



IMMULITE®

Anti-HBs

Total Antibodies to Hepatitis B Surface Antigen

DPC®

IMMULITE® Anti-HBs

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.

Performance characteristics have not been established for use of the IMMULITE Anti-HBs assay as an aid in determining the susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.

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 Anti-HBs 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: For *in vitro* diagnostic use with the IMMULITE automated immunoassay analyzer for the qualitative measurement of total antibodies to the hepatitis B surface antigen (anti-HBs) in human serum and plasma (heparinized or EDTA). Assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection for individuals prior to or following HBV vaccination, or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is *unknown*. The detection of anti-HBs is indicative of laboratory diagnosis of seroconversion from hepatitis B virus (HBV) infection.

Caution: Performance characteristics for the IMMULITE Anti-HBs 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 Anti-HBs or another legally-marketed anti-HBs assay.

Catalog Number: **LKAH1** (100 tests),
LKAH5 (500 tests)

Test Code: **aHB** Color: **Dark Pink**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of hepatitis-associated DNA viruses, 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 elevations, and symptoms. 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 during the incubation phase is HBsAg, and indicates an ongoing infection with HBV.^{1,2,4} Antibodies to HBsAg generally appears after HBsAg has been cleared from the blood stream, usually 6 months after infection, and its presence represents recovery and immunity. However, in a few patients known to have antibodies to HBsAg, subclinical infections have developed.⁵ The presence of HBsAg antibodies should not be used as the sole marker in determining a prior hepatitis B infection.

Principle of the Procedure

IMMULITE Anti-HBs is a solid-phase, two-step chemiluminescent enzyme immunoassay. The solid phase, a polystyrene bead enclosed within a Test Unit, is coated with purified HBsAg subtypes ad and ay.

An alkaline phosphatase-labeled HBsAg is added to the Test Unit. Chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase; the photon output, as measured by the luminometer, is related to the presence of antibodies to HBsAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples should not be used in this assay.

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.¹⁰

The IMMULITE Anti-HBs assay may be performed on human serum or plasma (heparinized 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: 50 µL serum or plasma (heparinized or EDTA). (See Alternate Sample Types section.) The sample cup must contain at least 100 µL more than the total volume required.

Storage: 2 days at room temperature (15°–28°C)¹⁰
3 days at 2–8°C.^{6,10}
For longer storage: at –20°C.⁷

Warnings and Precautions

For *in vitro* diagnostic use.

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

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 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.

Certain components contain HBsAg which has been inactivated by proven, documented methods. However, always handle them as if capable of transmitting infectious agents.

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

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

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Anti-HBs Test Units (LAH1)

Each barcode-labeled unit contains one bead coated with purified inactivated human HBsAg subtypes ad and ay. Stable at 2–8°C until expiration date.

LKAH1: 100 units. **LKAH5:** 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.

Anti-HBs Reagent Wedges (LAHA, LAHB)

With barcodes. **LAHA:** 6.5 mL consists of a protein-based phosphate buffer, with <0.1 g% sodium azide. **LAHB:** 6.5 mL alkaline phosphatase (bovine calf intestine) conjugated to purified inactivated HBsAg in buffer, with <0.1 g% 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.

LKAH1: 1 set. **LKAH5:** 5 sets.

Anti-HBs Adjustors (LAHL, LAHH)

Two vials (Low and High), 2 mL each, of human serum reactive to HBsAg in a buffer, with <0.1 g% sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

LKAH1: 1 set. **LKAH5:** 2 sets.

The adjustors are used to correlate the cps of the customer's instrument to that of the master curve instrument. The master curve of IMMULITE Anti-HBs is calibrated against WHO 1st IRP 26-1-7.

Anti-HBs Controls (LAHC1, LAHC2, LAHC3) (may be purchased separately.)

Three vials (Negative, Low Positive and Positive) containing 2 mL each. **LAHC1 (Negative Control):** One vial containing human serum nonreactive to HBsAg, with <0.1 g% sodium azide. **LAHC2, LAHC3 (Low Positive Control, Positive Control):** Two vials containing human serum reactive to HBsAg, with <0.1 g% sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

LKAH1: 1 set. **LKAH5:** 2 sets.

Refer to the control insert for concentration levels.

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.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Adjustment Interval: 4 weeks.

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

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 or outside of the acceptable 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 and intervals for testing.^{8,11}

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.

Interpretation of Results

The IMMULITE Anti-HBs calibration employs a stored master curve, generated by the four-parameter logistic mathematical model based on the dose-CPS (counts per minute) relationship during the calibration process.

DPC's IMMULITE Anti-HBs is calibrated against WHO 1st IRP 261-77. The Immunization Practice Advisory Committee (ACIP) of U.S. Center for Disease Control and Prevention (CDC) recommends a cutoff of 10 mIU/mL to determine the HBV immune status of an individual¹². The test results should be interpreted in the following way.

Positive: A result greater than or equal to 11 mIU/mL (WHO 1st IRP 26-1-77) indicates that antibodies to HBsAg are present and detected in the patient sample.⁹ This usually indicates protection against infection.

Negative: A result less than 9 mIU/mL indicates that antibodies to HBsAg are not detected in the patient sample, or are below the protective level for immunity.

Retest Zone: A result less than 11 mIU/mL and greater than or equal to 10

mIU/mL is indeterminate and must be retested in duplicate. If all three results (original and two retests) are greater than or equal to 10 mIU/mL, the specimen is positive for anti-HBs.

A result less than 10 mIU/mL and greater than or equal to 9 mIU/mL is indeterminate and must be retested in duplicate. If all three results (original and two retests) are less than 10 mIU/mL, the specimen is negative for anti-HBs.

The results obtained with the IMMULITE Anti-HBs assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

A specimen that has been retested but cannot be resolved as above remains indeterminate. An indeterminate result (9-11 mIU/mL) should not be reported until it is retested and resolved. The immune status of the individual should be further assessed by associated risk factors and the use of additional diagnostic information, or another sample may be collected and tested.

For the determination of seroconversion, two sera or plasma (heparinized or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection. The acute phase sample should be stored and tested in parallel with the convalescent sample.

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

Testing for anti-HBs antibodies alone is not sufficient in determining previous infections.

This assay does not differentiate between a vaccine-induced immune response and an immune response induced by natural infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infections, the total anti-HBc assay may be performed.

If mIU/mL values are reported, it is recommended that the following be included with the report to the physician:

The following results were obtained with the IMMULITE Anti-HBs EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported total anti-HBs level cannot be correlated to an endpoint titer. The clinical significance of values reported greater than or equal to 11 mIU/mL and less than 9 mIU/mL have not been determined, other than the individual is presumed to be immune (≥ 11) or nonimmune to HBV infection (<9).

Limitations

The ability of the IMMULITE Anti-HBs 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. The results from this or another diagnostic kit should be used and interpreted only in the context of the overall clinical picture.

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

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

Individuals acutely infected with the hepatitis B virus will exhibit anti-HBs

approximately two weeks after the disappearance of HBsAg. This antibody response will reach peak levels after several months and gradually decline over a period of years. The majority of persons who have been vaccinated against HBV will also have detectable levels of anti-HBs.

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.

Caution: Minimal Expected Values for an asymptomatic population have not been established for this assay. The user is required to establish adequate Expected Values for their population(s).

Apparently Healthy Subjects (Study 1)

IMMULITE Anti-HBs				
Age	Gender	Total	Pos.	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	2	50.0%%
	Female	5	0	0.0%
20 - 29	Male	7	3	42.9%
	Female	13	4	30.8%
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	5	35.7%
	Female	22	4	18.2%
	All	36	9	25.0%

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Other subjects

Subject	Total n	Male	Female	Mean Age	Age Range	Pos. by IML Anti-HBs	
						n	%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at high risk ¹ (Study 1)	3	0	3	33	30-36	0	0.0%
Pregnant at low risk ¹ (Study 1)	16	0	16	31	21-42	7	43.8%
Pregnant at low risk ¹ (Study 4)	199	0	199	28	17-41	48	24.1%
Pregnant at low risk ¹ (Europe)	13	Not available				2	15.4%

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

Performance Characteristics

I. Clinical Performance

Four clinical studies with a total of 769 subjects were conducted to assess the performance of the IMMULITE Anti-HBs 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 Anti-HBs. 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.

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 crossreactive 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. of patients	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 Anti-HBs results were compared to Kit A, a reference assay for the determination of anti-HBs.

Reference Serological Characterization	Kit A						Total
	+			-			
	IML Anti-HBs						
	+	Ind	-	+	Ind	-	
HBV Acute infection	0	0	0	0	0	69	69
HBV Chronic infection	2	0	3	0	0	2	7
HBV Early recovery	17	0	0	3	0	20	40
HBV Vaccine response	26	0	1	0	0	0	27
Not previously infected	0	0	0	1	2	117	120
Recovered	16	0	1	0	0	0	17
Uninterpretable	0	0	0	0	0	1	1
Total	61	0	5	4	2	209	281

HBV Acute Infection
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 100.0% (69/69)
 95% CI = 94.8 to 100.0%

HBV Chronic Infection
 Positive agreement = 40.0% (2/5)
 95% CI = 5.3 to 85.3%
 Negative agreement = N/A (2/2)
 95% CI = N/A

w/ly N/A

Early Recovery
 Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = 87.0% (20/23)
 95% CI = 66.4 to 97.2%

HBV Vaccine Response
 Positive agreement = 96.3% (26/27)
 95% CI = 81.0 to 99.9%
 Negative agreement = N/A (0/0)
 95% CI = N/A

Not previously Infected
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 97.5% (117/120)
 95% CI = 92.9 to 99.5%

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

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

Total
 Positive agreement = 92.4% (61/66)
 95% CI = 83.2 to 97.5%
 Negative agreement = 97.2% (209/215)
 95% CI = 94.0 to 99.0%
 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 Anti-HBs results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+		-		Ind		
	+	Ind	-	+	Ind	-	
HBV Acute infection	0	0	0	0	0	17	17
HBV Chronic infection	1	0	1	0	0	0	2
HBV Early recovery	31	0	2	0	1	16	50
HBV Vaccine response	31	0	1	0	0	0	32
Not previously infected	0	0	0	2	4	101	107
Uninterpretable	1	0	0	0	0	1	1
Total	64	0	4	2	5	134	209

HBV Acute Infection
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%

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

IMMULITE Anti-HBs

Early Recovery
 Positive agreement = 93.9% (31/33)
 95% CI = 79.8 to 99.3%
 Negative agreement = 94.1% (16/17)
 95% CI = 71.3 to 99.9%

HBV Vaccine Response
 Positive agreement = 96.9% (31/32)
 95% CI = 83.8 to 99.9%
 Negative agreement = N/A (0/0)
 95% CI = N/A

Not previously Infected
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 94.4% (101/107)
 95% CI = 88.2 to 97.9%

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

Total
 Positive agreement = 94.1% (64/68)
 95% CI = 85.6 to 98.4%
 Negative agreement = 95.0% (134/141)
 95% CI = 90.0 to 98.0%
 Total agreement = 94.7% (198/209)
 95% CI = 90.8 to 97.3%

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	HBsAg	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	+	-	+	+	+	+

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

Total
 Positive agreement = 25% (2/8)
 95% CI = 3.2 to 65.1%
 Negative agreement = 98.6% (70/71)
 95% CI = 92.4 to 100.0%
 Total agreement = 91.1% (72/79)
 95% CI = 82.6 to 96.4%

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:

Based on the above classifications the IMMULITE Anti-HBs results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+		-		Total	Total	
	+	Ind	-	+			
HBV Acute infection	1	0	3	0	1	66	71
HBV Chronic infection	0	0	2	0	0	4	6
Recovered	1	0	0	0	0	0	1
Uninterpretable	0	0	1	0	0	0	1
Total	2	0	6	0	1	70	79

HBV Acute Infection
 Positive agreement = N/A (1/4)
 95% CI = N/A
 Negative agreement = 98.5% (66/67)
 95% CI = 92.0 to 100.0%

HBV Chronic Infection
 Positive agreement = N/A (0/2)
 95% CI = N/A
 Negative agreement = N/A (4/4)
 95% CI = N/A

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

Characterization based on single point specimens	No. 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 Anti-HBs results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			-			
	IML Anti-HBs						
	+	Ind	-	+	Ind	-	
Early recovery	4	0	0	0	0	2	6
HBV vaccine response	42	0	0	0	0	0	42
Not previously infected	0	0	0	2	0	149	151*
Total	46	0	0	2	0	151	199

* One specimen was not tested for IMMULITE Anti-HBs.

Early Recovery

Positive agreement = N/A (4/4)
 95% CI = N/A
 Negative agreement = N/A (2/2)
 95% CI = N/A

HBV Vaccine Response

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

Not Previously Infected

Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 98.7% (149/151)
 95% CI = 95.3 to 99.8%

Total

Positive agreement = 100.0% (46/46)
 95% CI = 92.3 to 100.0%
 Negative agreement = 98.7% (151/153)
 95% CI = 95.4 to 99.8%
 Total agreement = 99.0% (197/199)
 95% CI = 96.4 to 99.9%

Clinical Performance with Individuals Who Have Received Hepatitis B Vaccine

In a European site, a retrospective study was conducted to evaluate a total of 94 serum specimens from subjects who had received hepatitis B vaccine. The specimens were tested by IMMULITE Anti-HBs and an FDA-approved or licensed anti-HBs assay (Kit B), with the following results.

Specimen	Kit B						Total
	+			-			
	IML Anti-HBs						
	+	Ind	-	+	Ind	-	
HBV vaccine response	94	0	0	0	0	0	94

IMMULITE Anti-HBs

Positive agreement = 100.0% (94/94)
 95% CI = 96.2 to 100.0%
 Negative agreement = N/A
 95% CI = N/A
 Total agreement = 100.0% (94/94)
 95% CI = 96.2 to 100.0%

Clinical Performance with Matched Pre- and Post-Vaccination Specimens

In Study 1 in the U.S., pre- and post-vaccination specimens from 17 individuals who had received recombinant HBV vaccine were tested by the IMMULITE Anti-HBs assay and the reference assay (Kit A). The results are shown below:

Specimens	Kit A						Total
	+			-			
	IML Anti-HBs						
	+	Ind	-	+	Ind	-	
Pre-vaccination	0	0	0	0	0	17	17
Post-vaccination	17	0	0	0	0	0	17
Total	17	0	0	0	0	17	34

HBV Pre-Vaccination

Positive agreement = N/A
 95% CI = N/A
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%

HBV Post-Vaccination

Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = N/A
 95% CI = N/A

Total

Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Total agreement = 100.0% (34/34)
 95% CI = 89.7 to 99.9%

II. Analytical Performance

See Tables and Graphs for data representative of the assay's performance. The following results are expressed in mIU/mL, based on WHO 1st IRP 26-1-77. (Unless otherwise noted, all data were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

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.)

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EDTA and heparin samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBs and one lot of IMMULITE 2000 Anti-HBs. The median total variance of coefficients (EDTA, 4.2%; heparin, 4.5%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBs.

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 40 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, 20 and 40 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1000, 2000 and 3000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Alternate Sample Types: The measurement of specimens is not significantly affected by the presence of heparinized and EDTA anti-coagulants, as shown in a study that included 46 specimens collected into plain, heparinized and EDTA vacutainer tubes. By regression: (See "Alternate Sample Types Graphs".)

(Heparin) = 1.04 (Serum) + 10 mIU/mL
r = 0.97

(EDTA) = 1.11 (Serum) – 2 mIU/mL
r = 0.93

Means:
318 mIU/mL (Serum)
340 mIU/mL (Heparin)
351 mIU/mL (EDTA)

Around the cutoff (10 mIU/mL), heparinized specimens demonstrated a 112% recovery and EDTA specimens demonstrated a 103% recovery.

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 Anti-HBs and a

commercially available enzyme immunoassay for anti-HBs (Kit A), with the following results.

Sample type	Kit A				Total
	+		-		
	+	- ¹	+	- ¹	
HAV	17	2	0	28	47
HCV	21	2	1	47	71
HDV	2	0	0	19	21
HEV	5	0	0	5	10
Non-viral liver diseases ²	7	0	0	47	54
Autoimmune diseases	4	1	1	19	25
CMV	3	0	0	10	13
EBV	6	0	1	11	18
Syphilis	5	0	0	5	10
Toxoplasma	7	0	0	13	20
HSV	13	0	1	33	47
Parvovirus B19	2	1	2	11	16
HIV	24	0	1	26	51
Influenza vaccine recipient	10	0	0	15	25
Transplant recipient	4	1	0	9	14
Dialysis	10	0	0	23	33
Intravenous drug abuser	2	0	0	3	5
Others ³	0	0	0	6	6
Total	142	7	7	330	486

¹ Includes Indeterminate cases.

² Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

³ Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, six specimens from *H. pylori* positive patients, four from antinuclear antibody (ANA) positive patients, and 27 from patients with positive rheumatoid factor (RF) were tested by IMMULITE Anti-HBs. IMMULITE Anti-HBs test results were negative for all six *H. pylori* specimens, negative for 3/4 ANA specimens, and negative for 24/27 RF specimens.

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References

- 1 Locarnini SA, Gust ID. Hepadnaviridae: hepatitis B virus and the delta virus. In: Balows A, et al, editors. *Laboratory diagnosis of infectious diseases: principles and practices*. New York: Springer-Verlag, 1988: 750-96.
- 2 Follett EAC. Diagnosis of hepatitis B infection. In: Young H, McMillan A, editors. *Immunological diagnosis of sexually transmitted diseases*. New York: Marcel Dekker, 1988: 433-49.
- 3 Hollinger FB, Dienstag JL. Hepatitis B and D viruses. In: Lennette EH, et al, editors. *Manual of clinical microbiology*. 6th ed. Washington, D.C.: American Society for Microbiology, 1995:1033-49.
- 4 Nowicki MJ, Balistreri WF. Hepatitis A to E: building up the alphabet. *Contemporary Peds* 1992: 118-28.
- 5 Zuckerman AJ, et al. Hepatitis B virus and hepatitis D virus. In: *Principles and practice of clinical virology*. 2nd ed. New York: John Wiley & Sons, 1992: 153-72.
- 6 Tietz NW, editor. *Clinical guide to laboratory tests*. 3rd ed. Philadelphia: WB Saunders, 1995:354-60.
- 7 Tietz NW, editor. *Clinical guide to laboratory tests*. 3rd ed. Philadelphia: W.B. Saunders, 1995:322-4.
- 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 Recommendations of the Immunization Practices Advisory Committee Update on Hepatitis B Prevention. *MMWR*. 1987: 36(23): 353-366.
- 10 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.
- 11 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.
- 12 CDC. Sensitivity of the Test for Antibody to Hepatitis B Surface Antigen-United States. *MMWR* 1993;42(36);707-710.

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 (mIU/mL)

Site 1

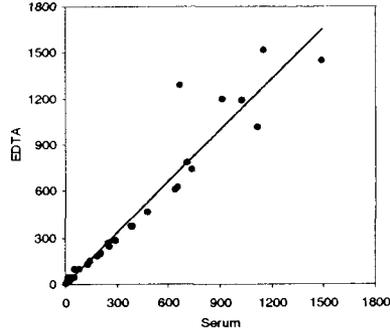
	<u>Intra-assay</u>			<u>Total</u>	
	Mean	SD	CV	SD	CV
1	8.69	0.52	5.9%	0.82	9.4%
2	8.95	0.51	5.7%	0.63	7.0%
3	14.6	0.71	4.9%	0.87	6.0%
4	64	4.77	7.4%	5.10	7.9%
5	123	4.52	3.7%	6.95	5.7%
6	488	23.2	4.7%	38.6	7.9%
7	864	39	4.5%	48	5.5%

Site 2

	<u>Intra-assay</u>			<u>Total</u>	
	Mean	SD	CV	SD	CV
1	8.71	0.56	6.4%	0.73	8.4%
2	9.82	0.46	4.6%	0.88	8.9%
3	15.8	0.42	2.7%	0.96	6.1%
4	75	3.84	5.2%	4.92	6.6%
5	131	3.68	2.8%	8.07	6.1%
6	512	27.6	5.4%	38.1	7.4%
7	887	23	2.6%	40	4.5%

Site 3

	Mean	<u>Intra-assay</u>			<u>Total</u>	
		SD	CV	SD	CV	
1	6.50	0.44	6.7%	0.82	12.6%	
2	8.35	0.34	4.1%	0.85	10.2%	
3	14.1	0.51	3.6%	1.07	7.6%	
4	68	4.00	5.9%	5.53	8.2%	
5	124	4.17	3.4%	6.49	5.2%	
6	433	25.7	5.9%	35	8.1%	
7	881	25	2.8%	49	5.6%	



Lot-to-Lot and Site-to-Site

	Mean	<u>Lot-to-Lot</u>			<u>Site-to-Site</u>	
		SD	CV	SD	CV	
1	7.34	0.92	12.6%	1.57	21.4%	
2	8.72	0.80	9.2%	1.11	12.8%	
3	14.6	1.04	7.1%	1.34	9.2%	
4	69	5.17	7.5%	6.62	9.6%	
5	125	7.17	5.7%	7.85	6.3%	
6	431	50	11.6%	59	13.7%	
7	847	82	9.6%	82	9.7%	

DPC[®]

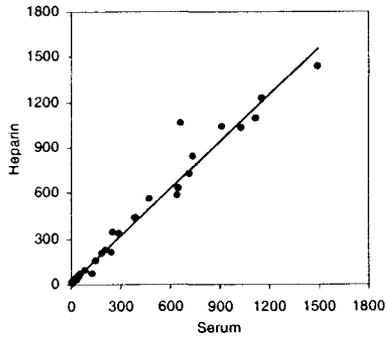
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

2002-07-24 (ISO 8601)

December 2, 2002

PILKAH - 7

Alternate Sample Types Graphs



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Anti-HBs

Total Antibodies to Hepatitis B Surface Antigen

DPC®

IMMULITE® 2000 Anti-HBs

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.

Performance characteristics have not been established for use of the IMMULITE Anti-HBs assay as an aid in determining the susceptibility to HBV infection prior to or following vaccination in infants, children, or adolescents.

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 Anti-HBs 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: For *in vitro* diagnostic use with the IMMULITE 2000 automated immunoassay analyzer for the qualitative measurement of total antibodies to the hepatitis B surface antigen (anti-HBs) in human serum and plasma (heparinized or EDTA). Assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection for individuals prior to or following HBV vaccination, or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is unknown. The detection of anti-HBs is indicative of laboratory diagnosis of seroconversion from hepatitis B virus (HBV) infection.

Caution: Performance characteristics for the IMMULITE 2000 Anti-HBs 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 Anti-HBs or another legally-marketed anti-HBs assay.

Catalog Number: **L2KAH2** (200 tests),
L2KAH6 (600 tests)

Test Code: **aHB** Color: **Dark Pink**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of hepatitis-associated DNA viruses, 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 elevations, and symptoms. 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 during the incubation

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phase is HBsAg, and indicates an ongoing infection with HBV.^{1,2,4} Antibodies to HBsAg generally appear after HBsAg has been cleared from the blood stream, usually 6 months after infection, and its presence represents recovery and immunity. However, in a few patients known to have antibodies to HBsAg, subclinical infections have developed.⁵ The presence of HBsAg antibodies should not be used as the sole marker in determining a prior hepatitis B infection.

Principle of the Procedure

IMMULITE 2000 Anti-HBs is a solid-phase, two-step chemiluminescent enzyme immunoassay. The solid phase, a polystyrene bead, is coated with purified HBsAg subtypes ad and ay.

An alkaline phosphatase-labeled HBsAg is added to the Reaction Tube. Chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase; the photon output, as measured by the luminometer, is related to the presence of antibodies to HBsAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples should not be used in this assay.

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.¹⁰

The IMMULITE 2000 Anti-HBs assay may be performed on human serum or plasma (heparinized 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: 50 µL serum or plasma (heparinized or EDTA). (See Anticoagulants section.)

Storage: 2 days at room temperature (15°C–28°C)¹⁰
3 days at 2–8°C^{6,10}
For longer storage: at –20°C.⁷

Warnings and Precautions

For *in vitro* diagnostic use.

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

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 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.

Certain components contain HBsAg which has been inactivated by proven, documented methods. However, always handle them as if capable of transmitting infectious agents.

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

Water: Use distilled or deionized water for the wash step and 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.

Anti-HBs Bead Pack (L2AH12)

With barcode. 200 beads coated with purified inactivated human HBsAg subtypes ad and ay, with desiccant. Stable at 2–8°C until expiration date.

L2KAH2: 1 Pack. **L2KAH6:** 3 Packs.

Anti-HBs Reagent Wedge (L2AHA2)

With barcode. 11.5 mL of a protein-based buffer, with <0.1 g% sodium azide. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to purified inactivated HBsAg in buffer, with <0.1 g% sodium azide. Stable at 2–8°C until expiration date.

L2KAH2: 1 Wedge. **L2KAH6:** 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.

Anti-HBs Adjustors (LAHL, LAHH)

Two vials (Low and High), 2 mL each, of human serum reactive to HBsAg in a buffer, with <0.1 g% sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

L2KAH2: 1 set. **L2KAH6:** 2 sets.

The adjustors are used to correlate the cps of the customer's instrument to that of the master curve instrument. The master curve of IMMULITE 2000 Anti-HBs is calibrated against WHO 1st IRP 26-1-7.

Anti-HBs Controls (LAHC1, LAHC2, LAHC3) (may be purchased separately.)

Three vials (Negative, Low Positive and Positive) containing 2 mL each. **LAHC1 (Negative Control):** One vial containing human serum nonreactive to HBsAg, with <0.1 g% sodium azide. **LAHC2, LAHC3 (Low Positive Control, Positive**

Control): Two vials containing human serum reactive to HBsAg, with <0.1 g% sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

L2KAH2: 1 set. **L2KAH6:** 2 sets.

Refer to the control insert for concentration levels.

Aliquot Labels with barcodes are supplied with the kit, for use with Adjustors and Controls. Before use, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

Kit Components Supplied Separately

L2SUBM: Chemiluminescent Substrate

L2PWSM: Probe Wash

L2KPM: Probe Cleaning Kit

L2RXT: Reaction Tubes (disposable)

Also Required

Distilled or deionized water and test tubes.

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 Controls supplied with the kit should be used as quality control material to monitor assay performance.

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 or outside of the acceptable 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 and intervals for testing.^{8,11}

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.

Interpretation of Results

The IMMULITE 2000 Anti-HBs calibration employs a stored master curve, generated by the four-parameter logistic mathematical model based on the dose-CPS (counts per minute) relationship during the calibration process.

DPC's IMMULITE 2000 Anti-HBs is calibrated against WHO 1st IRP 261-77. The Immunization Practice Advisory Committee (ACIP) of U.S. Center for Disease Control and Prevention (CDC) recommends a cutoff of 10 mIU/mL to determine the HBV immune status of an individual¹². The test results should be interpreted in the following way.

Positive: A result greater than or equal to 11 mIU/mL (WHO 1st IRP 26-1-77) indicates that antibodies to HBsAg are present and detected in the patient sample.⁹ This usually indicates protection against infection.

Negative: A result less than 9 mIU/mL indicates that antibodies to HBsAg are not detected in the patient sample, or are below the protective level for immunity.

Retest Zone: A result less than 11 mIU/mL and greater than or equal to 10 mIU/mL is indeterminate and must be retested in duplicate. If all three results (original and two retests) are greater than or equal to 10 mIU/mL, the specimen is positive for anti-HBs.

A result less than 10 mIU/mL and greater than or equal to 9 mIU/mL is indeterminate and must be retested in duplicate. If all

three results (original and two retests) are less than 10 mIU/mL, the specimen is negative for anti-HBs.

The results obtained with the IMMULITE 2000 Anti-HBs assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

A specimen that has been retested but cannot be resolved as above remains indeterminate. An indeterminate result (9-11 mIU/mL) should not be reported until it is retested and resolved. The immune status of the individual should be further assessed by associated risk factors and the use of additional diagnostic information, or another sample may be collected and tested.

For the determination of seroconversion, two sera or plasma (heparinized or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection. The acute phase sample should be stored and tested in parallel with the convalescent sample.

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

Testing for anti-HBs antibodies alone is not sufficient in determining previous infections.

This assay does not differentiate between a vaccine-induced immune response and an immune response induced by natural infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infections, the total anti-HBc assay may be performed.

If mIU/mL values are reported, it is recommended that the following be included with the report to the physician:

The following results were obtained with the IMMULITE 2000 Anti-HBs EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported total anti-HBs level cannot be correlated to an endpoint titer. The clinical significance of values reported greater than or equal to 11 mIU/mL and less than 9 mIU/mL have not been determined, other than the individual is presumed to be immune (≥ 11) or nonimmune to HBV infection (<9).

Limitations

The ability of the IMMULITE 2000 Anti-HBs 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. The results from this or another diagnostic kit should be used and interpreted only in the context of the overall clinical picture.

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

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

Individuals acutely infected with the hepatitis B virus will exhibit anti-HBs approximately two weeks after the disappearance of HBsAg. This antibody response will reach peak levels after several months and gradually decline over a period of years. The majority of persons who have been vaccinated against HBV will also have detectable levels of anti-HBs.

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.

Caution: Minimal Expected Values for an asymptomatic population have not been established for this assay. The user is required to establish adequate Expected Values for their population(s).

Apparently Healthy Subjects (Study 1)

IMMULITE 2000 Anti-HBs				
Age	Gender	Total	Pos.	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	2	50.0%
	Female	5	0	0.0%
20 - 29	Male	7	3	42.9%
	Female	13	4	30.8%
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	5	35.7%
	Female	22	4	18.2%
	All	36	9	25.0%

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Other subjects

Subject	Total n	Male	Female	Mean Age	Age Range	Pos. by IML 2000 Anti-HBs	
						n	%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at high risk ¹ (Study 1)	3	0	3	33	30-36	0	0.0%
Pregnant at low risk ¹ (Study 1)	16	0	16	31	21-42	7	43.8%
Pregnant at low risk ¹ (Study 4)	199	0	199	28	17-41	48	24.1%
Pregnant at low risk ¹ (Europe)	13	Not available				2	15.4%

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

Performance Characteristics

I. Clinical Performance

Four clinical studies with a total of 769 subjects were conducted to assess the performance of the IMMULITE 2000 Anti-HBs 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 Anti-HBs. 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.

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 crossreactive 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. of patients	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	-	+	-	-	-	-

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

Reference Serological Characterization	Kit A						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
HBV Acute infection	0	0	0	0	1	68	69
HBV Chronic infection	1	1	3	0	0	2	7
HBV Early recovery	17	0	0	3	1	19	40
HBV Vaccine response	26	0	1	0	0	0	27
Not previously infected	0	0	0	3	0	117	120
Recovered	16	0	1	0	0	0	17
Uninterpretable	0	0	0	0	0	1	1
Total	60	1	5	6	2	207	281

HBV Acute Infection
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 98.6% (68/69)
 95% CI = 92.2 to 100.0%

HBV Chronic Infection
 Positive agreement = N/A (1/4)
 95% CI = N/A
 Negative agreement = N/A (2/2)
 95% CI = N/A

Early Recovery
 Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = 82.6% (19/23)
 95% CI = 61.2 to 95.0%

HBV Vaccine Response
 Positive agreement = 96.3% (26/27)
 95% CI = 81.0 to 99.9%
 Negative agreement = N/A (0/0)
 95% CI = N/A

Not previously Infected
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 97.5% (117/120)
 95% CI = 92.9 to 99.5%

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

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

Total
 Positive agreement = 90.9% (60/66)
 95% CI = 81.3 to 96.6%
 Negative agreement = 96.3% (207/215)
 95% CI = 92.8 to 98.4%
 Total agreement = 95.0% (267/281)
 95% CI = 91.8 to 97.3%

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	+	-	-	+

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Based on the above classifications the IMMULITE 2000 Anti-HBs results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
HB Acute infection	0	0	0	0	0	17	17
HB Chronic infection	1	0	1	0	0	0	2
HB Early recovery	31	0	2	0	1	16	50
HBV Vaccine response	31	0	1	0	0	0	32
Not previously infected	0	0	0	2	1	104	107
Uninterpretable	1	0	0	0	0	0	1
Total	64	0	4	2	2	137	209

HBV Acute Infection
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%

HBV 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 = 93.9% (31/33)
 95% CI = 79.8 to 99.3%
 Negative agreement = 94.1% (16/17)
 95% CI = 71.3 to 99.9%

HBV Vaccine Response
 Positive agreement = 96.9% (31/32)
 95% CI = 83.9 to 99.9%
 Negative agreement = N/A (0/0)
 95% CI = N/A

Not previously Infected
 Positive agreement = N/A (0/0)
 95% CI = N/A
 Negative agreement = 97.2% (104/107)
 95% CI = 92.0 to 99.4%

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

Total
 Positive agreement = 94.1% (64/68)
 95% CI = 85.6 to 98.4%
 Negative agreement = 97.2% (137/141)
 95% CI = 92.9 to 99.2%
 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 Anti-HBs 2000 results were compared to Kit A.

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Reference Serological Characterization	Kit A						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
HBV Acute infection	0	0	4	1	0	66	71
HBV Chronic infection	0	1	1	0	0	4	6
Recovered	1	0	0	0	0	0	1
Uninterpretable	0	0	1	0	0	0	1
Total	1	1	6	1	0	70	79

HBV Acute Infection
 Positive agreement = N/A (0/4)
 95% CI = N/A
 Negative agreement = 98.5% (66/67)
 95% CI = 92.0 to 100.0%

HBV Chronic Infection
 Positive agreement = N/A (0/1)
 95% CI = N/A
 Negative agreement = N/A (4/4)
 95% CI = N/A

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

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

Total
 Positive agreement = 12.5% (1/8)
 95% CI = 0.00 to 52.7%
 Negative agreement = 98.6% (70/71)
 95% CI = 92.4 to 100.0%
 Total agreement = 89.9% (71/79)
 95% CI = 81.0 to 95.5%

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 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	subject cls	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 Anti-HBs results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
Early recovery	4	0	0	0	0	2	6
HBV vaccine response	42	0	0	0	0	0	42
Not previously infected	0	0	0	2	0	149	151*
Total	46	0	0	2	0	151	199

* One specimen was not tested for IMMULITE 2000 Anti-HBs.

Early Recovery
 Positive agreement = N/A (4/4)
 95% CI = N/A
 Negative agreement = N/A (2/2)
 95% CI = N/A

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

Not Previously Infected
 Positive agreement = N/A (0/0)
 95% CI = 99.0%
 Negative agreement = 98.7% (149/151)
 95% CI = 95.3 to 99.8%

Total
 Positive agreement = 100.0% (46/46)
 95% CI = 92.3 to 100.0%
 Negative agreement = 98.7% (151/153)
 95% CI = 95.4 to 99.8%
 Total agreement = 99.0% (197/199)
 95% CI = 96.4 to 99.9%

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Clinical Performance with Individuals Who Have Received Hepatitis B Vaccine

In a European site, a retrospective study was conducted to evaluate a total of 92 serum specimens from subjects who had received hepatitis B vaccine. The specimens were tested by IMMULITE 2000 Anti-HBs and an FDA-approved or licensed anti-HBs assay (Kit B), with the following results.

Specimen	Kit B						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
HBV vaccine response	92	0	0	0	0	0	92

Positive agreement = 100.0% (92/92)
 95% CI = 96.1 to 100.0%
 Negative agreement = N/A
 95% CI = N/A
 Total agreement = 100.0% (92/92)
 95% CI = 96.1 to 100.0%

Clinical Performance with Matched Pre- and Post-Vaccination Specimens

In Study 1 in the U.S., pre- and post-vaccination specimens from 17 individuals who had received recombinant HBV vaccine were tested by the IMMULITE 2000 Anti-HBs assay and the reference assay (Kit A). The results are shown below:

Specimens	Kit A						Total
	+			-			
	IML 2000 Anti-HBs						
	+	Ind	-	+	Ind	-	
Pre-vaccination	0	0	0	0	0	17	17
Post-vaccination	17	0	0	0	0	0	17
Total	17	0	0	0	0	17	34

HBV Pre-Vaccination
 Positive agreement = N/A
 95% CI = N/A
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%

HBV Post-Vaccination
 Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = N/A
 95% CI = N/A

Total
 Positive agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Negative agreement = 100.0% (17/17)
 95% CI = 80.5 to 100.0%
 Total agreement = 100.0% (34/34)
 95% CI = 89.7 to 99.9%

II. Analytical Performance

See Tables and Graphs for data representative of the assay's performance. The following results are expressed in mIU/mL, based on WHO 1st IRP 26-1-77. (Unless otherwise noted, all data were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Precision: 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. (See "Precision" tables.)

Caution: The IMMULITE 2000 Anti-HBs lot-to-lot precision has not been evaluated.

EDTA and heparin samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBs and one lot of IMMULITE 2000 Anti-HBs. The median total variance of coefficients (EDTA, 4.2%; heparin, 4.5%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBs.

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 40 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, 20 and 40 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1000, 2000 and 3000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Alternate Sample Types: The measurement of specimens is not significantly affected by the presence of heparinized and EDTA anti-coagulants, as shown in a study that included 46 specimens collected into plain, heparinized and EDTA vacutainer tubes.

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By regression: (See "Alternate Sample Types Graphs".)

(Heparin) = 1.03 (Serum) + 14 mIU/mL
r = 0.97

(EDTA) = 1.05 (Serum) + 16 mIU/mL
r = 0.88

Means
350 mIU/mL (Serum)
376 mIU/mL (Heparin)
384 mIU/mL (EDTA)

Around the cutoff (10 mIU/mL), heparinized specimens demonstrated a 117% recovery and EDTA specimens demonstrated a 106% recovery.

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 Anti-HBs and a commercially available enzyme immunoassay for Anti-HBs (Kit A), with the following results.

Sample type	Kit A				Total
	+		-		
	+	- ¹	+	- ¹	
HAV	17	2	0	28	47
HCV	21	2	1	47	71
HDV	2	0	0	19	21
HEV	5	0	0	5	10
Non-viral liver diseases ²	7	0	0	47	54
Autoimmune diseases	4	1	1	19	25
CMV	3	0	1	9	13
EBV	6	0	1	11	18
Syphilis	5	0	0	5	10
Toxoplasma	7	0	0	13	20
HSV	13	0	1	33	47
Parvovirus B19	2	1	2	11	16
HIV	24	0	1	26	51
Influenza vaccine recipient	10	0	1	14	25
Transplant recipient	4	1	0	9	14
Dialysis	10	0	0	23	33
Intravenous drug abuser	2	0	0	3	5
Others ³	0	0	0	6	6
Total	142	7	9	328	486

¹ Includes indeterminate cases.

² Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

³ Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, six specimens from *H. pylori* positive patients, three from antinuclear antibody (ANA) positive patients, and 26 from patients with positive rheumatoid factor (RF) were tested by IMMULITE 2000 Anti-HBs. IMMULITE 2000 Anti-HBs test results were negative for all six *H. pylori* specimens and all three ANA specimens.

IMMULITE 2000 Anti-HBs results were negative for 23/26 and positive for 3/26 RF specimens.

References

- 1 Locarnini SA, Gust ID. Hepadnaviridae: hepatitis B virus and the delta virus. In: Balows A, et al, editors. Laboratory diagnosis of infectious diseases: principles and practices. New York: Springer-Verlag, 1988: 750-96.
- 2 Follett EAC. Diagnosis of hepatitis B infection. In: Young H, McMillan A, editors. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker, 1988: 433-49.
- 3 Hollinger FB, Dienstag JL. Hepatitis B and D viruses. In: Lennette EH, et al, editors. Manual of clinical microbiology. 6th ed. Washington, D.C.: American Society for Microbiology, 1995:1033-49.
- 4 Nowicki MJ, Balistreri WF. Hepatitis A to E: building up the alphabet. Contemporary Peds 1992: 118-28.
- 5 Zuckerman AJ, et al. Hepatitis B virus and hepatitis D virus. In: Principles and practice of clinical virology. 2nd ed. New York: John Wiley & Sons, 1992: 153-72.
- 6 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders, 1995:354-60.
- 7 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: W.B. Saunders, 1995:322-4.
- 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 Recommendations of the Immunization Practices Advisory Committee Update on Hepatitis B Prevention. MMWR. 1987: 36(23): 53-366.
- 10 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.
- 11 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.

- 12 CDC. Sensitivity of the Test for Antibody to Hepatitis B Surface Antigen-United States. MMWR 1993;42(36);707-710.

Technical Assistance

In the United States, contact DPC's Technical Services department. Tel: 800.372.178s2 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 (mIU/mL)

Site 1

	Mean	Intra-assay		Total	
		SD	CV	SD	CV
1	8.05	0.58	7.2%	0.82	10.2%
2	9.04	0.32	3.6%	0.61	6.8%
3	15.3	0.46	3.0%	1.95	12.7%
4	73	4.38	6.0%	6.07	8.3%
5	130	3.87	3.0%	6.89	5.3%
6	504	22.2	4.4%	34.4	6.8%
7	863	19	2.2%	37.0	4.3%

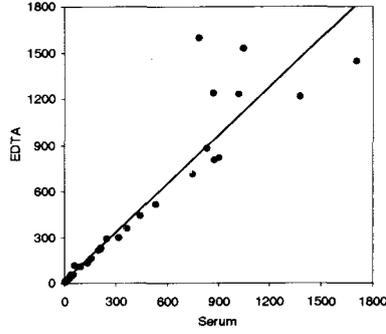
Site 2

	Mean	Intra-assay		Total	
		SD	CV	SD	CV
1	7.93	0.40	5.1%	0.67	8.4%
2	8.68	0.30	3.5%	0.57	6.6%
3	14.6	0.47	3.2%	0.87	6.0%
4	72	5.15	7.1%	6.25	8.7%
5	129	4.28	3.3%	6.71	5.2%
6	476	34.0	7.1%	38.3	8.0%
7	865	21.0	2.5%	43	4.9%

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Site 3

	Intra-assay			Total	
	Mean	SD	CV	SD	CV
1	7.46	0.47	6.3%	0.92	12.4%
2	8.72	0.59	6.8%	0.79	9.0%
3	14.1	0.71	5.0%	1.70	12.1%
4	75	4.24	5.6%	5.45	7.2%
5	129	6.95	5.4%	7.70	6.0%
6	546	50.3	9.2%	76.7	14.1%
7	907	29	3.2%	48	5.3%



Site-to-Site Precision

	Mean	Site-to-Site	
		SD	CV
1	7.82	0.85	10.9%
2	8.81	0.68	7.7%
3	14.7	1.64	11.2%
4	73.6	6.07	8.2%
5	129	7.09	5.5%
6	508	60.1	11.8%
7	878	47.0	5.4%



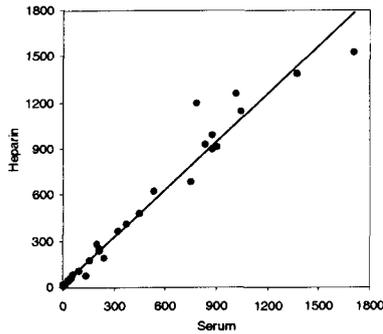
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

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Alternate Sample Types Graphs



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