcode-labeled unit contains one bead coated with murine monoclonal anti-HBs manufactured at DPC. Stable at 2-8°C until expiration date.
LKHB1: 100 units. **LKHB5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

HBsAg Reagent Wedges (LHBA, LHBB)
With barcodes. **LHBA:** 6.5 mL of a protein-based buffer, with preservative. **LHBB:** 6.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-HBs in buffer, with < 0.1 g/dL sodium azide. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKHB1: 1 set. **LKHB5:** 5 sets.

HBsAg Adjustor (LHBR)
One vial (4 mL) containing human serum with inactivated ad and ay hepatitis B viral antigens, with < 0.1 g/dL sodium azide. The Adjustor serves as the assay's Cutoff. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
LKHB1: 1 vial. **LKHB5:** 2 vials.

HBsAg Controls (LHBC1, LHBC2, LHBC3) (may be purchased separately.)
Three vials (Negative, Low Positive and Positive) containing 4 mL each. **LHBC1 (Negative Control):** human serum without HBsAg, with < 0.1 g/dL sodium azide.
LHBC2, LHBC3 (Low Positive Control, Positive Control): Human serum with inactivated ad and ay hepatitis B viral antigens, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
LKHB1: 1 set. **LKHB5:** 2 sets.

For the current control ratio ranges, please refer to the Control insert.

Kit Components Supplied Separately

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM : Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

Also Required
Sample transfer pipets, distilled or deionized water.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 6 of the IMMULITE Operator's Manual.

See Section 4 of the IMMULITE Operator's Manual for: preparation, setup, adjustment, assay and quality control procedures.

Sample cup must contain at least 250 µL more than the total volume required. Each Sample cup holder can be followed by up to four test units.

Note that both Reagent Wedges A and B must be loaded on the carousel to run this assay.

Adjustment Interval: 4 weeks.

Quality Control Samples: The control(s) supplied with the kit should be used as quality control material to monitor the performance of the assay.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

For the current control ratio ranges, please refer to the Control insert.

If control results fall outside the stated range, patient results must not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

It is recommended that the user refer to *NCCLS: Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations.[8,12]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

Calculation of Cutoff and S/CO Ratio:

The Master Cutoff of the assay was determined in an ROC analysis from 287 HBsAg positive and negative endogeous patient specimens to achieve optimal sensitivity and specificity of the assay.

The cutoff is set equal to the average counts per second (mean cps) of the Adjustor (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.)

Calculation of a signal/cutoff (s/co) ratio is done by using the following formula:

$$\text{S/CO Ratio} = \frac{\text{Sample or Control cps}}{\text{Mean Adjustor cps} \times \text{P1}}$$

Calculation and reporting of qualitative results (positive/negative) are handled automatically by the IMMULITE.

The result is reported as "Positive" if the sample's counts are above the cutoff, and "Negative" if below the cutoff.

> Although the IMMULITE automatically reports a result of "Positive" before this report is placed in the individuals records it should annotated as "Presumptively positive, confirmation testing to follow."

Interpretation of Results

A result of **"Positive"** (ratio of ≥1.0) indicates that HBsAg is presumptively present. Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.[9]

Specimens found to be initially reactive for HBsAg must be reassayed in duplicate to verify that the initially reactive result is repeatable.

If one or both of the duplicates of the reassayed sample are reactive, the patient sample must be tested using the IMMULITE HBsAg Confirmatory Assay (LKCH), a procedure to confirm the presence of HBsAg based on the neutralization of HBsAg-reactive samples with anti-HBs. 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.

A result of **"Negative"** (ratio of <1.0) indicates that HBsAg was not detected in the patient sample. The patient appears to not be infected with HBV.

A negative result does not indicate that the patient is not infected with HBV. The patient sample should be tested for the presence of other serological markers.

It is essential that results be confirmed before patients are informed of the test results.

When HBsAg is used as a stand alone assay for screening pregnant women to identify neonates who are at risk for acquiring HBV during the perinatal period, supplemental testing such as the IMMULITE HBsAg Confirmatory Kit must be used to confirm the result.

The magnitude of an IMMULITE HBsAg assay result cannot be correlated to an endpoint titer.

It is recommended that the following be included with the report to the physician:

> The following results were obtained with the IMMULITE HBsAg EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported HBsAg level cannot be correlated to an endpoint titer.

Liquid based anticoagulants may lower the signal/cutoff (s/c) values in some HBsAg presumptively reactive samples. High negative results obtained on samples collected with anticoagulants should be interpreted accordingly. Additional testing may be required.

Limitations

The ability of the IMMULITE HBsAg assay to detect HBV mutants has not been determined.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative test result does not exclude the possibility of infection with hepatitis B virus. HBsAg may be undetectable both in early infection and late after infection. In some cases HBsAg tests do not detect certain HBV mutant strains.[10]

Assay performance characteristics have not been established for any specimen matrices other than serum or heparin, EDTA, and sodium citrate anticoagulated plasma.

The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. False results may be obtained with any diagnostic test.

Lipemic, hemolyzed or grossly contaminated samples may give erroneous results and therefore should not be used.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Expected Values

Demographics and expected prevalence rates for different categories of subjects, each of whom provided one specimen, from three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4) and one in Europe, are summarized in the following tables.

Apparently Healthy Subjects (Study 1)

IMMULITE HBsAg				
Age	Gender	Total	Pos.	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	0	0.0%
	Female	5	0	0.0%
20 - 29	Male	7	0	0.0%
	Female	13	1	7.7%
30 - 39	Male	2	0	0.0%
	Female	2	0	0.0%
40 - 49	Male	1	0	0.0%
	Female	1	0	0.0%
50 - 59	Male	0	0	—
	Female	0	0	—
60 - 69	Male	0	0	—
	Female	1	0	0.0%
70 - 79	Male	0	0	—
	Female	0	0	—
80 - 89	Male	0	0	—
	Female	0	0	—
90 - 99	Male	0	0	—
	Female	0	0	—
Totals	Male	14	0	0.0%
	Female	22	1	4.5%
	All	36	1	2.8%

Other Subjects

Subject	Total	Male	Female	Age	Age Range	Pos. by IML HBsAg	
						n	%
Apparently Healthy (Europe)	1,730	Not available				9	1%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0%
Pregnant at high risk[1] (Study 1)	3	0	3	33	30-36	3	100%
Pregnant at low risk[1] (Study 1)	16	0	16	31	21-42	1	6%
Pregnant at low risk[1] (Study 4)	197	0	197	28	17-41	0	0%
Pregnant at low risk[1] (Europe)	27	Not available				0	0%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Five clinical studies with a total of 3,268 subjects were conducted to assess the performance of the IMMULITE HBsAg assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE HBsAg. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years, ranging from 21 to 85 years. Distributions of the ethnicity of the subjects are shown in the following table.

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HB Acute Infection
Positive agreement = 97.1% (67/69)
95% CI = 89.9 to 99.6%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (7/7)
95% CI = 59.0 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (40/40)
95% CI = 91.2 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.2% (119/120)
95% CI = 95.4 to 100.0%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 94.1% (16/17)
95% CI = 71.3 to 99.9%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 97.4% (74/76)
95% CI = 90.8 to 99.7%
Negative agreement = 99.0% (203/205)
95% CI = 96.5 to 99.9%
Total agreement = 98.6% (277/281)
95% CI = 96.4 to 99.6%

Study 2: Conducted in the northeastern United States, this study included 209 patients, consisting of 104 males and 103 females. Gender for two patients was not reported. These patients had an average age of 47 years, ranging from newborn to 93 years. Distributions of the ethnicity of the patients are shown in the following table.

Ethnicity	*N*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	0.7%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially cross-reactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	No. of patients	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE HBsAg results were compared to Kit A.

Reference Serological Characterization	Kit A				Total
	+		–		
	IML HBsAg				
	+	–	+	–	
HB Acute infection	12	5	0	0	17
HB Chronic infection	1	1	0	0	2
HB Early recovery	0	0	0	50	50
HBV Vaccine response	0	0	0	32	32
Not previously infected	0	0	2	105	107
Uninterpretable	1	0	0	0	1
Total	14	6	2	187	209

HB Acute Infection
Positive agreement = 70.6% (12/17)
95% CI = 44.0 to 89.7%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = N/A (1/2)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (50/50)
95% CI = 92.9 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 98.1% (105/107)
95% CI = 93.4 to 99.8%

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 70.0% (14/20)
95% CI = 45.7 to 88.1%
Negative agreement = 98.9% (187/189)
95% CI = 96.2 to 99.9%
Total agreement = 96.2% (201/209)
95% CI = 92.6 to 98.3%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	–	+/-	+	+	–
Acute	8	+	+/-	+	+	+	–
Acute	1	+	–	+	+/-	–	–
Acute	35	+	+	+/-	+	–	–
Acute	4	+	+	+	+	–	+/-
Chronic	1	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	–
Chronic	1	+	+	+/-	+	–	+
Chronic	1	+	+	+	+	+	+
Recovered	1	–	–	–	+/-	–	+
Uninterpretable	1	+	–	+	+	+	+

Based on the above classifications the IMMULITE HBsAg results were compared to Kit A.

Reference Serological Characterization	Kit A				Total
	+		–		
	IML HBsAg				
	+	–	+	–	
HB Acute infection	70	1	0	0	71
HB Chronic infection	6	0	0	0	6
Recovered	0	0	0	1	1
Uninterpretable	1	0	0	0	1
Total	77	1	0	1	79

HB Acute Infection
Positive agreement = 100.0% (70/70)
95% CI = 94.9 to 100.0%
Negative agreement = N/A (1/1)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Recovered
Positive agreement = N/A (0/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 98.7% (77/78)
95% CI = 93.1 to 100.0%
Negative agreement = N/A (1/1)
95% CI = N/A
Total agreement = 98.7% (78/79)
95% CI = 93.1 to 100.0%

Study 4: Conducted in the southern United States, this study included retrospectively collected specimens from 200 pregnant subjects. These subjects had an average age of 28 years, ranging from 17 to 41 years.

A total of 200 specimens were tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated three unique patterns:

Characterization based on single point specimens	# of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	–	+/-	+	+
Early recovery	2	–	–	+	–
HBV vaccine response	42	–	–	–	+
Not previously infected	152*	–	–	–	–

Based on the above classifications the IMMULITE HBsAg results were compared to Kit A.

Reference Serological Characterization	Kit A				Total
	+		–		
	IML HBsAg				
	+	–	+	–	
Early recovery	0	0	0	6	6
HBV vaccine response	0	0	0	42	42
Not previously infected	0	0	1	148	149*
Total	0	0	1	196	197

* Three specimens were not tested for IMMULITE HBsAg.

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (42/42)
95% CI = 91.6 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.3% (148/149)
95% CI = 96.3 to 100.0%

Total
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.5% (196/197)
95% CI = 97.2 to 100.0%
Total agreement = 99.5% (196/197)
95% CI = 97.2 to 100.0%

Study 5: In a clinical study conducted in Europe, IMMULITE HBsAg was compared to Kit B, a commercially available HBsAg electrochemiluminescence immunoassay. Presented below are the comparisons between IMMULITE HBsAg and Kit B on a

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total of 2,499 specimens (one specimen per subject).

Kit B				
+		−		
IML HBsAg				
+	−	+	−	Total
412	12	26	2,049	2,499

Positive agreement = 97.2% (412/424)
95% CI = 95.1 to 98.5%
Negative agreement = 98.7% (2049/2075)
95% CI = 98.2 to 99.2%
Total agreement = 98.5% (2461/2499)
95% CI = 97.9 to 98.9%

II. Analytical Performance

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed as a signal-to-cutoff ratio. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Analytical Sensitivity: Based on studies with serial dilution of WHO International Standard for Hepatitis B Surface Antigen (NIBSC 80/549), the threshold sensitivity (last positive dilution) for IMMULITE HBsAg is 0.063 IU/mL.

The 95% confidence interval at this level (0.063 IU/mL) is 0.040 - 0.086 IU/mL.

Precision: Serum samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates at three US sites. The same design was used for three lots and at three sites. (See "Precision" tables.)

EDTA, heparin, and sodium citrate samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE HBsAg and one lot of IMMULITE 2000 HBsAg. The median total variance of coefficients (EDTA, 5.2%; heparin, 7.2%; sodium citrate, 5.9%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 HBsAg.

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: May have an effect on the assay, causing an increase in values.

Lipemia: May have an effect on the assay, causing an increase in values. The use of an ultracentrifuge is recommended to clear lipemic samples.

Alternate Sample Type: The measurement of HBsAg is not significantly affected by the presence of heparin, sodium citrate, or EDTA anti-coagulants, as shown in a study that included 60 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression: (in cutoff-to-signal ratio)

(Heparin) = 1.03 (Serum) + 1.10
r = 1.00

(NaCitrate) = 1.03 (Serum) + 0.74
r = 1.00

(EDTA) = 1.01 (Serum) + 0.41
r = 1.00

Means:
168 (Serum)
174 (Heparin)
173 (NaCitrate)
170 (EDTA)

(See "Alternate Sample Types Graphs".)

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and at one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE HBsAg and a commercially available enzyme immunoassay for HBsAg (Kit A), with the following results:

	Kit A				
	+		−		
	IMMULITE HBsAg				
Sample type	+	−	+	−	Total
HAV	5	0	0	42	47
HCV	4	0	0	68	72
HDV	11	0	0	5	16
HEV	0	0	0	10	10
Non-viral liver diseases[1]	0	4	1	49	54
Autoimmune diseases	0	1	0	24	25
CMV	1	0	1	11	13
EBV	1	0	1	17	19
Syphilis	0	0	0	11	11
Toxoplasma	2	0	0	18	20
HSV	1	2	1	43	47
Parvovirus B19	0	0	0	15	15
HIV	8	0	1	42	51
Influenza vaccine recipient	0	0	0	25	25
Transplant recipient	0	0	0	14	14
Dialysis	0	1	1	31	33
Intravenous drug abuser	0	0	0	5	5
Others[2]	2	0	0	4	6
Total	35	8	7	433	483

[1] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[2] Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, eight specimens from antinuclear antibody (ANA) positive patients and 30 from patients with positive rheumatoid factor (RF) were tested by IMMULITE HBsAg. IMMULITE HBsAg test results were all negative for these 38 specimens.

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8 NCCLS. *Internal Quality Control: Principles and Definitions; Approved Guideline.* NCCLS document C24-A (ISBN 1-56238-112-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1991.

9 Kloster B, Kramer R, Eastlund T, Grossman B, Zarvan B. Hepatitis B surface antigenemia in blood donors following vaccination. Transfusion. 35:475-477; 1995.

10 Carmen WF. The clinical significance of surface antigen variants of hepatitis B virus. *Journal of Viral Hepatitis.* 4 (Suppl. 1):11-20; 1997.

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12 Code of Federal Regulations, Title 42, Part 493.1201-493.1285, Subpart K- *Quality Control for Tests of Moderate Complexity*, Volume 3. U.S. Government Printing Office; 1993.

Technical Assistance

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The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

Tables and Graphs

Precision (ratio)

Site 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.44	0.033	7.5%	0.039	8.9%
2	0.44	0.056	12.6%	0.073	16.5%
3	1.19	0.072	6.0%	0.078	6.5%
4	1.23	0.087	7.1%	0.165	13.4%
5	1.44	0.102	7.1%	0.126	8.7%
6	2.96	0.173	5.9%	0.191	6.4%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.41	0.043	10.5%	0.051	12.2%
2	0.42	0.059	14.1%	0.079	18.9%
3	1.18	0.067	5.7%	0.118	10.0%
4	1.20	0.059	4.9%	0.156	13.0%
5	1.37	0.059	4.3%	0.180	13.1%
6	2.86	0.163	5.7%	0.356	12.5%

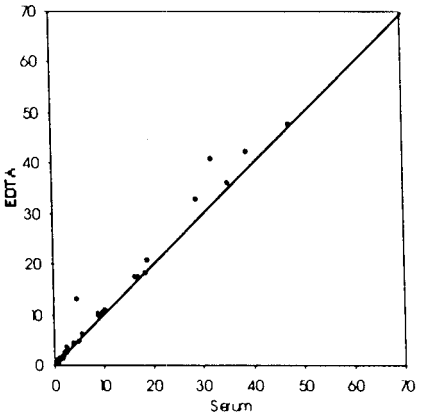
Site 3

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.44	0.051	11.8%	0.071	16.3%
2	0.42	0.044	10.6%	0.056	13.4%
3	1.14	0.089	7.8%	0.122	10.7%
4	1.13	0.086	7.6%	0.166	14.7%
5	1.43	0.062	4.3%	0.146	10.2%
6	2.86	0.128	4.5%	0.322	11.3%

Lot-to-Lot and Site-to-Site

		Lot-to-Lot		Site-to-Site	
	Mean	SD	CV	SD	CV
1	0.47	0.062	13.2%	0.063	13.4%
2	0.47	0.069	14.7%	0.069	14.9%
3	1.17	0.095	8.1%	0.099	8.5%
4	1.19	0.157	13.2%	0.165	13.8%
5	1.50	0.181	12.1%	0.182	12.1%
6	2.85	0.266	9.3%	0.273	9.6%

Alternate Sample Types Graphs (ratio)



Regression equation?

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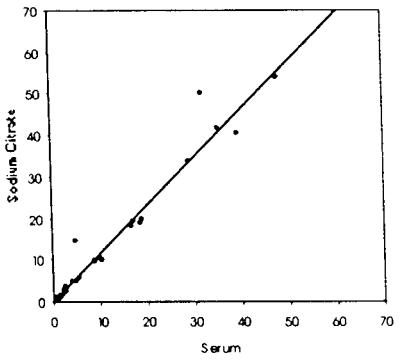

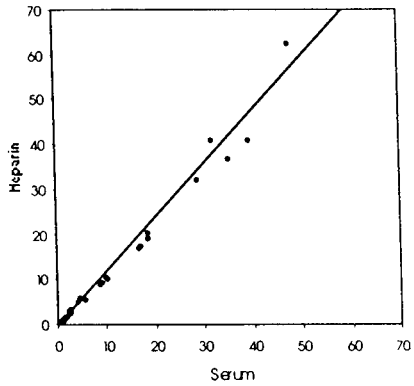

Needs regression equations

Note: Three specimens fall outside the range depicted in the graphs above.

Diagnostic Products Corporation
5700 West 96th Street
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2002-07-26 (ISO 8601)

December 2, 2002

PILKHB – 6

IMMULITE®

HBsAg Confirmatory Kit

For Confirming the Detection of Hepatitis B Surface Antigen

DPC®

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IMMULITE® HBsAg Confirmatory Kit

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established for testing of newborns.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE HBsAg Confirmatory is intended for *in vitro* diagnostic use in conjunction with the IMMULITE HBsAg or the IMMULITE 2000 HBsAg assays – for the confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum or plasma (EDTA, heparinized, citrate) that were repeatedly positive when tested by IMMULITE or IMMULITE 2000 HBsAg.

Caution: Performance characteristics for the IMMULITE HBsAg Confirmatory Kit were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE HBsAg Confirmatory Kit or another legally-marketed HBsAg confirmatory assay.

Catalog Number: **LKCH**

Summary and Explanation of the Test

In assays for HBsAg wherein the signal (instrument response) is directly related to the antigen concentration, the presence of HBsAg in a sample can be confirmed by demonstrating a significant reduction in signal following specific antibody neutralization. The signal reduction reflects a reduction in binding of HBsAg to the solid-phase antibodies in the presence of blocking antibodies. A sufficiently decreased signal relative to that of a control sample confirms a positive HBsAg result.

Principle of the Procedure

The IMMULITE HBsAg Confirmatory Kit is used in conjunction with the IMMULITE HBsAg assay or the IMMULITE 2000 HBsAg assay to confirm the presence of HBsAg in a patient sample that has tested repeatedly reactive for HBsAg.

In the confirmatory procedure, an undiluted sample and a 1:500 dilution of the sample are each divided into two aliquots. (Occasionally, a still higher dilution is required to demonstrate specific antibody neutralization.) One aliquot is combined with a blocking reagent (containing goat anti-HBs), the other with a control reagent (lacking the antibody).

For HBsAg-positive samples, suitably diluted, in the aliquots containing the blocking reagent, most of the HBsAg present in solution binds to the blocking antibody and does not bind to the coated bead; whereas, in the aliquots containing the control reagent, any HBsAg present in the sample remains free to bind to the coated bead.

The original patient sample is confirmed positive for the presence of HBsAg if the signal from *either* the undiluted *or* diluted aliquots of the "blocked" (antibody-neutralized) aliquots is at least 50% less than the signal from the corresponding "unblocked" (control) aliquot.

Specimen Collection and Preparation

Collect and store serum or plasma samples as instructed in the IMMULITE HBsAg or IMMULITE 2000 HBsAg package insert.

Storage: 2 days at room temperature (15°–28°C).[2]
3 days at 2–8°C.[1,2]
For longer storage: at –20°C.[3]

Minimum Volume Required: 1 mL.

Warnings and Precautions

For *in vitro* diagnostic use.

Assay performance characteristics have not been established for testing of newborns.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; and for antibodies to hepatitis C.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories, 1993.*

Sodium azide, at concentrations less than 0.1 g/dL, has been added to certain components as a preservative. Upon disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Materials Supplied

HBsAg Confirmatory Assay Reagent 1 (LCH1)
(Blocking Reagent)
One amber glass vial (1.2 mL) of polyclonal goat antibody against HBsAg in a protein-based buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

HBsAg Confirmatory Assay Reagent 2 (LCH2)
(Control Reagent)
One amber glass vial (1.2 mL) of a protein-based buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquoted) at –20°C.

HBsAg Confirmatory Assay Sample Diluent
(LCHZ4)
For the serial dilution of patient samples. Two bottles (100 mL each) of protein-based buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquoted) at –20°C.

HBsAg Positive Control (LHBC3) (may be purchased separately.)
One amber glass vial (4 mL) of human serum with inactivated ad and ay hepatitis B viral antigens, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

Kit Components Supplied Separately

IMMULITE HBsAg (LKHB) or IMMULITE 2000 HBsAg (L2KHB) — and other materials as required for these assays

Also Required
Test tubes; pipets for delivering 5 µL, 50 µL, 500 µL and 2.5 mL; sample transfer pipets

Assay Procedure

See flowchart.

1 Prepare off-line dilution(s). Dilute each serum or plasma specimen, using HBsAg Confirmatory Assay Sample Diluent and suitably labeled test tubes, as follows:

1-in-1, i.e. undiluted — all samples, *including* the HBsAg Positive Control.

1:500 — all samples, *except* the HBsAg Positive Control.
Prepare a dilution of approximately 1:500, e.g. by adding **5 µL** serum or plasma to **2.5 mL** Sample Diluent. Mix thoroughly. *Use within 2 hours.*

1:25,000 — only when indicated. For patient samples which have failed to *confirm* the 1-in-1 or 1:500 dilutions (i.e. for samples which may have exceptionally high HBsAg levels), also prepare a dilution of approximately 1:25000, e.g. by adding **50 µL** of the 1:500 dilution prepared above to **2.5 mL** Sample Diluent. Mix thoroughly.

2 For *each* of these dilutions, prepare "blocked" and "unblocked" samples in suitably labeled test tubes, as follows:

Blocked Sample.
Into one tube, add **500 µL** of the sample (diluted or undiluted) to **50 µL** of HBsAg Confirmatory Assay

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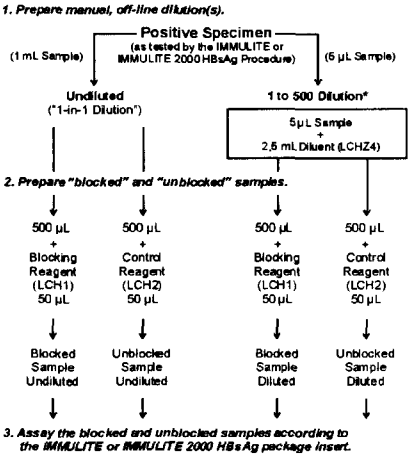
Reagent 1 (Blocking Reagent). Mix thoroughly.

Unblocked Sample.
Into a second tube, add **500 µL** of the sample (diluted or undiluted) to **50 µL** of HBsAg Confirmatory Assay Reagent 2 (Control Reagent). Mix thoroughly.

3 Assay the blocked and unblocked samples using the IMMULITE HBsAg or IMMULITE 2000 HBsAg, following instructions in the assay's package insert.

Flowchart

See text for details.



*The 1-to-500 dilution is not appropriate for the HBsAg Positive Control.

Quality Control

The HBsAg Positive Control, supplied with the IMMULITE HBsAg or IMMULITE 2000 HBsAg kit, is required and is used as quality control material to monitor assay performance of the IMMULITE HBsAg Confirmatory Kit. (Note that the 1:500 dilution specified in the Assay Procedure above is not appropriate for this control.)

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

If control results fall outside the stated range patient results should not be reported. Investigate and determine the cause for the unacceptable control results.

When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

It is recommended that the user refer to *NCCLS: Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations.[4]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Interpretation of Results

The patient sample should have previously tested presumptively positive for HBsAg by the IMMULITE HBsAg assay or by the IMMULITE 2000 HBsAg assay. The HBsAg Confirmatory Kit is not intended for HBsAg negative specimens.

Calculation of percent signal (expressed in cps) reduction is done by using the following formula:

$$\%\ \text{signal (cps) reduction} = \frac{\text{signal unblocked} - \text{signal blocked}}{\text{signal unblocked} - \text{signal negative control}} \times 100$$

Results of the assay are valid only if the HBsAg Positive Control demonstrates a signal reduction of ≥ 50%.

If the percent signal reduction of a patient sample for any dilution (1:1, 1:500 or 1:25,000) is ≥ 50%, the patient sample is *confirmed positive* for HBsAg. The patient appears to be infected with HBV.

Otherwise, the original HBsAg result is *not confirmed positive* for HBsAg. The patient appears to not be infected with HBV.

If the signal (cps) of a neat patient specimen is less than the average signal of the adjustors (i.e., a negative specimen), the formula above must not be used.

A negative result does not indicate that the patient is not infected with HBV. The patient sample should be tested for the presence of other serological markers.

The HBsAg results determined for a given specimen with assays from different manufacturers can vary due to differences

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in assay methods and reagent specificity. Therefore, it is recommended that the results reported by the laboratory to the physician include: "The following results were obtained with the IMMULITE HBsAg Confirmatory Kit. Results obtained from other manufacturers' assay methods may not be used interchangeably."

Limitations

The results of the test must be taken within the context of the patient's clinical history, symptomology and other laboratory findings.

The magnitude of an IMMULITE HBsAg assay result cannot be correlated to an endpoint titer.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

Assay performance characteristics have not been established for any specimen matrices other than serum or heparin, EDTA, and sodium citrate anticoagulated plasma.

Clinical Performance

In a European study, specimens with positive HBsAg results by IMMULITE HBsAg were further tested by the IMMULITE HBsAg Confirmatory procedure. The results are shown in the following table.

Specimens	HBsAg Confirmatory
273 positive by IMMULITE HBsAg	267 confirmed positive
	6 not confirmed*

* The six specimens not confirmed all had extremely high counts (CPS). Further dilution of these specimens (not done) would probably have confirmed the positivity of these specimens.

In one clinical study in the United States, 200 specimens from apparently healthy pregnant women with an average age of 28 years were tested by the IMMULITE 2000 HBsAg assay, Kit A, and the IMMULITE HBsAg Confirmatory procedure. The results are shown in the following table.

Specimens	HBsAg Confirmatory
200 negative by IMMULITE 2000 HBsAg and Kit A	200 not confirmed positive

In two other clinical studies in the United States, test results of 19 specimens (6 females, 13 males, with an average age of 47 years) were found to be discordant either between IMMULITE HBsAg and a commercially available HBsAg assay (Kit A), or between IMMULITE 2000 HBsAg and Kit A. These specimens were retested by all three assays and the IMMULITE HBsAg Confirmatory procedure. The results are shown in the following table.

Specimens	Final Determination*	HBsAg Confirmatory
19 specimens with discordant HBsAg results	17 negative	17 not confirmed positive
	2 positive	1 confirmed positive 1 not confirmed positive

* Negative if 2/3 or 3/3 results were negative. Positive if 2/3 or 3/3 results were positive.

In an additional study conducted in-house, 38 specimens that had tested positive by IMMULITE HBsAg were further tested by the IMMULITE HBsAg Confirmatory procedure and a commercially available HBsAg Confirmatory assay (Kit A).

Kit A				
+		−		
IML HBsAg Confirmatory				
+	−	+	−	Total
37	0	1*	0	38

Positive agreement = 100.0% (37/37)
95% CI = 90.5 to 100%
Negative agreement = N/A (0/1)
95% CI = N/A
Total agreement = 97.4% (37/38)
95% CI = 86.2 to 99.9%

* This specimen had a signal reduction of 59% by IMMULITE HBsAg Confirmatory and 48% by Kit A.

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Performance with Plasma Specimens

In a study conducted in the northwestern United States, blood specimens from 18 chronic hepatitis B patients at different stages of disease were drawn into plain, heparinized, sodium citrate and EDTA vacutainer tubes. All serum and all plasma specimens were tested by IMMULITE HBsAg and IMMULITE 2000 HBsAg. Positive results were further tested by IMMULITE HBsAg Confirmatory and all were confirmed positive.

Specimens	IML HBsAg Results	IML 2000 HBsAg Results	HBsAg Confirmatory
18 Serum	17/18 positive	17/18 positive	All 17 Confirmed
18 Heparinized	17/18 positive	17/18 positive	All 17 Confirmed
18 Sodium citrate	17/18 positive	17/18 positive	All 17 Confirmed
18 EDTA	17/18 positive	17/18 positive	All 17 Confirmed

References

1)Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: W.B. Saunders, 1995:322-4. 2)NCCLS: *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline-Second Edition.* NCCLS document H18-A2 (ISBN 1-56238-388-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1999. 3)National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998. 4)NCCLS: *Internal Quality Control: Principles and Definitions; Approved Guideline.* NCCLS document C24-A (ISBN 1-56238-112-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1991. 5)Kloster B, Kramer R, Eastlund T, Grossman B, Zarvan B. Hepatitis B surface antigenemia in blood donors following vaccination. *Transfusion.* 35:475-477; 1995. 6)Carmen WF. The clinical significance of surface antigen variants of hepatitis B virus. *Journal of Viral Hepatitis.* 4 (Suppl. 1):11-20; 1997.

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5700 West 96th Street
Los Angeles, CA 90045-5597

2002-07-26 (ISO 8601)

December 2, 2002

PILKCH – 7

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Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially cross-reactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

Characterization based on single point specimens	No	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	2	+	–	–	–	–	–
Acute	1	+	+	–	–	–	–
Acute	32	+	–	+/-	+	+	–
Acute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE HBsAg results were compared to Kit A, a reference assay for the determination of HBsAg.

Reference Serological Characterization	Kit A				Total
	+		–		
	IML HBsAg				
	+	–	+	–	
HB Acute infection	67	2	0	0	69
HB Chronic infection	7	0	0	0	7
HB Early recovery	0	0	0	40	40
HBV Vaccine response	0	0	0	27	27
Not previously infected	0	0	1	119	120
Recovered	0	0	1	16	17
Uninterpretable	0	0	0	1	1
Total	74	2	2	203	281

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IMMULITE 2000

HBsAg

Hepatitis B Surface Antigen

DPC

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IMMULITE® 2000 HBsAg

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established for testing of newborns.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE 2000 HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE 2000 HBsAg is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE 2000 automated immunoassay analyzer for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma (EDTA, heparinized, citrate). It is intended for *in vitro* diagnostic use for the laboratory diagnosis of acute and chronic hepatitis B virus infections in conjunction with other serological and clinical information. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates who are at high risk of acquiring HBV during the perinatal period.

Caution: Performance characteristics for the IMMULITE 2000 HBsAg were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE 2000 HBsAg or another legally-marketed HBsAg assay.

Catalog Number: **L2KHB2** (200 tests), **L2KHB6** (600 tests)

Test Code: **HBS** Color: **Orange**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of Hepadnaviridiae, a DNA virus, and is found world-wide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevation, and syptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear in serum is hepatitis B surface antigen (HBsAg). Presence of this antigen indicates an ongoing infection with HBV, and is detectable in the acutely ill and in chronic carriers.[1,2,4]

Principle of the Procedure

IMMULITE 2000 HBsAg is a solid-phase, two-step chemiluminescent enzyme immunoassay. The solid phase, a polystyrene bead, is coated with an antibody directed against the hepatitis B surface antigen (anti-HBs).

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The patient specimen is added to the Reaction Tube containing a coated bead. An alkaline phosphatase-labeled anti-HBs antibody is also added to the Reaction Tube. After the wash and incubation steps, chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase. The photon output, as measured by the luminometer, is related to the presence of HBsAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence, the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result.[8]

The IMMULITE 2000 HBsAg assay may be performed on human serum or plasma (heparinized, sodium citrate, or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens, which are not tested within 24 hours, should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested.

Multiple freeze-thaw cycles are not recommended.

Volume Required: 100 µL serum, or plasma (heparinized, sodium citrate or EDTA).

Storage: 2 days at room temperature (15°–28°C).[11]
3 days at 2–8°C.[6,11]
For longer storage: at –20°C.[7]

Warnings and Precautions

For *in vitro* diagnostic use.

Assay performance characteristics have not been established when the IMMULITE 2000 HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens. Pooled specimens must not be used.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

The HBsAg Adjustor, HBsAg Low Positive and HBsAg Positive Controls contain HBsAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

The components have been tested with FDA-approved methods and were found to contain no infectious agents such as HIV 1, HIV 2 and HCV. These components have also been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories, 1993.*

Follow universal precautions, and handle all components as if capable of

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transmitting infectious agents. Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water for the wash and to avoid system contamination.

Materials Supplied

The principal components — Bead Pack, Reagent Wedge, and Adjustor — represent a matched set. Barcodes encode information about the test, including expiration dates, component lot numbers, and cutoff parameters.

HBsAg Bead Pack (L2HB12)
With barcode. 200 beads coated with murine monoclonal anti-HBs manufactured at DPC. Stable at 2-8°C until the expiration date marked on the label.
L2KHB2: 1 Pack. **L2KHB6:** 3 Packs.

HBsAg Reagent Wedge (L2HBA2)
With barcodes. 11.5 mL of a protein-based buffer, with preservative. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-HBs in buffer, with < 0.1 g/dL sodium azide. Store refrigerated: stable at 2–8°C until the expiration date marked on the wedge.
L2KHB2: 1 Wedges. **L2KHB6:** 3 Wedges.

Before use, tear off the top of the label at the perforations without damaging the barcode on the main label. Remove the foil seal from the top of the Reagent Wedge, and snap the sliding cover down into the ramps on the reagent lid.

HBsAg Adjustor (LHBR)
One vial (4 mL) containing human serum with inactivated ad and ay hepatitis B viral antigens, with < 0.1 g/dL sodium azide. The Adjustor serves as the assay's Cutoff. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
L2KHB2: 1 vial. **L2KHB6:** 2 vials.

HBsAg Controls (LHBC1, LHBC2, LHBC3) (may be purchased separately.) Three vials (Negative, Low Positive and Positive) containing 4 mL each. **LHBC1 (Negative Control):** human serum without HBsAg, with < 0.1 g/dL sodium azide. **LHBC2, LHBC3 (Low Positive Control, Positive Control):** Human serum with inactivated ad and ay hepatitis B viral antigens, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
L2KHB2: 1 set. **L2KHB6:** 2 sets.

For the current control ratio ranges, please refer to the Control insert.

Aliquot Labels with barcodes are supplied with the kit, for use with the Adjustor and Controls. Before use, place the appropriate Aliquot Labels on standard test tubes, so the barcodes can be read by the barcode reader on the IMMULITE 2000.

Kit Components Supplied Separately

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
L2RXT: Reaction Tubes (disposable)

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 3 of the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual Section 6 for routine operation procedures (preparation, setup, assay, and quality control) and Section 4 for the adjustment procedure.

Adjustment Interval: 4 weeks.

Quality Control Samples: The control(s) supplied with the kit should be used as quality control material to monitor the performance of the assay.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The

user should provide alternate control material for a plasma matrix.

For the current control ratio ranges, please refer to the Control insert.

If control results fall outside the stated range, patient results must not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

It is recommended that the user refer to *NCCLS: Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations.[8,12]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

Calculation of Cutoff and S/CO Ratio:

The Master Cutoff of the assay was determined in an ROC analysis from 287 HBsAg positive and negative endogeous patient specimens to achieve optimal sensitivity and specificity of the assay.

The cutoff is set equal to the average counts per second (mean cps) of the Adjustor (from the most recent adjustment) multiplied by Curve Parameter 1. (See the "Low Adjustor CPS" and "Curve Parameter 1" fields in the IMMULITE 2000 Kit Information screen, which can be accessed from the menu via Data Entry: Kit Entry.)

Calculation of a signal/cutoff (s/co) ratio is done by using the following formula:

$$\text{S/CO Ratio} = \frac{\text{Sample or Control cps}}{\text{Mean Adjustor cps} \times \text{P1}}$$

Calculation and reporting of qualitative (positive/negative) and s/co ratio results are handled automatically by the IMMULITE 2000.

The result is reported as "Positive" if the sample's counts are above the cutoff, and "Negative" if below the cutoff.

> Although the IMMULITE automatically reports a result of "Positive" before this report is placed in the individuals records it should annotated as "Presumptively positive, confirmation testing to follow."

Interpretation of Results

A result of "**Positive**" (ratio of ≥1.0) indicates that HBsAg is presumptively present. Individuals recently vaccinated for hepatitis B may give a transient positive result for HBsAg because of its presence in the vaccine.[9]

Specimens found to be initially reactive for HBsAg must be reassayed in duplicate to verify that the initially reactive result is repeatable.

If one or both of the duplicates of the reassayed sample are reactive, the patient sample must be tested using the IMMULITE HBsAg Confirmatory Assay (LKCH), a procedure to confirm the presence of HBsAg based on the neutralization of HBsAg-reactive samples with anti-HBs. 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.

A result of "**Negative**" (ratio of <1.0) indicates that HBsAg was not detected in the patient sample. The patient appears to not be infected with HBV.

A negative result does not indicate that the patient is not infected with HBV. The patient sample should be tested for the presence of other serological markers.

It is essential that results be confirmed before patients are informed of the test results.

In instances where HBsAg is used as a stand alone assay (for example in pregnant women being screened to identify neonates who are at risk for acquiring HBV during the perinatal period),

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it is suggested that supplemental testing such as the IMMULITE HBsAg Confirmatory Kit be used to confirm the result.

The magnitude of a IMMULITE 2000 HBsAg assay result cannot be correlated to an endpoint titer.

When HBsAg is used as a stand alone assay for screening pregnant women to identify neonates who are at risk for acquiring HBV during the perinatal period, supplemental testing such as the IMMULITE HBsAg Confirmatory Kit must be used to confirm the result.

The magnitude of an IMMULITE HBsAg assay result cannot be correlated to an endpoint titer.

It is recommended that the following be included with the report to the physician:

> The following results were obtained with the IMMULITE 2000 HBsAg EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported HBsAg level cannot be correlated to an endpoint titer.

Liquid based anticoagulants may lower the signal/cutoff (s/c) values in some HBsAg presumptively reactive samples. High negative results obtained on samples collected with anticoagulants should be interpreted accordingly. Additional testing may be required.

Limitations

The ability of the IMMULITE HBsAg assay to detect HBV mutants has not been determined.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

HBsAg results should only be used and interpreted in the context of the overall clinical picture. A negative test result does not exclude the possibility of infection with hepatitis B virus. HBsAg may be undetectable both in early infection and late after infection. In some cases HBsAg tests do not detect certain HBV mutant strains.[10]

Lipemic, hemolyzed or grossly contaminated samples may give erroneous results.

Assay performance characteristics have not been established for any specimen matrices other than serum, or heparinized, EDTA, or sodium citrate plasma.

The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. False results may be obtained with any diagnostic test.

Lipemic, hemolyzed or grossly contaminated samples may give erroneous results and therefore should not be used.

Lipemic, hemolyzed or grossly contaminated samples may give erroneous results.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Expected Values

Demographics and expected prevalence rates for different categories of subjects, each of whom provided one specimen, from three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4) and one in Europe, are summarized in the following tables.

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Apparently Healthy Subjects (Study 1)

IMMULITE 2000 HBsAg				
Age	Gender	Total	Pos.	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	0	0.0%
	Female	5	0	0.0%
20 - 29	Male	7	0	0.0%
	Female	13	2	15.4%
30 - 39	Male	2	0	0.0%
	Female	2	0	0.0%
40 - 49	Male	1	0	0.0%
	Female	1	0	0.0%
50 - 59	Male	0	0	—
	Female	0	0	—
60 - 69	Male	0	0	—
	Female	1	0	0.0%
70 - 79	Male	0	0	—
	Female	0	0	—
80 - 89	Male	0	0	—
	Female	0	0	—
90 - 99	Male	0	0	—
	Female	0	0	—
Totals	Male	14	0	0.0%
	Female	22	2	9.1%
	All	36	2	5.6%

Other Subjects

Subject	Total *n*	Male	Female	Median *n*	Age Range	Pos. by IML 2000 HBsAg	
						n	%
Apparently Healthy (Europe)	1,743	Not available				3	0%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0%
Pregnant at high risk[1] (Study 1)	3	0	3	33	30-36	3	100%
Pregnant at low risk[1] (Study 1)	16	0	16	31	21-42	1	6%
Pregnant at low risk[1] (Study 4)	200	0	200	28	17-41	0	0%
Pregnant at low risk[1] (Europe)	25	Not available				0	0%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Five clinical studies with a total of 2,968 subjects were conducted to assess the performance of the IMMULITE 2000 HBsAg assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE 2000 HBsAg. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years, ranging from 21 to 85

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years. Distributions of the ethnicity of the subjects are shown in the following table.

Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially cross-reactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

Characterization based on single point specimens	No	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	2	+	–	–	–	–	–
Acute	1	+	+	–	–	–	–
Acute	32	+	–	+/-	+	+	–
Acute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE 2000 HBsAg results were compared to Kit A, a reference assay for the determination of HBsAg.

Reference Serological Characterization	Kit A				Total
	+		–		
	IML 2000 HBsAg				
	+	–	+	–	
HB Acute infection	67	2	0	0	69
HB Chronic infection	7	0	0	0	7
HB Early recovery	0	0	2	38	40
HBV Vaccine response	0	0	0	27	27
Not previously infected	0	0	6	114	120
Recovered	0	0	1	16	17
Uninterpretable	0	0	0	1	1
Total	74	2	9	196	281

HB Acute Infection
Positive agreement = 97.1% (67/69)
95% CI = 89.9 to 99.6%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (7/7)
95% CI = 59.0 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 95.0% (38/40)
95% CI = 83.1 to 99.4%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 95.0% (114/120)
95% CI = 89.4 to 98.1%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 94.1% (16/17)
95% CI = 71.3 to 99.9%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 97.4% (74/76)
95% CI = 90.8 to 99.7%
Negative agreement = 95.6% (196/205)
95% CI = 91.8 to 98.0%
Total agreement = 96.1% (270/281)
95% CI = 93.1 to 98.0%

Study 2: Conducted in the northeastern United States, this study included 209 patients, consisting of 104 males and 103 females. Gender for two patients was not reported. These patients had an average age of 47 years, ranging from newborn to 93 years. Distributions of the ethnicity of the patients are shown in the following table.

Ethnicity	*N*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	1.4%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially cross-reactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	No. of patients	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE 2000 HBsAg results were compared to Kit A.

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Reference Serological Characterization	Kit A				Total
	+		−		
	IML 2000 HBsAg				
	+	−	+	−	
HB Acute infection	11	6	0	0	17
HB Chronic infection	1	1	0	0	2
HB Early recovery	0	0	1	49	50
HBV Vaccine response	0	0	0	32	32
Not previously infected	0	0	1	106	107
Uninterpretable	1	0	0	0	1
Total	13	7	2	187	209

HB Acute Infection
Positive agreement = 64.7% (11/17)
95% CI = 38.3 to 85.8%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = N/A (1/2)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 98.0% (49/50)
95% CI = 89.4 to 99.9%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.1% (106/107)
95% CI = 94.9 to 100.0%

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 65.0% (13/20)
95% CI = 40.8 to 84.6%
Negative agreement = 98.9% (187/189)
95% CI = 96.2 to 99.9%
Total agreement = 95.7% (200/209)
95% CI = 92.0 to 98.0%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	−	+/-	+	+	−
Acute	8	+	+/-	+	+	+	−
Acute	1	+	−	+	+/-	−	−
Acute	35	+	+	+/-	+	−	−
Acute	4	+	+	+	+	−	+/-
Chronic	1	+	−	−	+	−	−
Chronic	3	+	+/-	−	+	+	−
Chronic	1	+	+	+/-	+	−	+
Chronic	1	+	+	+	+	+	+
Recovered	1	−	−	−	+/-	−	+
Uninterpretable	1	+	−	+	+	+	+

Based on the above classifications the IMMULITE 2000 HBsAg results were compared to Kit A.

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Reference Serological Characterization	Kit A				Total
	+		−		
	IML 2000 HBsAg				
	+	−	+	−	
HB Acute infection	71	0	0	0	71
HB Chronic infection	6	0	0	0	6
Recovered	0	0	0	1	1
Uninterpretable	1	0	0	0	1
Total	78	0	0	1	79

HB Acute Infection
Positive agreement = 100.0% (71/71)
95% CI = 94.9 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Recovered
Positive agreement = N/A (0/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 98.7% (78/79)
95% CI = 93.1 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A
Total agreement = 98.7% (78/79)
95% CI = 93.1 to 100.0%

Study 4: Conducted in the southern United States, this study included retrospectively collected specimens from 200 pregnant subjects. These subjects had an average age of 28 years, ranging from 17 to 41 years.

A total of 200 specimens were tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated three unique patterns:

Characterization based on single point specimens	# of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	−	+/-	+	+
Early recovery	2	−	−	+	−
HBV vaccine response	42	−	−	−	+
Not previously infected	152	−	−	−	−

Based on the above classifications the IMMULITE 2000 HBsAg results were compared to Kit A.

Reference Serological Characterization	Kit A				Total
	+		−		
	IML 2000 HBsAg				
	+	−	+	−	
Early recovery	0	0	0	6	6
HBV vaccine response	0	0	0	42	42
Not previously infected	0	0	0	152	152
Total	0	0	0	200	200

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (42/42)
95% CI = 91.6 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (152/152)
95% CI = 97.6 to 100.0%

Total
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (200/200)
95% CI = 98.2 to 100.0%
Total agreement = 100.0% (200/200)
95% CI = 98.2 to 100.0%

Study 5: In a clinical study conducted in Europe, IMMULITE 2000 HBsAg was compared to Kit B, a commercially available HBsAg electrochemiluminescence immunoassay. Presented below are the comparisons

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between IMMULITE 2000 HBsAg and Kit B on a total of 2,199 specimens (one specimen per subject).

Kit B				
+		–		
IML 2000 HBsAg				
+	–	+	–	Total
118	12	15	2,054	2,199

Positive agreement = 90.8% (118/130)
95% CI = 84.4 to 95.1%
Negative agreement = 98.0% (2054/2096)
95% CI = 97.3 to 98.6%
Total agreement = 98.8% (2172/2199)
95% CI = 98.2 to 99.2%

II. Analytical Performance

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed as a signal-to-cutoff ratio. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Analytical Sensitivity: Based on studies with serial dilution of WHO International Standard for Hepatitis B Surface Antigen (NIBSC 80/549), the threshold sensitivity (last positive dilution) for IMMULITE 2000 HBsAg is 0.063 IU/mL.

The 95% confidence interval at this level (0.063 IU/mL) is 0.040 - 0.086 IU/mL.

Precision: Serum samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates at three US sites. In the same studies, the IMMULITE HBsAg assay was tested for three lots and at three sites. The IMMULITE HBsAg lot-to-lot and site-to-site precision data is provided.

Caution: ***The IMMULITE 2000 HBsAg lot-to-lot precision has not been evaluated.***

(See "Precision" tables.)

EDTA, heparin, and sodium citrate samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE HBsAg and one lot of IMMULITE 2000 HBsAg. The median total variance of coefficients (EDTA, 5.2%; heparin, 7.2%; sodium citrate, 5.9%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 HBsAg.

Alternate Sample Types: The measurement of HBsAg is not significantly affected by the presence of heparinized, sodium citrate, or EDTA anti-coagulants, as shown in a study that included 60 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression: (in signal-to-cutoff ratio)

(Heparin) = 0.99 (Serum) + 1.93
r = 1.00

(NaCitrate) = 0.96 (Serum) + 0.83
r = 1.00

(EDTA) = 0.98 (Serum) + 1.52
r = 1.00

Means:
219 (Serum)
218 (Heparin)
211 (NaCitrate)
216 (EDTA)

(See "Alternate Sample Types Graphs".)

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: May have an effect on the assay, causing an increase in values.

Lipemia: May have an effect on the assay, causing an increase in values. The use of an ultracentrifuge is recommended to clear lipemic samples.

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and at one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE 2000 HBsAg and a commercially available HBsAg enzyme immunoassay (Kit A), with the following results:

	Kit A				
	+		−		
	IMMULITE 2000 HBsAg				
Sample type	+	−	+	−	Total
HAV	5	0	0	42	47
HCV	3	1	5	63	72
HDV	11	0	0	5	16
HEV	0	0	0	10	10
Non-viral liver diseases[1]	0	4	3	47	54
Autoimmune diseases	0	1	0	24	25
CMV	1	0	1	11	13
EBV	1	0	0	18	19
Syphilis	0	0	0	11	11
Toxoplasma	2	0	0	18	20
HSV	1	2	2	42	47
Parvovirus B19	0	0	0	15	15
HIV	8	0	1	42	51
Influenza vaccine recipient	0	0	1	24	25
Transplant recipient	0	0	0	14	14
Dialysis	0	1	1	31	33
Intravenous drug abuser	0	0	1	4	5
Others[2]	2	0	0	4	6
Total	34	9	15	425	483

[1] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[2] Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, seven specimens from antinuclear antibody (ANA) positive patients and 24 from patients with positive rheumatoid factor (RF) were tested by IMMULITE 2000 HBsAg. IMMULITE 2000 HBsAg test results were all negative for these 31 specimens.

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Technical Assistance

In the United States, contact DPC's Technical Services department. Tel: 800.372.1782 or 973.927.2828 Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

Tables and Graphs

Precision (ratio)

Site 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.55	0.060	11.1%	0.074	13.6%
2	0.53	0.042	7.8%	0.061	11.4%
3	1.16	0.091	7.8%	0.098	8.4%
4	1.20	0.074	6.2%	0.177	14.8%
5	1.47	0.102	7.0%	0.214	14.6%
6	2.56	0.149	5.8%	0.243	9.5%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.46	0.041	8.9%	0.052	11.2%
2	0.48	0.059	12.0%	0.066	13.7%
3	1.18	0.063	5.4%	0.093	7.9%
4	1.18	0.059	5.0%	0.152	12.9%
5	1.45	0.092	6.4%	0.161	11.1%
6	2.80	0.149	5.3%	0.244	8.7%

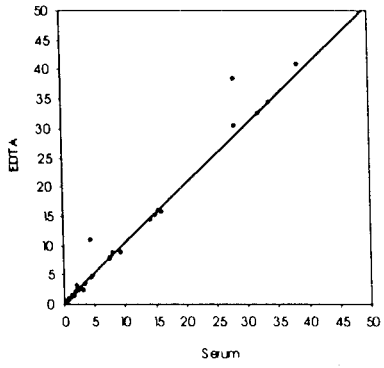
Site 3

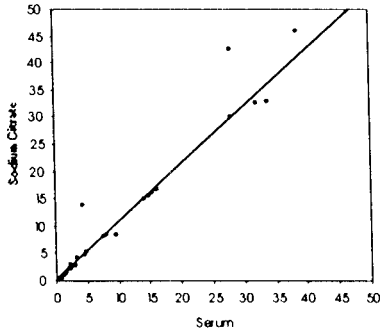
		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.50	0.038	7.6%	0.059	11.7%
2	0.50	0.051	10.2%	0.071	14.2%
3	1.17	0.126	10.8%	0.171	14.7%
4	1.18	0.053	4.5%	0.144	12.2%
5	1.48	0.091	6.1%	0.129	8.7%
6	2.70	0.117	4.3%	0.406	15.0%

Site-to-Site Precision

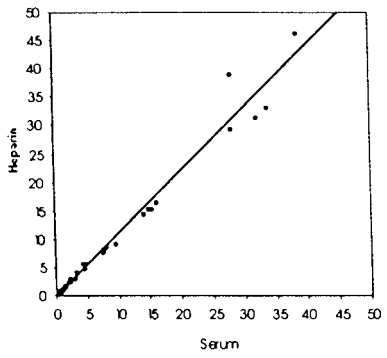
		Site-to-Site	
	Mean	SD	CV
1	0.50	0.070	13.9%
2	0.51	0.069	13.7%
3	1.17	0.126	10.7%
4	1.18	0.158	13.3%
5	1.47	0.172	11.7%
6	2.69	0.321	11.9%

Alternate Sample Types Graphs (ratio)





Regression equations



Note: Three specimens fall outside the range depicted in the graphs above.

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