

Summary of Safety and Effectiveness Data

I. General Information

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

Device Trade Name: MONOLISA™ Anti-HBc EIA

Applicant: Bio-Rad Laboratories
6565 185th Avenue NE
Redmond, WA 98052
Phone: 425 881-8300
Fax: 425 498-1651

PMA Number: P060031

Date of Panel Recommendation: N/A

Date of Notice of Approval to the Applicant: April 27, 2007

II. Indications for Use

The MONOLISA™ Anti-HBc EIA is an enzyme immunoassay intended for use in the qualitative detection of total antibodies (IgG/IgM) to hepatitis B core antigen (anti-HBc) in human serum and plasma (potassium EDTA, sodium citrate, ACD (acid citrate dextrose), lithium heparin and sodium heparin). Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection.

III. Contraindications

None known.

IV. Brief Description of Test

The MONOLISA™ Anti-HBc EIA is a qualitative enzyme immunoassay for the detection of total antibody to hepatitis B core antigen in human serum and plasma. The assay is based on the indirect antibody sandwich principle. In the assay procedure, patient specimens and controls are incubated with microwells coated with recombinant HBc antigen. If antibodies to HBc are present in a specimen or control, they bind to the antigen. Excess sample is removed by a wash step. The anti-human conjugate is then added to the microwells and allowed to incubate. The conjugate binds to any antigen-antibody complexes 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, 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, 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 present in the specimen. Absorbance value readings for patient specimens are compared to a cutoff value.

Components of the MONOLISA™ Anti-HBc EIA:

- **Anti-HBc Microwell Strip Plate:** Plates containing 96 wells coated with purified recombinant HBc antigen. Preservative: ProClin (trace).
- **Wash Solution (30X):** Contains sodium chloride and Tween 20.
- **Specimen Diluent:** Buffer with protein stabilizers and sample indicator dye. Preservative: 0.1% ProClin 300™, 0.005% Gentamicin, and < 0.001% Thimerosal.
- **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 with antibodies to HBc and HBs; 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:** 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:** Peroxidase-labeled goat antibody directed against human IgG and IgM, in a buffer containing protein stabilizers and dye. Preservative: 0.1% ProClin 300™ and 0.005% Gentamicin Sulfate.
- **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.

V. Warnings, Precautions, and Contraindications

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

VI. Alternative Practices and Procedures

Determination of the presence of anti-HBc 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-HBc EIA has not been commercially marketed in countries outside of the U.S.

VIII. Potential Adverse Effects of the Device on Health

The MONOLISA™ Anti-HBc 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 is not considered a public health risk, since individuals would be tested with other methodologies if signs and symptoms indicate 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 also 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.

IX. Summary of Preclinical Studies

A. Analytical Sensitivity

The sensitivity of the MONOLISA™ Anti-HBc EIA was evaluated by preparing serial dilutions of the Paul Ehrlich Institute (PEI) anti-HBc Total reference unit. Three kit lots were used in testing and the calculated concentration at endpoint was approximately 0.4, 0.5, and 0.5 Units/mL on the three lots. In the testing of the Paul Ehrlich Anti-HBc IgM Standard on three kit lots, MONOLISA™ Anti-HBc EIA detected concentrations of approximately 15, 16, and 17 Units/mL at endpoint.

B. Cross-Reactivity

The specificity of the MONOLISA™ Anti-HBc EIA assay was evaluated during the analysis of 415 serum samples from individuals with unrelated medical conditions, representing 21 potentially cross-reacting conditions. Each sample was tested once on the MONOLISA™ Anti-HBc EIA. Any sample that was reactive on the MONOLISA™ Anti-HBc EIA was further tested on a reference anti-HBc assay. The results of each sample tested on the MONOLISA™ Anti-HBc EIA are summarized in Table 1.

Table 1
Potentially Cross-Reactive Medical Conditions

| Clinical Condition | N = | MONOLISA™ Anti-HBc Result | | | | Total |
|--------------------------------------|------------|---------------------------|------------------|--------------------|----------|------------|
| | | NR | BRD ¹ | Reactive | | |
| | | | | Reference anti-HBc | | |
| | | | | R | NR | |
| Autoimmune Diseases ² | 20 | 20 | 0 | 0 | 0 | 20 |
| Cytomegalovirus (CMV) | 20 | 19 | 0 | 1 | 0 | 20 |
| Elevated Liver Enzymes/Cancer | 6 | 6 | 0 | 0 | 0 | 6 |
| Epstein Barr Virus (EBV) | 20 | 20 | 0 | 0 | 0 | 20 |
| H. pylori positive | 10 | 10 | 0 | 0 | 0 | 10 |
| Hepatitis A Infection (HAV) | 20 | 18 | 1 | 1 | 0 | 20 |
| Hepatitis C Infection (HCV) | 20 | 13 | 0 | 7 | 0 | 20 |
| Hepatitis D Infection (HDV) | 10 | 0 | 0 | 10 | 0 | 10 |
| Herpes Simplex Virus (HSV) | 20 | 18 | 0 | 2 | 0 | 20 |
| HIV-1 | 20 | 15 | 0 | 4 | 1 | 20 |
| HIV-2 | 20 | 5 | 0 | 12 | 3 | 20 |
| HTLV-I/II | 20 | 15 | 0 | 5 | 0 | 20 |
| Influenza Vaccine Recipients | 20 | 15 | 0 | 5 | 0 | 20 |
| Non-Viral liver disease ³ | 20 | 17 | 1 | 2 | 0 | 20 |
| Parvovirus B19 | 20 | 20 | 0 | 0 | 0 | 20 |
| Pregnant (bHCG positive) | 50 | 50 | 0 | 0 | 0 | 50 |
| Rheumatoid Factor (RF) | 19 | 19 | 0 | 0 | 0 | 19 |
| Rubella | 20 | 18 | 0 | 2 | 0 | 20 |
| SLE / ANA Positive | 20 | 16 | 0 | 3 | 1 | 20 |
| Syphilis | 20 | 18 | 0 | 2 | 0 | 20 |
| Toxoplasmosis | 20 | 18 | 2 | 0 | 0 | 20 |
| TOTAL | 415 | 350 | 4 | 56 | 5 | 415 |

¹ BRD = Borderline

² Scleroderma, Sjogren's, MCTD etc.

³ Primary Biliary Cirrhosis

Of the 415 samples from 21 unrelated medical conditions tested, 350/415 (84.3%) were non-reactive on the MONOLISA™ Anti-HBc EIA. Of the 61 samples that were reactive, 56/61 (91.8%) were also positive on the reference anti-HBc assay. Five (5) samples were negative on the reference anti-HBc and reactive on the MONOLISA™ Anti-HBc EIA, and were from the following conditions: 3 HIV-2 positive, 1 HIV-1 positive, and 1 SLE positive. Samples that were borderline on the MONOLISA™ Anti-HBc EIA were two that were Toxoplasmosis positive, 1 Non-viral liver disease, and 1 HAV positive.

C. Interfering Substances

The MONOLISA™ Anti-HBc 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. Matrix Equivalency Study

The performance of the MONOLISA™ Anti-HBc EIA with various anticoagulants was evaluated by testing paired serum and anticoagulant specimens. The specimens tested included those with no antibody and those with levels of antibody that are near the assay cutoff. All samples that were nonreactive in serum were also nonreactive when collected into the anticoagulants. Results from the specimens which contained antibody are summarized in the following table.

| Collection Tube Type | Distribution of % Difference to Serum | | |
|----------------------|---------------------------------------|----------------|------------|
| | 0% to < 10% | > 10% to < 20% | > 20% |
| Na Citrate | 50% (10/20) | 40% (8/20) | 10% (2/20) |
| ACD | 65% (13/20) | 30% (6/20) | 5% (1/20) |
| Potassium EDTA | 65% (13/20) | 35% (7/20) | 0 |
| Lithium Heparin | 50% (10/20) | 45% (9/20) | 5% (1/20) |
| Sodium Heparin | 65% (13/20) | 25% (5/20) | 10% (2/20) |

E. Stability Studies

1. Kit Stability

A functional stability study of the MONOLISA™ Anti-HBc 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 EIA, at multiple time points throughout the shelf life of the kit.

2. Interchangeability of Common Reagents

The MONOLISA™ Anti-HBc EIA contains four common reagents that may be used interchangeably with the same components in other lots of the Anti-HBc EIA: Wash Solution Concentrate, Chromogen, Substrate Buffer, and Stopping Solution. Matrix studies performed with the MONOLISA™ Anti-HBc 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.

F. Microbiology Studies

Antimicrobial preservatives have been added to the components in the Anti-HBc 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 EIA kit components in the presence of microbial organisms. One set of Anti-HBc EIA components that were inoculated with microorganisms was tested in comparison to a reference second set of Anti-HBc 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 12-month shelf life when microbial contamination is present.

G. Reproducibility

A 10-member panel consisting of 10 diluted patient 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 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, and are summarized in Tables 2 and 3.

Table 2
MONOLISA™ Anti-HBc 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 Serum | 60 | 3.54 | 0.125 | 3.5 | 0.211 | 6.0 | 0.250 | 7.1 |
| | 2 | CO+20%(Serum) | 60 | 1.52 | 0.061 | 4.0 | 0.108 | 7.1 | 0.124 | 8.2 |
| | 3 | CO-20%(Serum) | 59 | 0.88 | 0.061 | 6.9 | 0.060 | 6.8 | 0.086 | 9.7 |
| | 4 | Neg (Serum) | 58 | 0.14 | 0.007 | 5.0 | 0.012 | 8.8 | 0.017 | 12.4 |
| | 5 | CO+20%(EDTA) | 60 | 1.64 | 0.091 | 5.5 | 0.105 | 6.4 | 0.139 | 8.5 |
| | 6 | CO-20%(EDTA) | 60 | 0.93 | 0.048 | 5.1 | 0.075 | 8.0 | 0.088 | 9.5 |
| | 7 | Neg (EDTA) | 60 | 0.22 | 0.019 | 8.8 | 0.015 | 6.9 | 0.025 | 11.3 |
| | 8 | CO+20%(LiHeparin) | 60 | 1.66 | 0.127 | 7.7 | 0.098 | 5.9 | 0.161 | 9.7 |
| | 9 | CO-20%(Li Heparin) | 60 | 0.91 | 0.044 | 4.9 | 0.070 | 7.6 | 0.083 | 9.0 |
| | 10 | Neg (Li Heparin) | 60 | 0.08 | 0.005 | 5.5 | 0.007 | 9.0 | 0.009 | 11.2 |
| Site #2 | 1 | Pos Serum | 60 | 3.42 | 0.199 | 5.8 | 0.201 | 5.9 | 0.283 | 8.3 |
| | 2 | CO+20%(Serum) | 60 | 1.49 | 0.057 | 3.8 | 0.104 | 7.0 | 0.123 | 8.3 |
| | 3 | CO-20%(Serum) | 60 | 0.90 | 0.044 | 4.8 | 0.060 | 6.7 | 0.074 | 8.2 |
| | 4 | Neg (Serum) | 60 | 0.15 | 0.007 | 4.5 | 0.019 | 13.1 | 0.021 | 14.1 |
| | 5 | CO+20%(EDTA) | 60 | 1.58 | 0.083 | 5.3 | 0.094 | 5.9 | 0.125 | 7.9 |
| | 6 | CO-20%(EDTA) | 60 | 0.89 | 0.041 | 4.7 | 0.060 | 6.7 | 0.073 | 8.2 |
| | 7 | Neg (EDTA) | 60 | 0.21 | 0.020 | 9.4 | 0.023 | 10.8 | 0.035 | 16.7 |
| | 8 | CO+20%(LiHeparin) | 60 | 1.60 | 0.072 | 4.5 | 0.099 | 6.2 | 0.131 | 8.2 |
| | 9 | CO-20%(Li Heparin) | 60 | 0.87 | 0.035 | 4.0 | 0.073 | 8.4 | 0.085 | 9.8 |
| | 10 | Neg (Li Heparin) | 60 | 0.07 | 0.003 | 4.4 | 0.013 | 18.1 | 0.013 | 18.7 |
| Site #3 | 1 | Pos Serum | 60 | 3.42 | 0.199 | 5.8 | 0.201 | 5.9 | 0.283 | 8.3 |
| | 2 | CO+20%(Serum) | 60 | 3.46 | 0.131 | 3.8 | 0.173 | 5.0 | 0.220 | 6.4 |
| | 3 | CO-20%(Serum) | 60 | 1.52 | 0.079 | 5.2 | 0.078 | 5.2 | 0.112 | 7.4 |
| | 4 | Neg (Serum) | 60 | 0.89 | 0.043 | 4.9 | 0.052 | 5.9 | 0.074 | 8.3 |
| | 5 | CO+20%(EDTA) | 60 | 0.16 | 0.008 | 4.9 | 0.024 | 15.2 | 0.027 | 16.9 |
| | 6 | CO-20%(EDTA) | 60 | 1.62 | 0.108 | 6.6 | 0.089 | 5.5 | 0.147 | 9.1 |
| | 7 | Neg (EDTA) | 60 | 0.98 | 0.135 | 13.8 | 0.000 | 0.0 | 0.135 | 13.8 |
| | 8 | CO+20%(LiHeparin) | 60 | 0.22 | 0.023 | 10.4 | 0.022 | 9.8 | 0.031 | 14.3 |
| | 9 | CO-20%(Li Heparin) | 60 | 1.63 | 0.092 | 5.7 | 0.091 | 5.6 | 0.130 | 8.0 |
| | 10 | Neg (Li Heparin) | 60 | 0.92 | 0.064 | 7.0 | 0.058 | 6.3 | 0.088 | 9.6 |

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
MONOLISA™ Anti-HBc EIA Reproducibility Results (Positive, Low Positive, and High 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 | 3.47 | 0.155 | 4.5 | 0.196 | 5.6 | 0.036 | 1.0 | 0.038 | 1.1 | 0.255 | 7.3 |
| CO+20% (Serum) | 180 | 1.51 | 0.066 | 4.4 | 0.098 | 6.5 | 0.000 ⁵ | 0.0 | 0.000 ⁵ | 0.0 | 0.118 | 7.8 |
| CO-20% (Serum) | 179 | 0.89 | 0.050 | 5.6 | 0.058 | 6.5 | 0.004 | 0.5 | 0.000 ⁵ | 0.0 | 0.076 | 8.6 |
| Neg (Serum) | 178 | 0.15 | 0.007 | 4.8 | 0.019 | 13.0 | 0.008 | 5.3 | 0.010 | 6.6 | 0.024 | 16.2 |
| CO+20% (EDTA) | 180 | 1.61 | 0.094 | 5.8 | 0.096 | 6.0 | 0.011 | 0.7 | 0.019 | 1.2 | 0.137 | 8.5 |
| CO-20% (EDTA) | 180 | 0.93 | 0.086 | 9.2 | 0.055 | 5.9 | 0.000 ⁵ | 0.0 | 0.044 | 4.7 | 0.111 | 11.9 |
| Neg (EDTA) | 180 | 0.22 | 0.021 | 9.6 | 0.020 | 9.3 | 0.010 | 4.6 | 0.000 ⁵ | 0.0 | 0.031 | 14.1 |
| CO+20% (Li Heparin) | 180 | 1.63 | 0.100 | 6.1 | 0.096 | 5.9 | 0.007 | 0.4 | 0.020 | 1.2 | 0.140 | 8.6 |
| CO-20% (Li Heparin) | 180 | 0.9 | 0.049 | 5.5 | 0.067 | 7.5 | 0.016 | 1.8 | 0.022 | 2.4 | 0.088 | 9.7 |
| Neg (Li Heparin) | 180 | 0.09 | 0.006 | 7.5 | 0.013 | 14.7 | 0.000 ⁵ | 0.0 | 0.018 | 20.6 | 0.023 | 26.4 |

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.

H. Qualitative Precision

A precision study was performed with the MONOLISA™ Anti-HBc EIA using panels prepared in serum, EDTA plasma, and sodium heparin. The 10 specimens were tested in triplicate, twice a day, for 20 days, and results are summarized in Table 4.

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

| Panel Member | N | Mean | Within Run | | Between Day | | Between Run | | Total | |
|---------------------|-----|-------|------------|------|-------------|------|-------------|-----|-------|------|
| | | O.D. | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 Serum | 120 | 0.830 | 0.020 | 2.3 | 0.060 | 7.5 | 0.020 | 2.8 | 0.070 | 8.3 |
| 2 Serum | 120 | 0.330 | 0.030 | 7.9 | 0.030 | 8.3 | 0.010 | 4.5 | 0.040 | 12.3 |
| 3 Serum | 120 | 0.200 | 0.010 | 7.1 | 0.010 | 4.1 | 0.010 | 4.9 | 0.020 | 9.6 |
| 4 Serum | 120 | 0.040 | 0.010 | 19.4 | 0.000 | 8.7 | 0.000 | 6.1 | 0.010 | 22.1 |
| 5 EDTA Plasma | 120 | 0.430 | 0.030 | 7.2 | 0.020 | 4.9 | 0.020 | 3.7 | 0.040 | 9.4 |
| 6 EDTA Plasma | 120 | 0.230 | 0.010 | 3.6 | 0.020 | 7.6 | 0.010 | 3.1 | 0.020 | 9.0 |
| 7 EDTA Plasma | 120 | 0.050 | 0.000 | 9.3 | 0.010 | 10.8 | 0.000 | 0.0 | 0.010 | 14.3 |
| 8 NaHeparin Plasma | 120 | 0.370 | 0.030 | 8.9 | 0.010 | 3.7 | 0.030 | 8.8 | 0.050 | 13.0 |
| 9 NaHeparin Plasma | 120 | 0.210 | 0.010 | 5.9 | 0.010 | 2.7 | 0.000 | 0.0 | 0.010 | 6.5 |
| 10 NaHeparin Plasma | 120 | 0.020 | 0.000 | 18.3 | 0.000 | 5.6 | 0.000 | 6.9 | 0.000 | 20.3 |

X. Summary of Clinical Studies

A multi-center clinical trial was conducted to evaluate the performance of the MONOLISA™ Anti-HBc 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 1349 prospective asymptomatic subjects were tested with the MONOLISA™ Anti-HBc 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 was unknown. The subjects were male (70.2%) and female (29.8%) and ranged in age from 18 to 81 years.

The MONOLISA™ Anti-HBc 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-HBc EIA

| Age Range | Gender | MONOLISA™ Anti-HBc EIA Result | | | | | | Total N |
|---------------|--------|-------------------------------|---------------|------------|--------------|--------------|---------------|-------------|
| | | Reactive | | Borderline | | Non-reactive | | |
| | | N | % | N | % | N | % | |
| 10-19* | F | 0 | 0.0 % | 0 | 0.0 % | 6 | 100.0% | 6 |
| | M | 1 | 10.0 % | 0 | 0.0 % | 9 | 90.0 % | 10 |
| 20-29 | F | 7 | 6.7 % | 1 | 1.0 % | 96 | 92.3 % | 104 |
| | M | 20 | 16.4 % | 1 | 0.8 % | 101 | 82.8 % | 122 |
| 30-39 | F | 25 | 23.1 % | 2 | 1.9 % | 81 | 75.0 % | 108 |
| | M | 44 | 20.8 % | 2 | 0.9 % | 166 | 78.3 % | 212 |
| 40-49 | F | 36 | 34.0 % | 2 | 1.9 % | 68 | 64.2 % | 106 |
| | M | 135 | 38.8 % | 1 | 0.3 % | 212 | 60.9 % | 348 |
| 50-59 | F | 21 | 34.4 % | 1 | 1.6 % | 39 | 63.9 % | 61 |
| | M | 104 | 49.8 % | 4 | 1.9 % | 101 | 48.3 % | 209 |
| 60-69 | F | 3 | 27.3 % | 0 | 0.0 % | 8 | 72.7 % | 11 |
| | M | 19 | 51.4 % | 1 | 2.7 % | 17 | 45.9 % | 37 |
| 70-79 | F | 0 | 0.0 % | 0 | 0.0 % | 2 | 100.0% | 2 |
| | M | 2 | 40.0 % | 0 | 0.0 % | 3 | 60.0 % | 5 |
| 80-89 | F | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % | 0 |
| | M | 0 | 0.0 % | 0 | 0.0 % | 1 | 100.0% | 1 |
| Unknown | F | 1 | 33.3 % | 0 | 0.0 % | 2 | 66.7 % | 3 |
| | M | 3 | 75.0 % | 0 | 0.0 % | 1 | 25.0 % | 4 |
| Totals | | 421 | 31.2 % | 15 | 1.1 % | 913 | 67.7 % | 1349 |

*There were no subjects less than 18 years of age.

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 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 35 unique reference marker patterns observed in the MONOLISA™ Anti-HBc 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 | + | + | + | + | - | - |
| Acute infection | + | + | I | + | - | - |
| Acute infection | + | + | - | - | - | - |
| Acute infection | + | - | + | - | - | + |
| Acute infection | + | - | + | - | - | - |
| Acute infection | + | - | - | - | - | - |
| Chronic infection | + | + | + | + | - | + |
| Chronic infection | + | + | - | + | - | - |
| Chronic infection | + | + | - | + | - | + |
| Chronic infection | + | + | - | + | + | + |
| Chronic infection | + | + | - | + | + | - |
| Chronic infection | + | - | - | + | + | - |
| Chronic infection | + | - | - | + | + | + |
| Early recovery | - | - | + | + | + | + |
| Early recovery | - | - | + | + | + | - |
| Early recovery | - | - | - | + | - | - |
| Early recovery | - | - | - | + | + | - |
| Early recovery | - | - | - | + | I | - |
| Early recovery | - | - | I | + | + | + |
| HBV vaccine response | - | - | - | - | - | + |
| HBV vaccine response status indeterminate | - | - | - | - | - | I |
| Not previously infected with HBV | - | - | - | - | - | - |
| Recovered | - | - | - | + | I | I |
| Recovered | - | - | - | + | - | I |

*There were no subjects less than 18 years of age.

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 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 35 unique reference marker patterns observed in the MONOLISA™ Anti-HBc 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 | + | + | + | + | - | - |
| Acute infection | + | + | I | + | - | - |
| Acute infection | + | + | - | - | - | - |
| Acute infection | + | - | + | - | - | + |
| Acute infection | + | - | + | - | - | - |
| Acute infection | + | - | - | - | - | - |
| Chronic infection | + | + | + | + | - | + |
| Chronic infection | + | + | - | + | - | - |
| Chronic infection | + | + | - | + | - | + |
| Chronic infection | + | + | - | + | + | + |
| Chronic infection | + | + | - | + | + | - |
| Chronic infection | + | - | - | + | + | - |
| Chronic infection | + | - | - | + | + | + |
| Early recovery | - | - | + | + | + | + |
| Early recovery | - | - | + | + | + | - |
| Early recovery | - | - | - | + | - | - |
| Early recovery | - | - | - | + | + | - |
| Early recovery | - | - | - | + | I | - |
| Early recovery | - | - | I | + | + | + |
| HBV vaccine response | - | - | - | - | - | + |
| HBV vaccine response status indeterminate | - | - | - | - | - | I |
| Not previously infected with HBV | - | - | - | - | - | - |
| Recovered | - | - | - | + | I | I |
| Recovered | - | - | - | + | - | I |

| FDA Characterization based on single point specimen | HBsAg | HBeAg | Anti-HBc IgM | Total HBc | Anti-HBe | Anti-HBs |
|---|-------|-------|--------------|-----------|----------|----------|
| Recovered or Immune due to natural infection | - | - | - | + | - | + |
| Recovery | - | - | - | - | + | + |
| Recovery | - | - | - | + | I | + |
| Recovery | - | - | - | + | + | I |
| Recovery | - | - | - | + | + | + |
| Uninterpretable | + | - | - | - | + | - |
| Uninterpretable | - | + | - | - | - | + |
| Uninterpretable | - | + | - | - | - | - |
| Uninterpretable | - | - | - | - | + | - |
| Acute infection | + | + | + | + | - | - |
| Acute infection | + | + | I | + | - | - |
| Acute infection | + | + | - | - | - | - |

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

Comparison of Results

A comparison of the MONOLISA™ Anti-HBc EIA results with the reference anti-HBc assay for each specimen classification is shown in Table 7.

Table 7
FDA HBV Classification of High Risk Prospective Specimens
MONOLISA™ Anti-HBc EIA versus Reference Anti-HBc EIA

| Reference Serology Classification | Reference Anti-HBc Assay | | | | | | Totals |
|--|--------------------------|------------------|--------------|------------------------|------------------|--------------|-------------|
| | Reactive | | | Non-reactive | | | |
| | MONOLISA™ Anti-HBc EIA | | | MONOLISA™ Anti-HBc EIA | | | |
| | Reactive | BRD ¹ | Non-reactive | Reactive | BRD ¹ | Non-reactive | |
| Acute Infection | 6 | 0 | 0 | 1 | 0 | 7 | 14 |
| Chronic Infection | 76 | 1 | 1 | 0 | 0 | 0 | 78 |
| Early recovery | 101 | 0 | 4 | 0 | 0 | 0 | 105 |
| HBV vaccine response | 0 | 0 | 0 | 16 ² | 5 | 288 | 309 |
| HBV vaccine response (?) ³ | 0 | 0 | 0 | 1 | 1 | 29 | 31 |
| Not previously infected with HBV | 0 | 0 | 0 | 9 | 6 | 591 | 606 |
| Recovered | 12 | 0 | 0 | 0 | 0 | 0 | 12 |
| Recovered or Immune due to natural infection | 86 | 2 | 2 | 0 | 0 | 0 | 90 |
| Recovery | 169 | 0 | 0 | 2 | 1 | 0 | 172 |
| Uninterpretable | 0 | 0 | 0 | 1 | 0 | 7 | 8 |
| Total | 450 | 3 | 7 | 30 | 13 | 922 | 1425 |

¹BRD = Borderline

²12 of the 16 samples were reactive on a second reference assay, in agreement with the MONOLISA™ Anti-HBc EIA

³(?)= anti-HBs status indeterminate

Percent Agreement

The percent agreement between the MONOLISA™ Anti-HBc EIA and the reference anti-HBc 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-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 | (6/6) 100.0% | 60.9%, 100% | (7/8) 87.5% | 52.9%, 97.8% |
| Chronic Infection | 78 | (76/78) 97.4% | 91.1%, 99.3% | (0/0) NA | NA |
| Early recovery | 105 | (101/105) 96.2% | 90.6%, 98.5% | (0/0) NA | NA |
| HBV vaccine response | 309 | (0/0) NA | NA | (288/309) 93.2% | 89.8%, 95.5% |
| HBV vaccine response (?) | 31 | (0/0) NA | NA | (29/31) 93.5% | 79.3%, 98.2% |
| Not previously infected with HBV | 606 | (0/0) NA | NA | (591/606) 97.5% | 96%, 98.5% |
| Recovered | 12 | (12/12) 100.0% | 75.7%, 100% | (0/0) NA | NA |
| Recovered or Immune due to natural infection | 90 | (86/90) 95.6 % | 89.1%, 98.3% | (0/0) NA | NA |
| Recovery | 172 | (169/169) 100.0 % | 97.8%, 100% | (0/3) 0.0% | NA |
| Uninterpretable | 8 | (0/0) NA | NA | (7/8) 87.5% | 52.9%, 97.8% |
| Total | 1425 | (450/460) 97.8% | 96%, 98.8% | (922/965) 95.5% | 94%, 96.7% |

Of the 1425 samples tested, 16 samples gave borderline results with MONOLISA™ Anti-HBc EIA. The reference method has positive/negative results with a retest zone. Three (3) of the MONOLISA™ Anti-HBc EIA borderline samples were found to be positive by the reference method and 13 were negative by the reference method. Below are the calculations of percent agreement when the borderline results by MONOLISA™ Anti-HBc EIA are considered as positive results and when the borderline results by MONOLISA™ Anti-HBc EIA are considered as negative results.

| <u>MONOLISA™ Anti-HBc EIA</u> | <u>Positive Agreement</u> | <u>Negative Agreement</u> |
|--------------------------------|---------------------------|---------------------------|
| Borderline considered positive | 98.5% (453/460) | 95.5% (922/965) |
| Borderline considered negative | 97.8% (450/460) | 96.9% (935/965) |

Seroconversion Panels

The comparative sensitivity of the MONOLISA™ Anti-HBc EIA was determined by testing 5 commercially available Anti-HBV seroconversion panels and comparing to a reference Anti-HBc assay. 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-HBc | | Reference Anti-HBc EIA |
|------------|---------------------------------|-----------------|--------------------|----------|------------------------|
| | | | S/CO | Result | Result |
| PHM935A-11 | 35 | 20 | 0.07 | NR | NR |
| PHM935A-12 | 50 | | 0.08 | NR | NR |
| PHM935A-13 | 66 | | 2.11 | R | R |
| PHM935A-14 | 68 | | 1.57 | R | R |
| RP009-04 | 13 | 20 | 0.17 | NR | NR |
| RP009-05 | 29 | | 2.92 | R | R |
| RP009-06 | 31 | | 3.25 | R | R |
| RP009-07 | 36 | | 3.58 | R | R |
| RP016-05 | 23 | 20 | 0.10 | NR | NR |
| RP016-06 | 25 | | 0.12 | NR | NR |
| RP016-07 | 57 | | 0.24 | NR | R |
| RP016-08 | 60 | | 2.33 | R | R |
| 6278-08 | 26 | 11 | 0.08 | NR | NR |
| 6278-09 | 33 | | 0.32 | NR | NR |
| 6278-10 | 37 | | 1.07 | R | R |
| 6278-11 | 41 | | 2.13 | R | R |
| 6281-08 | 36 | 12 | 0.08 | NR | NR |
| 6281-09 | 41 | | 0.75 | NR | R |
| 6281-10 | 43 | | 2.15 | R | R |
| 6281-11 | 50 | | 3.64 | R | R |

In 3 of the 5 seroconversion panels, the MONOLISA™ Anti-HBc EIA detected reactive levels of hepatitis B core antibody at the same member as the reference anti-HBc test. The MONOLISA™ Anti-HBc EIA detected reactive levels of hepatitis B core antibody 1 member later than the reference anti-HBc test on 2 of 5 seroconversion panels.

Clinical Performance with Acute HBV Samples

Retrospective acute HBV samples from 85 individuals were tested with the MONOLISA™ Anti-HBc EIA and a reference Anti-HBc EIA. The results of the MONOLISA™ Anti-HBc EIA are compared to results of the reference anti-HBc method in Table 10.

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

| MONOLISA™ Anti-HBc Result | Reference Anti-HBc Result | | |
|------------------------------|---------------------------|--------------|-----------|
| | Reactive | Non-Reactive | Total |
| Reactive | 85 | 0 | 85 |
| Non-Reactive | 0 | 0 | 0 |
| Total | 85 | 0 | 85 |

The positive percent agreement with the reference method is 100% (85/85) with a 95% confidence interval of 95.7-100%.

Clinical Evaluation of the MONOLISA™ Anti-HBc 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 EIA and a reference Anti-HBc EIA. The results of the MONOLISA™ Anti-HBc EIA are compared to results of the reference anti-HBc method in Table 11.

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

| MONOLISA™ Anti-HBc Result | Reference Anti-HBc Result | | |
|------------------------------|---------------------------|--------------|------------|
| | Reactive | Non-Reactive | Total |
| Reactive | 117 | 0 | 117 |
| Non-Reactive | 0 | 3 | 3 |
| Total | 117 | 3 | 120 |

The positive percent agreement with the Reference Method is 100% (117/117) with a 95% confidence interval of 96.8-100%. The negative percent agreement with the Reference Method is 100% (3/3) with a 95% confidence interval of 43.9-100%.

XI. Conclusions Drawn from the Studies

Multi-centered clinical and non-clinical studies were conducted in the US to evaluate the MONOLISA™ Anti-HBc EIA. A method comparison was performed with a commercially available FDA-approved assay to detect total 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 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 35 unique reference marker patterns. The overall positive percent agreement between the MONOLISA™ Anti-HBc EIA and the reference assay was 97.8% (450/460) in the high risk, signs and symptoms,

and vaccinated populations. The overall negative percent agreement between the MONOLISA™ Anti-HBc EIA and the reference assay was 95.5% (922/965) in the same population.

In a study of 85 retrospective acute HBV samples, the positive agreement was 100 % (85/85) with the comparison method. In another study with 120 retrospective chronic HBV samples (HBsAg positive for more than 6 months), the positive percent agreement with the comparison method was 100% (117/117) and the negative agreement rate with the comparison method was 100% (3/3).

The ability of the MONOLISA™ Anti-HBc EIA to detect HBV infections was demonstrated with 5 seroconversion panel evaluations.

Precision and reproducibility of the MONOLISA™ Anti-HBc 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 (potassium EDTA, sodium citrate, ACD, lithium heparin and sodium heparin) with the MONOLISA™ Anti-HBc EIA.

The results from both the non-clinical and clinical studies indicate that the MONOLISA™ Anti-HBc EIA can be used safely and effectively for the qualitative determination of anti-HBc 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 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 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 EIA has been demonstrated for use in determining if 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 EIA assay for aiding in the diagnosis of acute and chronic HBV infection in suspected individuals has been demonstrated.

XII. 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.

XIII. CDRH Decision

Information submitted to the Agency by Bio-Rad Laboratories has demonstrated that the MONOLISA™ Anti-HBc EIA kit performs as intended for the detection of total antibodies to HBc. FDA issued an approval order on April 27, 2007.

The applicant's manufacturing facilities were inspected on May 5, May 31, and June 1, 2006 and found to be in substantial compliance with the Quality Systems Regulation as defined in 21 CFR 820.

XIV. 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.