

Summary of Safety and Effectiveness

I. General Information

Device Generic Name: IgM Antibody to Hepatitis B Core Antigen (Anti-HBc IgM) assay

Device Trade Name: MONOLISA™ Anti-HBc IgM EIA

Applicant: Bio-Rad Laboratories
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PMA Number: P060034

Date of Panel Recommendation: N/A

Date of Notice of Approval to the Applicant: May 31, 2007

II. Indications for Use

The MONOLISA™ Anti-HBc IgM EIA is a qualitative enzyme immunoassay for the detection of IgM antibodies to hepatitis B core antigen in human serum and plasma. The detection of IgM antibodies to hepatitis B core antigen is a significant marker for the presence of recent infection by the hepatitis B virus (HBV). 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.

III. Device Description

The MONOLISA™ Anti-HBc IgM EIA is a qualitative enzyme immunoassay for the detection of IgM antibody to hepatitis B core antigen in human serum and plasma. The assay is based on the IgM antibody capture format. In the assay procedure, patient specimens and controls are incubated with anti-human IgM antibodies coated on the microwells. If IgM antibodies to HBc are present in a specimen or control, they bind to the antibody. Excess sample is removed by a wash step. The conjugate is then added to the microwells and allowed to incubate. The conjugate binds to any antibody-IgM antibody complexes (specific for HBc) that are present in the microwells. Excess conjugate is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HBc IgM, the bound enzyme (HRP) causes the colorless tetramethylbenzidine (TMB) in the chromogen solution to change to blue. The blue color turns yellow after the addition of a stopping solution. If a sample does not contain anti-HBc IgM, the chromogen/substrate solution in the well remains colorless during the substrate incubation, and after addition of the stopping solution. The color intensity, measured spectrophotometrically, is proportional to the amount of anti-HBc IgM present in the specimen. Absorbance value readings for patient specimens are compared to a cutoff value.

Components of the MONOLISA™ Anti-HBc IgM EIA:

- **Anti-HBc IgM Microwell Strip Plate:** Plates containing 96 wells coated with anti-human IgM. Preservative: ProClin (trace).
- **Wash Solution (30X):** Contains sodium chloride and Tween 20.
- **Specimen Diluent:** Buffer with protein stabilizers and red dye. Preservative: 0.5% ProClin 300®.
- **Negative Control:** Human serum non-reactive for HBsAg and antibodies to HBc, HBs, HIV-1, HIV-2, and HCV. Preservatives: 0.005% Gentamicin and 0.16% ProClin 950®.
- **Positive Control:** Human serum prepared from infectious material positive for anti-HBc IgM and HBsAg, and negative for HIV and HCV antibodies; red dye. Preservative: 0.5% ProClin 300®.
- **Cutoff Calibrator:** Human serum non-reactive for HBsAg and antibodies to HBc, HBs, HIV-1, HIV-2, and HCV. Preservatives: 0.005% Gentamicin and 0.16% ProClin 950®.
- **Conjugate:** HBc recombinant conjugated to HRP, Lyophilized. Preservative: 0.1% ProClin 300®.
- **Conjugate Diluent:** Buffer with protein stabilizers and green dye. Preservatives: 0.5% ProClin® 300 and < 0.001% Thimerosal.
- **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.

IV. Warnings, Precautions, and Contraindications

- For *in vitro* diagnostic use only.
- 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.
- Other warnings and precautions for the MONOLISA™ Anti-HBc IgM EIA can be found in the associated product labeling.

V. Alternative Practices and Procedures

Determination of the presence of anti-HBc IgM antibodies 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, a diagnosis of infection with HBV can be determined.

VI. Marketing History

The MONOLISA™ Anti-HBc IgM EIA has not been commercially marketed in countries outside of the U.S.

VII. Potential Adverse Effects of the Device on Health

The MONOLISA™ Anti-HBc IgM EIA is intended for *in vitro* diagnostic use, and therefore there is no direct adverse effect on the patient. Failure of the product to perform as intended, or errors in the use of the product, may lead to a false result.

For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection. A false nonreactive result may be considered a public health risk if it leads to inappropriate therapy, and individuals are not tested with other methodologies to determine the presence of HBV infection. A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus, and may be due to antibody levels below the detection limits of this assay.

A false reactive result is not considered a public health risk, since the immune status of subjects should be evaluated based on a combination of their clinical status, related risk factors, and other diagnostic test results.

VIII. Summary of Preclinical Studies

A. Analytical Sensitivity

The sensitivity of the MONOLISA™ Anti-HBc IgM EIA was evaluated by preparing serial dilutions of the Paul Ehrlich Institute (PEI) anti-HBc IgM Reference Standard. In the testing of this standard on three kit lots, the MONOLISA™ Anti-HBc IgM EIA detected 19, 47 and 54 PEI Units/mL respectively.

In testing of a Low Titer and a Mixed Titer Anti-HBc IgM Performance Panel, all positive samples (37/37) were correctly identified as reactive by all three kit lots tested. The detection of anti-HBc IgM in 8 commercial seroconversion panels was equivalent or better than other approved assays.

B. Cross-Reactivity

The specificity of the MONOLISA™ Anti-HBc IgM EIA assay was evaluated by testing 357 characterized serum and plasma samples from 21 potentially cross-reacting sub-groups. Each sample was tested once on the MONOLISA™ Anti-HBc IgM EIA. Any sample that was reactive on the MONOLISA™ Anti-HBc IgM EIA was also tested on a reference Anti-HBc IgM assay. A summary of the results is given in Table 1.

Table 1
Potentially Cross-Reactive Medical Conditions

Clinical Condition	N =	MONOLISA™ Anti-HBc IgM Result					Total
		NR	Borderline	Reactive			
				Reference Anti-HBc IgM			
NR	R	NR					
Anti-CMV IgM Pos	20	18	0	1	0	1	20
Anti-EBV IgM Pos	20	20	0	0	0	0	20
Anti-HAV Pos	20	19	0	0	0	1	20
Anti-HBc Pos/IgM Neg	10	8	0	0	0	2	10
Anti-HCV Pos	20	20	0	0	0	0	20
Anti-HDV Pos	10	10	0	0	0	0	10
Anti-HIV-1 Pos	20	19	0	0	0	1	20
Anti-HIV-2 Pos	20	18	0	0	1	1	20
Anti-HSV-1 IgM Pos	14	14	0	0	0	0	14
Anti-HSV-2 IgM Pos	6	6	0	0	0	0	6
Anti-HTLV Pos	20	20	0	0	0	0	20
Anti-Rubella IgM Pos	20	19	1	0	0	0	20
Anti-Toxo IgM Pos	20	19	1	0	0	0	20
Elevated Liver Enzymes	3	3	0	0	0	0	3
Flu Vaccine	20	19	0	0	1	0	20
Hepatic cancer	4	4	0	0	0	0	4
Heterophile Ab Pos	10	10	0	0	0	0	10
Non-Viral Liver Disease	20	20	0	0	0	0	20
Parvovirus Pos	20	20	0	0	0	0	20
Rheumatoid factor Pos	20	16	2	1	0	1	20
SLE	20	20	0	0	0	0	20
Syphilis Pos	20	20	0	0	0	0	20
Total	357	342	4	2	2	7	357

Of the 357 samples from 21 unrelated medical conditions tested, 342/357 (95.8%) were non-reactive on the MONOLISA™ Anti-HBc IgM EIA. Of the 9 samples that were reactive, 2/9 (22.2%) were also positive on the reference Anti-HBc IgM assay. Of the 7 samples that were negative on the reference Anti-HBc IgM and reactive on the MONOLISA™ Anti-HBc IgM EIA, 2 were from the Anti-HBc positive/Anti-HBc IgM negative group, 1 was CMV IgM positive, 1 was HAV positive, 1 was HIV-1 positive, 1 was HIV-2 positive and 1 was RF positive. Six (6) samples (1 CMV IgM, 3 Rheumatoid factor, 1 Rubella IgM and 1 Toxoplasmosis positive) were borderline on the MONOLISA™ Anti-HBc IgM EIA.

C. Interfering substances

The MONOLISA™ Anti-HBc IgM EIA was evaluated for interference according to CLSI Document EP7. The following substances, and the upper levels that were tested, did not interfere with the performance of the assay.

Hemolyzed: 500 mg/dL of hemoglobin
Lipemic: 500 mg/dL of triglycerides

Icteric: 20 mg/dL of bilirubin
Proteinemic: 11 g/dL of protein

D. Stability Studies

1. Kit Stability

A functional stability study of the MONOLISA™ Anti-HBc IgM EIA test 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 the Anti-HBc IgM EIA, at multiple time points throughout the shelf life of the kit. Data from these studies support a 7-month dating period for the MONOLISA™ Anti-HBc IgM EIA test 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-HBc IgM EIA contains four common reagents that may be used interchangeably with the same components in other lots of the Anti-HBc IgM EIA: Wash Solution Concentrate, Chromogen, Substrate Buffer, and Stopping Solution. Matrix studies performed with the MONOLISA™ Anti-HBc IgM 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-HBc IgM EIA 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-HBc IgM EIA kit components in the presence of microbial organisms. One set of Anti-HBc IgM EIA components that were inoculated with microorganisms was tested in comparison to a reference second set of Anti-HBc IgM EIA 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 functionality of the EIA is not impaired and the reagents are stable for the 7-month shelf life when microbial contamination is present.

F. Reproducibility

A panel consisting of 13 diluted samples in various matrices (serum, EDTA, and lithium heparin) was tested in duplicate, once a day for 10 days on 3 lots of the MONOLISA™ Anti-HBc IgM EIA at each of the 3 trial sites. 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 & 3.

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

Test Site	Panel Member	N	Mean S/CO	Within Run ¹		Between Day ²		Total ³	
				SD	CV (%)	SD	CV (%)	SD	CV (%)
Site #1	1 Pos Defibrinated Plasma	60	6.01	0.289	4.8	0.281	4.7	0.781	13.0
	2 Neg Defibrinated Plasma	60	0.31	0.055	17.8	0.000	0.0	0.012	4.0
	3 Neg Defibrinated Plasma	60	0.30	0.045	14.8	0.000	0.0	0.000	0.0
	4 Pos Serum	60	2.80	0.090	3.2	0.061	2.2	0.677	24.2
	5 CO+20%(Serum)	60	1.65	0.025	1.5	0.071	4.3	0.474	28.8
	6 CO-20%(Serum)	60	1.02	0.062	6.1	0.035	3.5	0.289	28.4
	7 Neg (Serum)	60	0.44	0.047	10.6	0.048	10.8	0.075	16.9
	8 CO+20%(EDTA)	60	1.62	0.049	3.0	0.055	3.4	0.444	27.4
	9 CO-20%(EDTA)	60	1.05	0.026	2.5	0.045	4.3	0.269	25.7
	10 Neg (EDTA)	60	0.37	0.042	11.4	0.000	0.0	0.038	10.3
	11 CO+20%(Li Heparin)	60	1.50	0.025	1.6	0.059	3.9	0.361	24.2
	12 CO-20%(Li Heparin)	60	1.01	0.107	10.6	0.015	1.5	0.239	23.6
	13 Neg (Li Heparin)	60	0.39	0.022	5.5	0.017	4.4	0.052	13.4
Site #2	1 Pos Defibrinated Plasma	60	5.94	0.390	6.6	0.375	6.3	0.420	7.1
	2 Neg Defibrinated Plasma	60	0.30	0.078	26.3	0.037	12.4	0.036	12.0
	3 Neg Defibrinated Plasma	60	0.30	0.030	10.1	0.038	12.9	0.017	5.6
	4 Pos Serum	60	2.84	0.049	1.7	0.137	4.8	0.637	22.5
	5 CO+20%(Serum)	60	1.67	0.058	3.5	0.089	5.3	0.462	27.6
	6 CO-20%(Serum)	60	1.07	0.050	4.6	0.060	5.6	0.282	26.3
	7 Neg (Serum)	60	0.50	0.030	6.1	0.062	12.4	0.063	12.7
	8 CO+20%(EDTA)	60	1.67	0.042	2.5	0.122	7.3	0.429	25.8
	9 CO-20%(EDTA)	60	1.05	0.031	3.0	0.084	8.0	0.229	21.8
	10 Neg (EDTA)	60	0.36	0.025	7.1	0.047	13.2	0.028	7.7
	11 CO+20%(Li Heparin)	60	1.54	0.032	2.1	0.157	10.2	0.316	20.5
	12 CO-20%(Li Heparin)	60	0.99	0.044	4.4	0.116	11.7	0.174	17.5
	13 Neg (Li Heparin)	60	0.38	0.037	9.9	0.049	12.9	0.027	7.2
Site #3	1 Pos Defibrinated Plasma	60	6.11	0.405	6.6	0.649	10.6	0.686	11.2
	2 Neg Defibrinated Plasma	60	0.31	0.028	9.1	0.013	4.2	0.022	7.0
	3 Neg Defibrinated Plasma	59	0.30	0.020	6.9	0.012	4.0	0.017	5.8
	4 Pos Serum	60	2.89	0.077	2.6	0.135	4.7	0.710	24.5
	5 CO+20%(Serum)	60	1.67	0.031	1.9	0.084	5.0	0.476	28.6
	6 CO-20%(Serum)	60	1.07	0.029	2.7	0.051	4.7	0.301	28.2
	7 Neg (Serum)	60	0.49	0.015	3.0	0.027	5.6	0.097	19.9
	8 CO+20%(EDTA)	60	1.68	0.032	1.9	0.106	6.3	0.428	25.5
	9 CO-20%(EDTA)	60	1.07	0.025	2.3	0.080	7.5	0.237	22.2
	10 Neg (EDTA)	60	0.36	0.040	11.0	0.034	9.4	0.036	9.9
	11 CO+20%(Li Heparin)	60	1.51	0.042	2.8	0.069	4.6	0.344	22.8
	12 CO-20%(Li Heparin)	60	1.01	0.031	3.1	0.068	6.7	0.200	19.9
	13 Neg (Li Heparin)	59	0.38	0.028	7.5	0.040	10.7	0.045	11.9

¹ Within Run: variability of the assay performance from replicate to replicate.

² Between Day: 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
Summary of MONOLISA™ Anti-HBc IgM EIA Reproducibility Results
(Positive, Low Positive, and Negative) by Panel Member S/CO

Panel Member	N	Mean S/CO	Within Run ¹		Between Day ²		Between Lot ³		Between Site		Total ⁴	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Pos Serum	180	2.84	0.074	2.6	0.116	4.1	0.675	23.7	0.043	1.5	0.690	24.3
CO+20%(Serum)	180	1.66	0.041	2.4	0.082	4.9	0.471	28.3	0.000 ⁵	0.0	0.480	28.9
CO-20%(Serum)	180	1.05	0.049	4.6	0.050	4.7	0.291	27.6	0.026	2.5	0.300	28.5
Neg(Serum)	180	0.48	0.033	7.0	0.048	10.0	0.078	16.4	0.028	5.8	0.101	21.2
CO+20%(EDTA)	180	1.66	0.042	2.5	0.098	5.9	0.434	26.2	0.025	1.5	0.447	27.0
CO-20%(EDTA)	180	1.06	0.027	2.6	0.072	6.8	0.245	23.2	0.009	0.9	0.257	24.4
Neg(EDTA)	180	0.36	0.036	10.0	0.034	9.2	0.036	9.9	0.000 ⁵	0.0	0.061	16.9
CO+20%(Li Heparin)	180	1.52	0.034	2.2	0.105	6.9	0.341	22.5	0.014	0.9	0.359	23.7
CO-20%(Li Heparin)	180	1.00	0.069	6.9	0.078	7.8	0.205	20.5	0.005	0.5	0.231	23.0
Neg(Li Heparin)	179	0.38	0.030	7.8	0.038	9.9	0.042	11.1	0.005	1.3	0.064	16.8

¹ Within Run: variability of the assay performance from replicate to replicate.

² Between Day: variability of the assay performance from run to run.

³ Between Lot: variability of the assay performance from lot to lot.

⁴ Total variability of the assay performance includes within run, between run and between lot.

⁵ Negative variances were rounded to zero, per statistical convention.

G. Precision

A precision study was performed with the MONOLISA™ Anti-HBc IgM EIA using panels prepared in serum, EDTA plasma, and sodium heparin. The 13 specimens were tested in triplicate, twice a day, for 20 days, with one lot, and results are summarized in Table 4.

Table 4
MONOLISA™ Anti-HBc EIA 20-Day Precision Results in S/CO

Panel Member	N	Mean S/CO	Within Run ¹		Between Day ²		Between Run ³		Total ⁴	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1 Defibrinated Plasma	120	4.80	0.052	1.1	0.091	1.9	0.103	2.1	0.147	3.1
2 Defibrinated Plasma	120	0.33	0.058	17.8	0.034	10.3	0.000 ⁵	0.0	0.067	20.6
3 Defibrinated Plasma	120	0.32	0.025	7.9	0.011	3.4	0.012	3.9	0.030	9.5
4 Serum	120	2.28	0.041	1.8	0.050	2.2	0.028	1.2	0.070	3.1
5 Serum	120	1.27	0.030	2.3	0.033	2.6	0.000 ⁵	0.0	0.044	3.5
6 Serum	120	0.83	0.020	2.4	0.016	1.9	0.012	1.4	0.028	3.4
7 Serum	120	0.41	0.014	3.5	0.018	4.5	0.012	3.0	0.026	6.5
8 EDTA Plasma	120	1.38	0.030	2.2	0.036	2.6	0.005	0.4	0.047	3.4
9 EDTA Plasma	120	0.93	0.023	2.5	0.016	1.7	0.011	1.2	0.030	3.2
10 EDTA Plasma	120	0.39	0.017	4.3	0.025	6.3	0.000 ⁵	0.0	0.030	7.6
11 Na Heparin Plasma	120	1.29	0.030	2.3	0.046	3.6	0.000 ⁵	0.0	0.055	4.3
12 Na Heparin Plasma	120	0.94	0.025	2.7	0.033	3.5	0.000 ⁵	0.0	0.041	4.4
13 Na Heparin Plasma	120	0.44	0.016	3.6	0.014	3.2	0.004	0.8	0.021	4.9

¹ Within Run: variability of the assay performance from replicate to replicate.

² Between Day: variability of the assay performance from day to day.

³ Between Run: variability of the assay performance from run to run.

⁴ Total variability of the assay performance includes within run, between day and between run.

⁵ Negative variances were rounded to zero, per statistical convention.

IX. Summary of Clinical Studies

A multi-center clinical trial was conducted to evaluate the performance of the MONOLISA™ Anti-HBc IgM Enzyme Immunoassay (EIA) in human serum and plasma. A total of 1430 prospective subjects at high risk for viral hepatitis and/or showing signs/symptoms of HBV were included in the study. Of these 1430, 1352 were from the asymptomatic high risk population and 78 reported signs or symptoms of HBV.

Expected Values

A total of 1352 prospective asymptomatic subjects were tested with the MONOLISA™ Anti-HBc IgM EIA. All subjects (100%) were at high risk for viral hepatitis including intravenous drug users (N = 461), homosexual males (N = 143), sex workers (N = 172), prison history (N = 340), high risk sex partners (N = 165), high risk occupation/health care workers (N = 75), hemodialysis (N = 55), hemophiliacs (N = 3), and other (N = 481). Many had more than 1 high-risk behavior or risk factor. One hundred seventy-four (174, 12.9%) 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, (34.0%); 57 from Santa Ana, CA (4.2%); 72 from Miami, FL (5.3%); 344 from Cocoa, FL (25.4%); 254 from San Francisco, CA (18.8%); and 166 from Seattle, WA (12.3%). The group was Caucasian (36.6%), Black or African American (41.1%), Hispanic or Latino (13.2%), Asian (4.3%), Native Hawaiian or other Pacific Islander (0.7%), and American Indian or Alaska Native (2.3%), with the remaining 1.9% represented by multiple ethnic groups or unknown ethnicity. The subjects were male (70.2%) and female (29.8%) and ranged in age from 18 to 81 years.

The MONOLISA™ Anti-HBc IgM EIA results for the asymptomatic prospective population (N = 1352) are presented in Table 5 by gender and age range.

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

Age Range	Gender	MONOLISA™ Anti-HBc IgM EIA Result						Total
		Reactive		Borderline		Non-reactive		N
		N	%	N	%	N	%	
10-19	F	6	0.4 %	1	1.3 %	7	0.5 %	6
	M	10	0.7 %	1	1.3 %	11	0.8 %	10
20-29	F	104	7.7 %	9	11.5 %	113	7.9 %	104
	M	122	9.0 %	9	11.5 %	131	9.2 %	122
30-39	F	108	8.0 %	7	9.0 %	115	8.0 %	108
	M	212	15.7 %	13	16.7 %	225	15.7 %	212
40-49	F	107	7.9 %	5	6.4 %	112	7.8 %	107
	M	348	25.7 %	8	10.3 %	356	24.9 %	348
50-59	F	62	4.6 %	5	6.4 %	67	4.7 %	62
	M	210	15.5 %	11	14.1 %	221	15.5 %	210
60-69	F	11	0.8 %	3	3.8 %	14	1.0 %	11
	M	37	2.7 %	4	5.1 %	41	2.9 %	37
70-79	F	2	0.1 %	1	1.3 %	3	0.2 %	2
	M	5	0.4 %	0	0.0 %	5	0.3 %	5
80-89	F	0	0.0 %	0	0.0 %	0	0.0 %	0
	M	1	0.1 %	0	0.0 %	1	0.1 %	1
Unknown	F	3	0.2 %	1	1.3 %	4	0.3 %	3
	M	4	0.3 %	0	0.0 %	4	0.3 %	4
Totals		1352	100 %	78	100 %	1430	100 %	1352

Reference Markers

The HBV disease classification for each subject in the total prospective population (N = 1430) was previously determined by a serological assessment using a hepatitis marker profile consisting of commercially available FDA approved reference assays. 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 HBeAg (Anti-HBe), and total antibody to hepatitis B virus surface antigen (anti-HBs). All reference assays were tested according to the manufacturer's package insert instructions. Agreement of the MONOLISA™ Anti-HBc IgM EIA was assessed relative to the reference anti-HBc result and to HBV classification.

The data were analyzed following the assignment of specimen classification based upon the positive or reactive (+) / negative or non-reactive (-) / Indeterminate (I) patterns for the six HBV reference marker assays. Table 6 below summarizes how these classification patterns were derived. No other laboratory or clinical information was used in the disease classification process. There were 33 unique reference marker patterns observed in the MONOLISA™ Anti-HBc IgM EIA clinical study across the three clinical sites.

Table 6
Characterization of Prospective Specimens

FDA Characterization based on single point specimen	HBsAg	HBeAg	Anti-HBc IgM	Total HBc	Anti-HBe	Anti-HBs
Acute infection	+	+	+	+	-	-
	+	+	I	+	-	-
	+	+	-	-	-	-
	+	-	+	-	-	+
	+	-	+	-	-	-
	+	-	-	-	-	-
Chronic infection	+	+	+	+	-	+
	+	+	-	+	-	-
	+	+	-	+	-	+
	+	+	-	+	+	+
	+	+	-	+	+	-
	+	-	-	+	+	-
	+	-	-	+	+	+
Early recovery	-	-	+	+	+	+
	-	-	+	+	+	-
	-	-	-	+	-	-
	-	-	-	+	+	-
	-	-	-	+	I	-
	-	-	I	+	+	+
HBV vaccine response	-	-	-	-	-	+
HBV vaccine response (?)	-	-	-	-	-	I
Not previously infected with HBV	-	-	-	-	-	-
Recovered	-	-	-	+	I	I
	-	-	-	+	-	I
Recovered or Immune due to natural	-	-	-	+	-	+
Recovery	-	-	-	-	+	+
	-	-	-	+	I	+
	-	-	-	+	+	I
	-	-	-	+	+	+
Uninterpretable	+	-	-	-	+	-
	-	+	-	-	-	+
	-	+	-	-	-	-
	-	-	-	-	+	-

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

Comparison of Results by Specimen Classification

Table 7 compares the MONOLISA™ Anti-HBc IgM EIA results with the previously determined anti-HBc IgM reference assay results for each specimen classification. The data in the table are representative of the number of specimens in each result category.

Table 7
FDA HBV Classification of High Risk Prospective Samples
MONOLISA™ Anti-HBc IgM EIA versus FDA Approved Anti-HBc IgM EIA

Reference Serology Classification	Reference Anti-HBc IgM Assay									Totals
	Reactive			GRZ ¹			Non-Reactive			
	MONOLISA™ Anti-HBc IgM			MONOLISA™ Anti-HBc IgM			MONOLISA™ Anti-HBc IgM			
	R	BRD ²	NR	R	BRD ²	NR	R	BRD ²	NR	
Acute Infection	7	0	0	1	0	0	0	0	6	14
Chronic Infection	1	0	0	0	0	0	2	1	75	79
Early recovery	3	0	0	0	1	1	5	5	91	106
HBV vaccine response	0	0	0	0	0	0	4	5	300	309
HBV vaccine response (?)	0	0	0	0	0	0	0	0	31	31
Not previously infected with HBV	0	0	0	0	0	0	3	7	596	606
Recovered	0	0	0	0	0	0	1	0	11	12
Recovered or Immune due to natural infection	0	0	0	0	0	0	7	2	83	92
Recovery	0	0	0	0	0	0	16	8	149	173
Uninterpretable	0	0	0	0	0	0	0	0	8	8
Total	11	0	0	1	1	1	38	28	1350	1430

¹ GRZ = Gray Zone Reactive; ² BRD = Borderline

Percent Agreement

The percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the reference anti-HBc IgM assays for each specimen classification was determined, including the upper and lower 95% Wilson confidence bounds. The percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the anti-HBc IgM reference assay for the prospective population is presented in Table 8 for each HBV classification.

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

HBV Classification	N =	Positive Percent Agreement		95% Confidence Interval	Negative Percent Agreement		95% Confidence Interval
Acute Infection	14	(7/7)	100.0%	64.5%, 100%	(6/7)	85.7%	48.6%, 97.4%
Chronic Infection	79	(1/1)	100.0%	20.6%, 100%	(75/78)	96.2%	89.3%, 98.7%
Early recovery	106	(3/4)	75.0%	30%, 95.4%	(91/101)	90.1%	82.7%, 94.5%
HBV vaccine response	309	(0/0)	NA	NA	(300/309)	97.1%	94.6%, 98.5%
HBV vaccine response (?)	31	(0/0)	NA	NA	(31/31)	100.0%	89%, 100%
Not previously infected with HBV	606	(0/0)	NA	NA	(596/606)	98.3%	97%, 99.1%
Recovered	12	(0/0)	NA	NA	(11/12)	91.7%	64.6%, 98.5%
Recovered or Immune due to natural infection	92	(0/0)	NA	NA	(83/92)	90.2%	82.4%, 94.8%
Recovery	173	(0/0)	NA	NA	(149/173)	86.1%	80.2%, 90.5%
Uninterpretable	8	(0/0)	NA	NA	(8/8)	100.0%	67.5%, 100%
Total	1430	(11/12)	91.7%	64.6%, 98.5%	(1350/1417)	95.3%	94%, 96.3%

- 1 N=Total number of samples; refer to Table 3 for correlation of borderline samples. The one specimen that was indeterminate by both assays was not included in the percent agreement calculations. Positive or negative results from the MONOLISA™ Anti-HBc IgM 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-HBc IgM 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-HBc IgM EIA.

Of the 1430 samples tested, 29 samples gave borderline results with MONOLISA™ Anti-HBc IgM EIA. Three (3) of the 1430 samples were equivocal with the reference assay. One (1) of the MONOLISA™ Anti-HBc IgM EIA borderline samples was also equivocal by the reference method and 28 were negative by the reference method.

Percent agreement can also be determined by evaluating equivocal results as agreement with the reference assay. Below are the calculations of percent agreement when the borderline results by MONOLISA™ Anti-HBc IgM EIA are considered as positive results and when the borderline results by MONOLISA™ Anti-HBc IgM EIA are considered as negative results.

MONOLISA™ Anti-HBc IgM EIA	Positive Agreement	Negative Agreement
Borderline considered positive	100.0% (11/11)	95.3% (1350/1460)
Borderline considered negative	100.0% (11/11)	97.3% (1378/1416)

Seroconversion Panels

The comparative sensitivity of the MONOLISA™ Anti-HBc IgM EIA was determined by testing 6 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	# Members	MONOLISA™ Anti-HBc IgM		Reference anti-HBc IgM test
			S/CO	Result	Result
PHM935A-11	35	18	0.23	NR	NR
PHM935A-12	50		0.22	NR	NR
PHM935A-	66		1.83	R	NR

Panel ID	Day since 1 st bleed	# Members	MONOLISA™ Anti-HBc IgM		Reference anti-HBc IgM test
			S/CO	Result	Result
13					
PHM935A-14	68		3.02	R	R
RP009-06	31	20	0.45	NR	NR
RP009-07	36		2.20	R	NR
RP009-08	43		5.46	R	R
RP009-09	56		5.76	R	R
RP016-06	25	20	0.27	NR	NR
RP016-07	57		0.67	NR	NR
RP016-08	60		3.90	R	R
RP016-09	74		5.34	R	R
RP017-13	65	30	0.37	NR	NR
RP017-14	71		0.54	NR	NR
RP017-15	76		1.14	R	NR
RP017-16	78		2.26	R	R
RP017-27	179		1.94	R	R
RP017-28	181		1.72	R	NR
RP017-29	186		1.76	R	NR
RP017-30	188		1.51	R	NR
6278-08	26	11	0.28	NR	NR
6278-09	33		0.55	NR	NR
6278-10	37		0.98	BRD	NR
6278-11	41		3.97	R	R
6281-08	36	12	0.29	NR	NR
6281-09	41		1.30	R	NR
6281-10	43		3.41	R	R
6281-11	50		5.02	R	R

In 4 of the 6 (66%) seroconversion panels, the MONOLISA™ Anti-HBc IgM EIA detected reactive levels of hepatitis B core IgM antibody 1 member before the reference anti-HBc IgM test. In 2 of the 6 (33%) seroconversion panels, the MONOLISA™ Anti-HBc IgM EIA detected reactive levels of hepatitis B core IgM antibody at the same member as the reference anti-HBc IgM test. MONOLISA™ Anti-HBc IgM EIA appears to detect IgM for a longer period than the reference assay for qualitative determination of IgM antibody to Hepatitis B core

Clinical Performance with Acute HBV Samples

Retrospective acute HBV samples from 85 individuals were tested with the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All testing was according to the protocol and the manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 10.

Table 10
Acute HBV Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA

MONOLISA™ Anti-HBc IgM Result	Reference Anti-HBc IgM Result			
	Positive	Gray zone	Negative	Total
Reactive	66	13	4	83
Borderline	0	0	0	0
Non-Reactive	0	0	2	2
Total	66	13	6	85

The positive percent agreement with the reference method is 100% (66/66) with a 95% confidence interval of 94.5 - 100%. The negative percent agreement with the reference method is 10.5% (2/19) with a 95% confidence interval of 2.9 - 31.4%. Of the 17 discrepant results, the MONOLISA™ Anti-HBc IgM EIA was reactive on 13 samples with gray zone reactive results on the reference assay and 4 samples that were negative on the reference assay.

Clinical Evaluation of the MONOLISA™ Anti-HBc IgM EIA on Chronic HBV Samples

Retrospective chronic HBV samples (HBsAg positive for more than 6 months) from 120 individuals were tested with the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All testing was according to the protocol and the manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 11.

Table 11
Chronic HBV Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA

MONOLISA™ Anti-HBc IgM Result	Reference Anti-HBc IgM Result			
	Positive	Gray zone	Negative	Total
Reactive	1	3	3	7
Borderline	0	0	1	1
Non-Reactive	0	6	106	112
Total	1	9	110	120

The negative percent agreement with the reference method is 93.8% (106/113) with a 95% confidence interval of 87.8 - 97%. The positive percent agreement with the reference method is 14.3% (1/7) with a 95% confidence interval of 2.6 - 51.4%. Of the 13 discrepant results, the MONOLISA™ Anti-HBc IgM EIA was reactive on 3 and non-reactive on 6 of the 9 samples with gray zone reactive results on the reference assay. The MONOLISA™ Anti-HBc IgM EIA was reactive on 3 and borderline on 1 of the 4 samples that were negative on the reference assay.

Clinical Performance with Pre-HBV Vaccination Samples

Pre-vaccine samples from 38 individuals were tested on one lot of the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All testing was according to the protocol and the

manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 12.

Table 12
Pre-Vaccination Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA

MONOLISA™ Anti-HBc IgM Result	Reference Anti-HBc IgM Result			
	Positive	Gray zone	Negative	Total
Reactive	0	0	0	0
Borderline	0	0	0	0
Non-Reactive	0	0	38	38
Total	0	0	38	38

The negative percent agreement with the reference method is 100% (38/38) with a 95% confidence interval of 90.8 - 100%.

X. Conclusions Drawn from the Studies

Multi-centered clinical and non-clinical studies were conducted in the US to evaluate the MONOLISA™ Anti-HBc IgM EIA. A method comparison was performed with a commercially available FDA-approved assay to detect IgM antibodies to hepatitis B core antigen 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-HBc IgM EIA kits which are stored as indicated (2-8°C) are stable for the intended shelf-life of the kits.

Hepatitis B virus classification using the prospective population showed 33 unique reference marker patterns. The overall positive percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the reference assay was 91.7% (11/12) in the high risk, signs and symptoms, and vaccinated populations. The overall negative percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the reference assay was 95.3% (1350/1417) in the same population.

In a study of 85 retrospective acute HBV samples, the positive agreement was 100 % (66/66) with the comparison method. In another study with 120 retrospective chronic HBV samples (HBsAg positive for more than 6 months), the negative percent agreement with the comparison method was 93.8% (106/113).

Precision and reproducibility of the MONOLISA™ Anti-HBc IgM EIA was established for within-run, between-day, between-lot, and between sites.

Specimen collection tube study results support the use of human serum and plasma (EDTA, sodium and lithium heparin, ACD, or sodium citrate) with the MONOLISA™ Anti-HBc IgM EIA.

The results from both the non-clinical and clinical studies indicate that the MONOLISA™ Anti-HBc IgM EIA can be used safely and effectively for the qualitative determination of anti-HBc IgM 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-HBc IgM 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-HBc IgM 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-HBc IgM EIA has been demonstrated for use in determining if IgM antibodies to the hepatitis B core antigen are present in an individual's serum or plasma. A reasonable determination of effectiveness of the MONOLISA™ Anti-HBc IgM EIA assay for aiding in the diagnosis 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

The applicant's manufacturing facilities at Seattle and Woodinville were inspected on May 25 and May 31 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.