

SUMMARY OF SAFETY AND EFFECTIVENESS

I. General Information

Device Generic Name: Antibody to Hepatitis B Surface Antigen (Anti-HBs) assay

Device Trade Name: MONOLISA™ Anti-HBs EIA
MONOLISA™ Anti-HBs Calibrator Kit

Applicant: Bio-Rad Laboratories
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Date of Panel Recommendation: None

PMA Number: P050048

Date of Notice of Approval to the Applicant: August 25, 2006

II. Indications For Use

MONOLISA™ Anti-HBs EIA

The Bio-Rad MONOLISA™ Anti-HBs EIA is a qualitative and quantitative enzyme immunoassay for the detection of antibody to hepatitis B surface antigen in human serum and EDTA or citrated plasma. The assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection in 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 MONOLISA™ Anti-HBs Calibrator Kit is intended for quantitative determination of anti-HBs in human serum and EDTA or citrated plasma when used in conjunction with the MONOLISA™ Anti-HBs EIA.

MONOLISA™ Anti-HBs Calibrator Kit

The MONOLISA™ Anti-HBs Calibrator Kit is intended for quantitative determination of anti-HBs in human serum and EDTA or citrated plasma. The MONOLISA™ Anti-HBs Calibrator Kit is to be used only with the MONOLISA™ Anti-HBs EIA (Catalog # 25220).

III. CONTRAINDICATIONS: None known.

IV. WARNINGS AND PRECAUTIONS:

Warnings and precautions for the MONOLISA™ Anti-HBs EIA, and The MONOLISA™ Anti-HBs Calibrator Kit are stated in the respective product labeling.

Both the MONOLISA™ Anti-HBs EIA and The MONOLISA™ Anti-HBs Calibrator Kit are for in vitro diagnostic use only. Both kits are not intended for use in screening blood, plasma, or tissue donors. The effectiveness of the MONOLISA™ Anti-HBs EIA and the MONOLISA™ Anti-HBs Calibrator Kit for use in screening blood, plasma, or tissue donors has not been established. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations.

V. DEVICE DESCRIPTION:

Components of the MONOLISA™ Anti-HBs EIA:

- **Anti-HBs Microwell Strip Plate:** Plates containing 96 wells coated with human HBsAg (*ad* and *ay* sybtypes). Preservative: ProClin (trace).
- **Wash Solution (30X):** Contains sodium chloride and Tween 20.
- **Specimen Diluent:** Buffer with protein stabilizers, fetal calf serum, and sample indicator dye. Preservative: 0.1% ProClin 300™.
- **Negative Control:** Human serum non-reactive for HBsAg and antibodies to HBs, HIV-1, HIV-2, and HCV. Preservatives: 0.005% Gentamicin and 0.16% ProClin 950™ and Gentamicin Sulfate.
- **Positive Control:** Human serum with anti-HBs immunoglobulin; Non-reactive for HBsAg and for antibodies to HIV-1, HIV-2, and HCV. Preservative: 0.16% ProClin 950™ and 0.005% Gentamicin Sulfate.
- **Cutoff Calibrator:** Fetal calf serum, buffer with protein stabilizers, bovine serum albumin, anti-HBs immunoglobulin and dye. Preservative: 0.16% ProClin 950™.
- **Conjugate Concentrate:** Purified HBsAg (human) labeled with peroxidase in a buffer containing protein stabilizers and dye. Preservative: 0.5% ProClin 300™ and 0.005% Gentamicin Sulfate.
- **Conjugate Diluent:** Buffer with protein stabilizers. Preservative: 0.1% ProClin 300™.
- **Chromogen:** Contains tetramethylbenzidine (TMB).
- **Substrate Buffer:** Contains hydrogen peroxide, citric acid, and dimethylsulfoxide (DMSO).
- **Stopping Solution:** Contains 1N H₂SO₄
- **Plate Sealers:** Used to cover the plates during testing.

Components of the MONOLISA™ Anti-HBs Calibrator Kit:

- **0 mIU/mL Calibrator:** Fetal calf serum, buffer with protein stabilizers, bovine serum albumin, anti-HBs immunoglobulin, and dye. Preservative: 0.16% ProClin 950™.
- **100 mIU/mL Calibrator:** Fetal calf serum, buffer with protein stabilizers, bovine serum albumin, anti-HBs immunoglobulin, and dye. Preservative: 0.16% ProClin 950™.
- **400 mIU/mL Calibrator:** Fetal calf serum, buffer with protein stabilizers, bovine serum albumin, anti-HBs immunoglobulin, and dye. Preservative: 0.16% ProClin 950™.
- **1000 mIU/mL Calibrator:** Fetal calf serum, buffer with protein stabilizers, bovine serum albumin, anti-HBs immunoglobulin, and dye. Preservative: 0.16% ProClin 950™.

Assay Principal and Format

The MONOLISA™ Anti-HBs EIA is a quantitative enzyme immunoassay for the detection of antibody to hepatitis B surface antigen in human serum and EDTA or citrated plasma. The assay is based on the direct antibody sandwich principle, in which microwell strip plates (the solid phase) are coated with purified native *ad* and *ay* antigens of the hepatitis B virus.

Patient samples are evaluated for the presence of anti-HBs by interaction with the adsorbed antigen on the wells. After incubation of the samples in the microwells, the plates are washed. If antibodies to the virus are present, they bind to the antigen and are not removed by washing. The Conjugate reagent, containing peroxidase-labeled purified hepatitis B *ad* and *ay* antigens, is then added to the wells and will bind to the IgG, IgM, or IgA captured from the patient samples and bound on the solid phase. Unbound conjugate is removed by a wash step. Next, Working TMB Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of antibody that has been bound to the antigen-coated plate. The enzyme reaction is stopped by the addition of acid, which results in a color change to yellow. The optical absorbance of controls and specimens is determined with a spectrophotometer with wavelength set at 450 nm.

The kit contains a Negative Control, prepared from human serum negative for hepatitis B antibody, and a Positive Control, prepared from human serum negative for hepatitis B antibody spiked with purified anti-HBs. Also included in the kit is a cutoff calibrator (10 mIU/ml), prepared in a PBS-based buffer with protein stabilizers and spiked with purified anti-HBs, and it is calibrated against the WHO standard.

The MONOLISA™ Anti-HBs Calibrator Kit contains 4 levels of purified anti-HBs, prepared in a PBS-based buffer with protein stabilizers, and they are calibrated against the WHO standard. The kit is used to plot a calibration curve from the optical density value of each calibrator, for the determination of anti-HBs concentration in samples.

For more concentrated samples, plates are read at both 450nm and 405nm wavelengths. When read at 405nm, all absorbance values are reduced so that samples which are above the reader maximum at 450nm will often be within the linear range of the reader at 405nm. By using the second wavelength, the dynamic range of the assay can be extended to calibrate samples from 400-1000 mIU/mL, without diluting and testing them again.

VI. Alternative Practices and Procedures

Determination of the presence of anti-HBs in patients may be attained by using a number of commercially available, FDA licensed/approved, serological tests. When these test results are evaluated in conjunction with a physician's assessment and biochemical test results, susceptibility to HBV can be excluded.

VII. Marketing History

The MONOLISA™ Anti-HBs EIA and MONOLISA™ Anti-HBs Calibrator Kit have not been commercially marketed in countries outside of the U.S.

VIII. Potential Adverse Effects of the Device on Health

Failure of the product to perform as intended, or errors in the use of the product, may lead to a false result. A false nonreactive result is not considered a public health risk, as the individual would either be vaccinated unnecessarily, or would be erroneously diagnosed as having not recovered from an acute HBV infection. A false reactive result can be considered a public health risk because an individual would be considered either to have been previously vaccinated or to have been previously exposed to the virus, and would therefore be assumed to be immune to HBV. As such, the risk is that the individual would not be vaccinated and would be at a higher risk of infection if exposed to HBV, and would then also be at a subsequent risk of spreading infection to other non-immune individuals.

IX. Summary of Preclinical Studies

A. Analytical Sensitivity

The MONOLISA™ Anti-HBs EIA may be utilized as a quantitative assay when run in conjunction with the calibrators from the MONOLISA™ Anti-HBs Calibrator Kit. Studies that have been performed to test the analytical sensitivity of the assay used dilutions of the WHO anti-HBs Standard to demonstrate correlation with concentrations of anti-HBs ranging from 0 mIU/mL to 1000 mIU/mL. The dilutional linearity was evaluated using high-titer anti-HBs specimens to prepare a series of dilutions in negative plasma. The data provide assurance that the assay, when run with the Anti-HBs Calibrator Kit, yields valid quantitative results.

The limit of detection, the lowest amount of anti-HBs that can be detected with a 95% probability, was determined for the MONOLISA™ Anti-HBs EIA. The calculation of the limit was based on NCCLS/CLSI EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. The limit of detection was 4.14 mIU/mL at an OD of 0.034.

B. Cross-Reactivity

The specificity of the MONOLISA™ Anti-HBs EIA assay was evaluated during the analysis of 393 serum samples from individuals with unrelated medical conditions, representing 20 potentially cross-reacting conditions. All of the samples were anti-HBs negative on another commercially available Anti-HBs assay. The results of each sample tested on the MONOLISA™ Anti-HBs EIA are summarized in Table 1.

Table 1
Potentially Cross-Reactive Medical Conditions

Clinical Condition	Nonreactive	Reactive	Borderline	Total
Autoimmune Diseases ¹	20	0	0	20
Cytomegalovirus (CMV)	19	1 ²	0	20
Elevated liver enzymes	3	0	0	3
Epstein Barr Virus (EBV)	20	0	0	20
H. pylori positive	10	0	0	10
Hepatic Cancer	4	0	0	4
Hepatitis A Infection (HAV)	19	1 ²	0	20
Hepatitis C Infection (HCV)	18	2 ²	0	20
Hepatitis D Infection (HDV)	6	0	0	6
Herpes Simplex Virus (HSV)	20	0	0	20
HIV-1	19	1 ²	0	20
HIV-2	15	4 ³	1 ⁴	20
HTLV-I/II	19	1 ²	0	20
Influenza Vaccine Recipients	18	2 ²	0	20
Parvovirus B19	20	0	0	20
Pregnant (bHCG positive)	50	0	0	50
Rheumatoid Factor (RF)	20	0	0	20
Rubella	19	1 ²	0	20
SLE / ANA Positive	19	1 ²	0	20
Syphilis	20	0	0	20
Toxoplasmosis	20	0	0	20
TOTAL	378	14	1	393

¹ Scleroderma, Sjögren's, MCTD etc.

² Of the 10 medical condition specimens that were reactive on MONOLISA™ Anti-HBs EIA (excluding HIV-2 positive samples), 3 were reactive on the reference anti-HBs EIA, 5 were nonreactive, and 2 were QNS for additional testing.

³ Two specimens were reactive when tested with a reference anti-HBs EIA; 2 were QNS for additional testing.

⁴ Specimen was reactive when tested with a reference anti-HBs EIA.

Of the 393 specimens from 20 unrelated medical conditions that were tested, 378/393 (96.2%) were nonreactive on the MONOLISA™ Anti-HBs EIA. Fourteen (14) specimens were reactive: 4 HIV-2 reactive specimens, 2 influenza vaccine specimens, 2 HCV positive specimens and 1 each of 8 other conditions (CMV, HAV, HIV-1, HTLV, Rubella, and SLE).

C. Interfering substances

The MONOLISA™ Anti-HBs EIA was evaluated to determine if there were any effects on assay performance when tested with specimens containing potentially interfering substances, according to CLSI Document EP7. None of the interferents at the levels tested below produced a change in clinical interpretation or a significant $\geq 10\%$ change of the assay.

Hemolyzed: 500 mg/dL of hemoglobin

Lipemic: 1000 mg/dL of triglycerides

Icteric: 20 mg/dL of bilirubin

Proteinemic: 15 g/dL of protein

The MONOLISA™ Anti-HBs assay did not exhibit a high-dose hook effect in patient samples with levels of antibodies to HBsAg as high as 175,000 mIU/mL. The MONOLISA™ Anti-HBs EIA is designed using a two-step format, where a high-dose hook effect is not normally observed.

D. Stability Studies

1. Kit Stability

A functional stability study of the MONOLISA™ Anti-HBs EIA test kit and the MONOLISA™ Anti-HBs Calibrator Kit has demonstrated that kits which are stored at 2-8°C are stable for the intended shelf-life of the kits. Real-time studies were performed on three kit lots of both the EIA and Calibrator kits, at multiple time points throughout the shelf life of the kits. Data from these studies support a 12-month dating period for the MONOLISA™ Anti-HBs EIA test kit and the MONOLISA™ Anti-HBs Calibrator Kit, with the expiration of the assembled kit based on the component with the shortest dating period.

2. Interchangeability of Common Reagents

The MONOLISA™ Anti-HBs EIA contains four common reagents that may be used interchangeably with the same components in other lots of the Anti-HBs EIA: Wash Solution Concentrate, Chromogen, Substrate Buffer, and Stopping Solution. Matrix studies performed with the MONOLISA™ Anti-HBs EIA have evaluated different lots of each of these components in the kit, and demonstrated equivalent results. Therefore, any lot number of these reagents may be used with this assay provided they are not used beyond their labeled expiration date.

E. Microbiology Studies

Antimicrobial preservatives have been added to the components in the Anti-HBs EIA kit and Anti-HBs Calibrator Kit to protect the product from degradation and performance failure due to the presence of microbial contamination. Preservative effectiveness studies have been conducted in accordance with the protocol specified in the United States Pharmacopoeia (Microbiological Tests, <51> Antimicrobial Preservatives/Effectiveness) to assess the efficacy of these preservatives in suppressing microbial growth. These studies have demonstrated that the antimicrobial agents are present in concentrations required to inhibit the growth of adventitious agents.

A Microbial Challenge study has been performed to evaluate the functional stability of the Anti-HBs EIA kit and Anti-HBs Calibrator Kit components in the presence of microbial organisms. One set of Anti-HBs EIA and Anti-HBs Calibrator kit components that were inoculated with microorganisms was tested in comparison to a reference second set of Anti-HBs EIA and Anti-HBs Calibrator kit components that had not been inoculated. A variety of organisms from the environment were used in this challenge study. Each kit was stored at the recommended product storage of 2-8°C after inoculation and tested at multiple time points throughout kit expiration. These studies have demonstrated that the functionalities of the EIA and Calibrator products are not impaired and the reagents are stable for the 12-month shelf life when microbial contamination is present.

F. Reproducibility

A 7-member panel consisting of diluted patient samples in various matrices (serum and EDTA) was tested in duplicate, once a day for 10 days, on 3 lots of the MONOLISA™ Anti-HBs EIA.

The data from all 3 reagent lots were combined to obtain standard deviation (SD) and percent coefficient of variation (CV) for within run, between run, and total variance. The data were analyzed according to the principles described in CLSI EP5-A2 and ISO/TR 22971:2005. The data summary for this study is shown in Tables 2 and 3.

Table 2
MONOLISA™ Anti-HBs EIA Reproducibility Results
by Panel Member Signal to Cutoff (S/CO)

Test Site	Panel Member	N	Mean S/CO	Within Run ¹		Between Run ²		Total ³	
				SD	CV (%)	SD	CV (%)	SD	CV (%)
Site #1	1 Pos Serum	60	8.265	0.309	3.7	0.579	7.0	0.487	5.9
	2 ~12 mIU/mL (Serum)	60	1.349	0.051	3.8	0.104	7.7	0.085	6.3
	3 ~8 mIU/mL (Serum)	60	0.955	0.047	4.9	0.072	7.5	0.064	6.7
	4 Neg (Serum)	60	0.288	0.012	4.3	0.073	25.3	0.054	NA
	5 ~12 mIU/mL (EDTA)	60	1.435	0.032	2.2	0.090	6.3	0.078	5.5
	6 ~8 mIU/mL (EDTA)	60	1.044	0.033	3.2	0.070	6.7	0.063	6.1
	7 Neg (EDTA)	60	0.329	0.018	5.5	0.090	27.3	0.065	NA
Site #2	1 Pos Serum	60	8.008	0.259	3.2	0.984	12.3	0.696	8.7
	2 ~12 mIU/mL (Serum)	60	1.289	0.078	6.1	0.136	10.5	0.109	8.5
	3 ~8 mIU/mL (Serum)	60	0.950	0.089	9.3	0.225	23.6	0.165	17.4
	4 Neg (Serum)	60	0.285	0.093	32.7	0.197	69.1	0.149	NA
	5 ~12 mIU/mL (EDTA)	60	1.384	0.098	7.0	0.205	14.8	0.156	11.3
	6 ~8 mIU/mL (EDTA)	60	0.971	0.037	3.8	0.085	8.7	0.068	7.0
	7 Neg (EDTA)	60	0.292	0.058	19.9	0.149	50.9	0.117	NA
Site #3	1 Pos Serum	60	6.707	0.373	5.6	1.560	23.3	1.150	17.1
	2 ~12 mIU/mL (Serum)	60	1.034	0.055	5.3	0.243	23.5	0.176	17.0
	3 ~8 mIU/mL (Serum)	60	0.705	0.050	7.1	0.146	20.7	0.111	15.7
	4 Neg (Serum)	60	0.249	0.061	24.3	0.130	52.0	0.098	NA
	5 ~12 mIU/mL (EDTA)	60	1.124	0.093	8.2	0.299	26.6	0.218	19.4
	6 ~8 mIU/mL (EDTA)	60	0.763	0.078	10.2	0.181	23.7	0.139	18.2
	7 Neg (EDTA)	60	0.200	0.042	21.0	0.062	30.8	0.054	NA

NA = Not Applicable.

¹ Within Run: variability of the assay performance from replicate to replicate.² Between Run: variability of the assay performance from run to run.³ Total variability of the assay performance includes within run, between run and between lot.

Table 3
MONOLISA™ Anti-HBs EIA Reproducibility Results (Positive, Low Positive, and High Negative)
by Panel Member S/CO

Summary of Panel Members	N=	Mean S/CO	Between Lot		Between Site ¹		Total ²	
			SD	CV	SD	CV	SD	CV
Positive Serum	180	7.660	1.334	17.4	6.473	84.5	1.069	14.0
~12 mIU/mL (Serum)	180	1.223	0.194	15.9	1.295	105.9	0.188	15.3
~8 mIU/mL (Serum)	180	0.870	0.145	16.7	1.107	127.2	0.168	19.3
~12 mIU/mL (EDTA)	180	1.314	0.207	15.7	1.292	98.3	0.211	16.0
~8 mIU/mL (EDTA)	180	0.926	0.175	18.9	1.131	122.2	0.153	16.6

¹ Sites were nested within lots

² Total variability includes within run, between run, between lot, and between site.

G. Quantitative Precision

A precision study was performed with the MONOLISA Anti-HBs EIA using quantitative panels prepared in serum and EDTA plasma. Each 7-member panel spanned the linear range of the assay. The 14 specimens were tested in triplicate for 20 days, and results are summarized in Table 4.

Table 4
MONOLISA™ Anti-HBs EIA 20-Day Precision Results in mIU/mL

Panel Member	N	mIU/mL Mean	Within-Run		Between-Day		Total	
			SD	CV%	SD	CV%	SD	CV%
Serum ~10 mIU/mL	60	11.1	0.259	2.34	1.361	12.30	1.386	12.52
Serum ~25 mIU/mL	60	26.6	1.163	4.37	1.589	5.97	1.969	7.40
Serum ~85 mIU/mL	60	89.1	1.372	1.54	2.383	2.67	2.750	3.09
Serum ~350 mIU/mL	60	363.3	3.372	0.93	5.202	1.43	6.199	1.71
Serum ~500 mIU/mL	60	492.2	19.882	4.04	17.794	3.62	26.682	5.42
Serum ~750 mIU/mL	60	726.9	13.088	1.80	17.429	2.40	21.796	3.00
Serum ~950 mIU/mL	60	946.3	11.786	1.25	14.777	1.56	18.901	2.00
EDTA Plasma ~10 mIU/mL	60	12.4	0.596	4.80	1.263	10.17	1.396	11.24
EDTA Plasma ~25 mIU/mL	60	27.9	0.462	1.66	1.111	3.99	1.204	4.32
EDTA Plasma ~85 mIU/mL	60	92.5	1.055	1.14	2.281	2.47	2.513	2.72
EDTA Plasma ~350 mIU/mL	60	367.5	8.216	2.24	7.953	2.16	11.435	3.11
EDTA Plasma ~500 mIU/mL	60	496.0	11.173	2.25	19.743	3.98	22.685	4.57
EDTA Plasma ~750 mIU/mL	60	738.5	10.457	1.42	16.504	2.23	19.538	2.65
EDTA Plasma ~950 mIU/mL	60	940.1	20.127	2.14	13.425	1.43	24.193	2.57

IX. Summary of Clinical Studies

A multi-center clinical trial was conducted to evaluate the performance of the MONOLISA™ Anti-HBs EIA and the MONOLISA™ Anti-HBs Calibrator Kit in human serum and plasma. A total of 1452 prospective subjects at high risk for viral hepatitis and/or showing signs/symptoms of HBV were included in the study. Of these 1452, 1373 were from the asymptomatic high risk population and 79 reported signs or symptoms of HBV.

Expected Values

The expected values that can be seen with the MONOLISA™ Anti-HBs EIA, by gender and age range, were determined during the evaluation of 1373 prospective asymptomatic subjects. All subjects (100%) were at high risk for viral hepatitis including intravenous drug users (N = 476), homosexual males (N = 144), sex workers (N = 171), prison history (N = 340), high risk sex partners (167), high risk occupation/health care workers (N = 85), hemodialysis (N = 58), hemophiliacs (3), and other (N = 470). Many had more than 1 high risk behavior or risk factor. One hundred seventy six (12.8%) of these high risk subjects also reported having received a full course of injections of an HBV vaccine. Subjects in the asymptomatic prospective population were from the following geographic locations: 459 from Los Angeles, CA, (33.4%), 57 from Santa Ana, CA (4.1%), 72 from Miami, FL (5.2%), 345 from Cocoa, FL (25.1%), 273 from San Francisco, CA (19.9%), and 167 from Seattle, WA (12.2%). The group was Caucasian (36.5%), Black or African American (41.1%), Hispanic or Latino (13.3%), Asian (4.2%), Native Hawaiian or other Pacific Islander (0.7%), and American Indian or Alaska Native (2.2%), with the remaining 2.0% represented by multiple ethnic groups or was unknown. The subjects were male (70.1%) and female (29.9%) and ranged in age from 18 to 81 years.

The MONOLISA™ Anti-HBs EIA results for the asymptomatic prospective population, by gender and age range, are presented in Table 5.

Table 5
Expected Values by Gender and Age - MONOLISA™ Anti-HBs EIA

Age Range	Gender	MONOLISA™ Anti-HBs EIA Result						Total
		Reactive		Borderline		Non-reactive		
		N	%	N	%	N	%	
10-19	F	6	100.0%	0	0.0%	0	NA	6
	M	7	70.0%	0	0.0%	3	30.0%	10
20-29	F	44	42.3%	1	1.0%	59	56.7%	104
	M	48	39.0%	0	0.0%	75	61.0%	123
30-39	F	42	37.5%	4	3.6%	66	58.9%	112
	M	75	35.0%	1	0.5%	138	64.5%	214
40-49	F	40	37.4%	3	2.8%	64	59.8%	107
	M	162	45.8%	7	2.0%	185	52.3%	354
50-59	F	33	51.6%	4	6.3%	27	42.2%	64
	M	108	51.2%	8	3.8%	95	45.0%	211
60-69	F	5	41.7%	0	0.0%	7	58.3%	12
	M	23	57.5%	0	0.0%	17	42.5%	40
70-79	F	1	50.0%	0	0.0%	1	50.0%	2
	M	3	60.0%	0	0.0%	2	40.0%	5
80-89	F	0	NA	0	NA	0	NA	0
	M	0	NA	0	0.0%	1	100.0%	1
Unknown	F	2	66.7%	0	0.0%	1	33.3%	3
	M	1	20.0%	1	20.0%	3	60.0%	5
Totals		600	43.7%	29	2.1%	744	54.2%	1373

Reference Markers

The HBV disease classification for each subject in the total prospective population (N = 1452) was determined by a serological assessment using a hepatitis marker profile consisting of commercially available reference EIAs. The six HBV reference marker assays included HBsAg, hepatitis B virus e antigen (HBeAg), total antibody to hepatitis B virus core antigen (Anti-HBc, Total), IgM antibody to hepatitis B virus core antigen (Anti-HBc IgM), total antibody to HBe Ag (Anti-HBe), and total antibody to hepatitis B virus surface antigen (anti-HBs, qualitative or quantitative). All reference EIAs were tested according to the manufacturer's package insert instructions. Agreement of the

MONOLISA™ Anti-HBs EIA was assessed relative to the reference anti-HBs result and to HBV classification.

In the MONOLISA™ Anti-HBs EIA clinical study, across 3 clinical sites, there were 38 unique reference marker patterns observed. Table 6 summarizes the patterns and their associated classifications. No other laboratory or clinical information was used in the disease classification process.

Table 6
Characterization of Prospective Samples

FDA Characterization (single point specimen)	HBsAg	HBeAg	Anti-HBc IgM	Total HBc	Anti-HBe	Anti-HBs
Acute infection	+	+	+	+	-	-
Acute infection	+	+	-	-	-	-
Acute infection	+	-	+	-	-	+
Acute infection	+	-	-	-	-	-
Acute infection	+	-	+	-	-	-
Acute infection	+	+	I	+	-	-
Chronic infection	+	+	-	+	-	-
Chronic infection	+	+	-	+	-	+
Chronic infection	+	+	-	+	+	-
Chronic infection	+	+	+	+	-	+
Chronic infection	+	-	-	+	-	-
Chronic infection	+	-	-	+	-	+
Chronic infection	+	-	-	+	+	-
Chronic infection	+	-	-	+	+	+
Chronic infection	+	-	-	+	I	-
Chronic infection	+	+	-	+	+	+
Early recovery	-	-	-	+	-	-
Early recovery	-	-	-	+	+	-
Early recovery	-	-	I	+	+	+
Early recovery	-	-	-	+	I	-
Early recovery	-	-	+	+	-	+
Early recovery	-	-	+	+	+	-
Early recovery	-	-	+	+	+	+
Recovery	-	-	-	-	+	+
Recovery	-	-	-	+	+	+
Recovery	-	-	-	+	+	I
Recovery	-	-	-	+	I	+
Recovered	-	-	-	+	-	I
Recovered	-	-	-	+	I	I
Recovered or Immune due to natural infection	-	-	-	+	-	+

HBV vaccine response	-	-	-	-	-	+
HBV vaccine response (?)	-	-	-	-	-	I
Not previously infected with HBV	-	-	-	-	-	-
Uninterpretable	+	-	-	-	+	-
Uninterpretable	-	+	-	-	-	+
Uninterpretable	-	+	-	+	-	-
Uninterpretable	-	+	-	-	-	-
Uninterpretable	-	-	-	-	+	-

(-) = Negative / Non-reactive: (+) = Positive / Reactive: (I) = Indeterminate

Comparison of Results

A comparison of the MONOLISA™ Anti-HBs EIA results with the reference anti-HBs assay for each specimen classification is shown in Table 7. In clinical studies, specimens that had indeterminate results on the reference test were retested in duplicate per the manufacturer's instructions for use. Any specimens with 2/3 or 3/3 results within the indeterminate range were classified as indeterminate.

Table 7
Comparison of Results - High Risk Prospective Samples
MONOLISA™ Anti-HBs EIA versus Reference Anti-HBs EIAs

Reference HBV Classification	Reference Anti-HBs Result									Total
	Positive			Indeterminate			Negative			
	MONOLISA Anti-HBs			MONOLISA Anti-HBs			MONOLISA Anti-HBs			
	R	BRD ²	NR	R	BRD ²	NR	R	BRD ₂	NR	
Acute infection	1	0	0	0	0	0	0	0	13	14
Chronic infection	4	1	1	0	0	0	4 ¹	0	71	81
Early Recovery	3	1	0	0	0	0	10	5	88	107
Recovery	160	3	4	3	4	2	0	0	0	176
Recovered	0	0	0	5	6	1	0	0	0	12
Recovered or Immune due to natural infection	91	1	4	0	0	0	0	0	0	96
HBV vaccine response	307	1	8	0	0	0	0	0	0	316
HBV vaccine response (?)	0	0	0	16	8	7	0	0	1	32
Not previously infected with HBV	0	0	0	0	0	0	11	0	598 ¹	609
Uninterpretable	1	0	0	0	0	0	0	0	8	9
Total	567	7	17	24	18	10	25	5	779	1452

¹ Includes specimens that were NRR (not repeatedly reactive)

² BRD = Borderline ($\pm 10\%$ of cutoff value)

Overall, 567 samples were positive on both assays, 18 samples were indeterminate/borderline on both assays, and 779 samples were negative on both assays.

Percent Agreement

The percent agreement between the MONOLISA™ Anti-HBs EIA and the reference anti-HBs assays was evaluated for each specimen classification, including the upper and lower 95% Wilson confidence bounds. A summary of this analysis for the prospective population is presented for each HBV classification in Table 8.

Table 8
Percent Agreement
MONOLISA™ Anti-HBs EIA versus Reference Anti-HBs EIA

HBV Classification	N ¹	Positive Percent Agreement ²	95% Confidence Interval	Negative Percent Agreement ³	95% Confidence Interval
Acute Infection	14	100.0% (1/1)	20.7%, 100.0%	100.0% (13/13)	77.2%, 100.0%
Chronic Infection	81	66.7% (4/6)	30.0%, 90.3%	94.7% (71/75)	87.1%, 97.9%
Early Recovery	107	75.0% (3/4)	30.1%, 95.4%	85.4% (88/103)	77.4%, 91.0%
Recovery	176	94.7% (160/169)	90.2%, 97.2%	0.0% (0/3)	NA
Recovered	12	0.0% (0/1)	NA	0.0% (0/5)	NA
Past Infection	96	94.8% (91/96)	88.4%, 97.8%	NA (0/0)	NA
HBV Vaccine response	316	97.2% (307/316)	94.7%, 98.5%	NA (0/0)	NA
HBV vaccine response (?)	32	0.0% (0/7)	NA	5.9% (1/17)	1.0%, 27.0%
Not previously infected	609	NA (0/0)	NA	98.2% (598/609)	96.8%, 99.0%
Uninterpretable	9	100.0% (1/1)	20.7%, 100.0%	100.0% (8/8)	67.6%, 100.0%
Total	1452	94.3% (567/601)	92.2%, 95.9%	93.5% (779/833)	91.6%, 95.0%

¹ N=Total number of samples; refer to Table 3 for correlation of borderline samples. The eighteen specimens that were indeterminate by both assays were not included in the percent agreement calculations. Positive or negative results from the MONOLISA™ Anti-HBs EIA were considered as non-agreements in the calculation of percent positive agreement and percent negative agreement when the corresponding reference assay result was indeterminate/borderline.

² Compares number of samples positive on both assays to sum of all positive samples on the reference assay + samples indeterminate on the reference assay and negative on MONOLISA™ Anti-HBs EIA.

³ Compares number of samples negative on both assays to sum of all negative samples on the reference assay + samples indeterminate on the reference assay and positive on MONOLISA™ Anti-HBs EIA.

The positive percent agreement with the reference method is 94.3% (567/601) with a 95% confidence interval of 92.2 – 95.9%. The negative percent agreement with the reference method is 93.5% (779/833) with a 95% confidence interval of 91.6 – 95.0%.

Seroconversion Panels

The comparative sensitivity of the MONOLISA™ Anti-HBs EIA was determined by testing 4 commercially available Anti-HBV seroconversion panels and comparing the results to those in the associated certificates of analysis. Comparative results for only panel members near the point of seroconversion are presented in Table 9.

Table 9
HBV Seroconversion Panel Results

Panel ID	Day since 1 st bleed	Total # Members	MONOLISA™ Anti-HBs		Reference Anti-HBs EIA ¹	
			S/CO	Result	S/CO	Result
RP016-08	60	20	0.26	NR	0.50	NR
RP016-09	74		1.02	BRD²	1.47	R
RP016-10	79		1.95	R	2.43	R
RP016-11	81		3.72	R	2.43	R
6506-06	69	14	0.74	NR	0.8	NR
6506-07	83		1.75	R	1.1	R
6506-08	97		3.00	R	2.0	R
6514-09	112	17	0.57	NR	0.7	NR
6514-10	126		1.20	R	1.3	R
6514-11	140		1.91	R	1.4	R
6536-06	70	12	0.85	NR	0.7	NR
6536-07	84		1.15	R	0.9	NR
6536-08	98		1.42	R	1.3	R

¹ From Certificates of Analysis.

² BRD = Borderline

In 2 of the 4 seroconversion panels, the MONOLISA™ Anti-HBs EIA detected reactive levels of hepatitis B surface antibody at the same member as the reference anti-HBs EIA. In 1 of the 4 seroconversion panels the MONOLISA™ Anti-HBs EIA detected reactive levels of hepatitis B surface antibody 1 member before the reference anti-HBs EIA. One panel was borderline (S/CO = 1.02) on the MONOLISA™ Anti-HBs EIA at the first reactive bleed on the reference test.

Clinical Performance with Individuals Who Received a Full Course of Hepatitis B Vaccine

Retrospective studies were conducted to evaluate a total of 197 serum specimens from 197 subjects who had received a full course of 3 HBV vaccinations (SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® vaccine). Testing was compared to reference anti-HBs EIA. The MONOLISA™ Anti-HBs EIA demonstrated immunity in 141/197 specimens (71.6%; 95% confidence interval of 64.9% to 77.4%). The reference method demonstrated immunity in 134/197 specimens (68.0%; 95% confidence interval of 61.2% - 74.1%).

Table 10
Post-HBV Vaccination Results

MONOLISA™ Anti-HBs Result	Reference Anti-HBs Result			
	Immune	Indeterminate	Not-Immune	Totals
Immune	134	3	4	141
Borderline	0	2*	1	3
Not-Immune	0	1	52	53
Totals	134	6	57	197

* Two specimens that were indeterminate by both assays were not included in percent agreement calculations.

The positive percent agreement with the reference method is 99.3% (134/135) with a 95% confidence interval of 95.9 – 99.9%. The negative percent agreement with the reference method is 86.7% (52/60) with a 95% confidence interval of 75.8 – 93.1%.

Clinical Performance with Matched Pre- and Post-HBV Vaccination Specimens

In another study, matched sets of pre- and post-vaccination specimens from thirty-eight individuals who had received recombinant HBV vaccine (either SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® vaccine) were tested with the MONOLISA™ Anti-HBs EIA. The matched sets from each subject included four specimens. One specimen was a pre- vaccination specimen collected before receiving the first vaccination dose of HBV vaccine. The second and third specimens were collected right before the second vaccination dose and third vaccination dose respectively. A post vaccination specimen was collected a minimum of 2 weeks after receiving the full course of 3 injections.

Pre- Vaccination Samples

In pre-vaccination samples, one sample was reactive (immune) on the MONOLISA™ Anti-HBs EIA but nonreactive (not immune) on the reference assay. The negative percent agreement with the reference method is 97.4% (37/38) with a 95% confidence interval of 86.5 - 99.5%. Results are presented in Table 11 below.

Table 11
Pre-Vaccination Specimen Results
MONOLISA™ Anti-HBs EIA versus Reference EIA

MONOLISA™ Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
Immune	0	1	1
Borderline	0	0	0
Not Immune	0	37	37
Total	0	38	38

I = Immune, NI = Not Immune

Pre- Second Vaccination Samples

In samples drawn just prior to the second vaccination in the series, the MONOLISA™ Anti-HBs EIA demonstrated immunity in 4/38 (10.5%) of the samples. The reference method demonstrated immunity in 1/38 (2.6%) of the samples.

The positive percent agreement with the reference method is 100% (1/1) with a 95% confidence interval of 20.7 – 100%. The negative percent agreement with the reference method is 89.2% (33/37) with a 95% confidence interval of 75.3 – 95.7%. One sample was borderline on the MONOLISA™ Anti-HBs EIA assay. Results are presented in Table 12 below.

Table 12
Pre-Second Vaccination Specimen Results
MONOLISA™ Anti-HBs EIA versus Reference EIA

MONOLISA™ Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
Immune	1	3	4
Borderline	0	1	1
Not Immune	0	33	33
Total	1	37	38

I = Immune, NI = Not Immune

Pre- Third Vaccination Samples

The positive percent agreement with the reference method is 100% (15/15) with a 95% confidence interval of 79.6 - 100%. The negative percent agreement with the reference method is 82.6% (19/23) with a 95% confidence interval of 62.9 – 93.0%. One sample was borderline on the MONOLISA™ Anti-HBs EIA assay. Results are presented in Table 13 below.

Table 13
Pre-Third Vaccination Specimen Results
MONOLISA™ Anti-HBs EIA versus Reference EIA

MONOLISA™ Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
Immune	15	3	18
Borderline	0	1	1
Not Immune	0	19	19
Total	15	23	38

I = Immune, NI = Not Immune

Post Vaccination Samples

In samples drawn after the complete vaccination series (post vaccination), the positive percent agreement with the reference method is 97.0% (32/33) with a 95% confidence interval of 84.7 – 99.5%. The negative percent agreement with the reference method is 60% (3/5) with a 95% confidence interval of 23.1 – 88.2%. One sample that was immune and two that were not immune with the reference assay were borderline with the MONOLISA™ Anti-HBs EIA. Results are presented in Table 14 below.

Table 14
Post-Vaccination Results
MONOLISA™ Anti-HBs EIA versus Reference EIA

MONOLISA™ Anti-HBs Result	Reference Anti-HBs Result		
	I	NI	Total
Immune	32	0	32
Borderline	1	2	3
Not Immune	0	3	3
Total	33	5	38

I = Immune, NI = Not Immune

X. Conclusions Drawn from the Studies

Multi-centered clinical and non-clinical were conducted in the US to evaluate the MONOLISA™ Anti-HBs EIA. A method comparison was performed with a commercially available licensed assay to detect anti-HBs antibodies in specimens from an intended use diagnostic population.

The performance characteristics of the assay are not affected by potential cross-reacting substances that may be present in clinical samples, or by interfering substances (hemoglobin, lipemia, bilirubin, or elevated protein levels).

Stability studies have demonstrated that Anti-HBs EIA kits and Anti-HBs Calibrator Kits which are stored as indicated (2-8°C) are stable for the intended shelf-life of the kits.

Multi-centered clinical studies and in-house analytical studies were conducted in the US to evaluate the MONOLISA™ Anti-HBs EIA. A method comparison was performed with a commercially available licensed assay to detect anti-HBs antibodies in specimens from an intended use diagnostic population.

Hepatitis B virus classification using the prospective population showed 38 unique reference marker patterns. The overall positive percent agreement between the MONOLISA™ Anti-HBs EIA and the reference assay was 94.3% (567/601) in the high risk, signs and symptoms, and vaccinated populations. The overall negative percent agreement between the MONOLISA™ Anti-HBs EIA and the reference assay was 93.5% (779/833) in the same population.

In HBV vaccinated individuals, the positive agreement was 99.3% (134/135) and the negative percent agreement was 86.7% (52/60) with the comparison method. In another study, the Pre-vaccination HBV vaccine group recipients, the negative agreement with the comparison method was 97.4 (37/38) and in the post vaccination group the positive agreement rate with the comparison method was 97.0 % (32/33).

The ability of the MONOLISA™ Anti-HBs EIA to detect the anti-HBs antibodies was demonstrated in pediatric specimen testing.

Precision and reproducibility of the MONOLISA™ Anti-HBs EIA was established for within-run ($\leq 8.9\%$), within-day ($\leq 9.1\%$), within-lab ($\leq 11.4\%$), and between sites ($\leq 13.2\%$).

Tube Type Interference study results support the use of human serum and EDTA or citrated specimens in the MONOLISA™ Anti-HBs EIA.

The results from both the non-clinical and clinical studies indicate that the MONOLISA™ Anti-HBs EIA can be used safely and effectively for the qualitative and quantitative determination of anti-HBs antibodies in human serum and plasma. The assay may be used with other HBV serological markers to define the clinical status of patients known to be infected with HBV.

RISK BENEFIT ANALYSIS

As a diagnostic test, the MONOLISA™ Anti-HBs EIA involves the removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefits to HBV-infected individuals tested by the assay outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error.

The potential risks encountered with this *in vitro* diagnostic test are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

SAFETY

Based on the results of the preclinical and clinical laboratory studies, the MONOLISA™ Anti-HBs EIA, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and effective and pose minimal risk to the patient due to false test results.

EFFECTIVENESS

The effectiveness of the MONOLISA™ Anti-HBs EIA has been demonstrated for use in determining if antibodies to the HBs antigen of the hepatitis B virus are present in an individual's serum or plasma. A reasonable determination of effectiveness of the MONOLISA™ Anti-HBs EIA assay for aiding in the diagnosis of immunity and status of HBV infection in suspected individuals has been demonstrated.

XI. Panel Recommendations

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CDRH Decision

FDA issued an approval order on August 25, 2006.

The applicant's manufacturing facilities were inspected on May 17 – June 1, 2006 and found to be in substantial compliance with the Quality Systems Regulation as defined in 21 CFR 820.

XII. Approval Specifications

Directions for Use: See labeling.

Hazards to Health from Use of the Device: Refer to the Warnings, Precautions, and Contraindications in the device labeling.

Postapproval Requirements and Restrictions: See approval order.