BIO-RAD

MONOLISA™ Anti-HBc EIA

26186

Enzyme Immunoassay (EIA) for the Detection of Total Antibodies to Hepatitis B Nucleocapsid Antigen (core) in Human Serum and Plasma

For In Vitro Diagnostic Use

MONOLISA™ Anti-HBc EIA • 192 Tests

LEXICON

WASH	Wash Solution Concentrate (30X)
TMB SOLUTION	Chromogen: TMB Solution
SUB BUF	Substrate Buffer
STOP	Stopping Solution

CONTENTS

1 - NAME AND INTENDED 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.

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

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

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.

2 - SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is a major public health problem, with more than 400 million people infected worldwide.[1] Chronic hepatitis B is a leading cause of cirrhosis and liver cancer. The virus is transmitted efficiently by a number of routes, including passage from mother to child and percutaneous or permucosal exposure to infectious blood or body fluids.[2] Sexual contact, intravenous drug use, blood transfusion, tissue transplantation, and hemodialysis procedures may transmit the disease.[3,4]

The genetic organization, transcription, and replication of the virus are well understood.[5,6] The whole virion, or Dane particle, contains an envelope, consisting of a lipid bilayer and glycoproteins (surface antigens), and a core (nucleocapsid) that encloses a circular DNA genome. The viral DNA encodes at least seven proteins from four open reading frames [surface (S), core (C), polymerase (P), and the X gene (X)]. The proteins that are important diagnostically are surface antigen (HBsAg), core antigen (HBcAg) and e antigen (HBeAg), a hidden epitope released by disruption of the nucleocapsid.

During the early stages of primary infection by hepatitis B virus, HBV DNA, as well as HBsAg and HBeAg, are readily detectable. As the host mounts an immune response, the first antibodies to appear are antibodies to the core antigen (anti-HBc).[7-10] Anti-HBc IgM antibodies are the initial response to infection and remain detectable for several weeks, disappearing during convalescence. Anti-HBc IgG antibodies, which are produced later, continue for many years after recovery. The detection of antibodies to hepatitis B core antigen is a significant marker for the presence of past (anti-HBc total) or recent (anti-HBc IgM) infection by the hepatitis B virus.[11-17]

Patients with chronic hepatitis B virus infection usually show high levels of anti-HBc. The anti-HBc antibodies can persist for a long time and may be observed associated with HBsAg, associated with anti-HBs antibodies, or in some cases, alone. In the latter case, additional clinical evaluation may be necessary to determine whether an "anti-HBc only" pattern is caused by occult hepatitis B infection.[18]

3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The MONOLISA™ Anti-HBc EIA is an enzyme immunoassay (indirect format) for detection of total antibodies to hepatitis B core antigen. 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.

4 - REAGENTS

MONOLISA™ Anti-HBc EIA Product Description
Product No. 26186 (192 test kit)

Component	Contents	Preparation
R1 • Anti-HBc Microwell Strip Plates (2)	• Microwell strips in holder, coated with purified recombinant HBc antigen • Tabs are labeled "GG" • ProClin® (trace)	Use as supplied. Return unused strips to the pouch. Do not remove desiccant.
R2 • Wash Solution Concentrate (30X) 1 bottle (120 mL)	• Sodium Chloride • Tween 20	Dilute 1:30 with deionized water. Clinical laboratory reagent water Type I or Type II is acceptable.
R3 • Specimen Diluent 2 bottles (30 mL)	• PBS Buffer • Gentamicin, 0.005% • ProClin® 300, 0.1% • < 0.001% Thimerosal • Sample indicator dye	Use as supplied.
C0 • Anti-HBc Negative Control 1 vial (0.8 mL)	• Human serum; negative for antibodies to HBc, HBs, HIV and HCV; negative for HBsAg • Gentamicin, 0.005% • ProClin® 950, 0.16%	Dilute in Specimen Diluent as described.
C1 • Anti-HBc Positive Control 1 vial (0.8 mL)	• Human serum; positive for anti-HBc and anti-HBs; negative for HBsAg; negative for HIV and HCV antibodies • Gentamicin, 0.005% • ProClin® 950, 0.16%	Dilute in Specimen Diluent as described.
C2 • Anti-HBc Cutoff Calibrator 1 vial (1.5 mL)	• Human serum; negative for antibodies to HBc, HBs, HIV and HCV; negative for HBsAg • Gentamicin, 0.005% • ProClin® 950, 0.16%	Dilute in Specimen Diluent as described.
R4 • Anti-HBc Conjugate 1 bottle (30 mL)	• Peroxidase-labeled goat antibody directed against human IgG and IgM • Buffer with protein stabilizers • Gentamicin, 0.005% • ProClin® 300, 0.1%	Use as supplied.
R8 • Substrate Buffer 1 bottle (120 mL)	• Hydrogen Peroxide • Citric Acid/Sodium Acetate buffer • Dimethylsulfoxide (DMSO)	Use as supplied.
R9 • Chromogen (11X) 1 bottle (12 mL)	• Tetramethylbenzidine (TMB)*	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1 bottle (120 mL)	• 1N H_2SO_4 (Sulfuric Acid)	Use as supplied.
Plate Sealers	• Clear plastic sealers	Use as supplied.

*NOTE: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[19,20]

Store the kit at 2-8°C. Bring all reagents to room temperature (18-30°C) before use. Return reagents to 2-8°C immediately after use. Store all unused strips/plates in pouch and reseal. Do not remove desiccant. Store strip plates at 2-8°C.

5 – WARNINGS

For in vitro diagnostic use only.

1. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[21] Biosafety Level 2[22] or other appropriate biosafety practices[23,24] should be used for materials that contain or are suspected of containing infectious agents. The following human blood derivatives are found in this kit:
   1.1 Human source material used in the preparation of the Negative Control (C0) and Cutoff Calibrator (C2) has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core antigen, hepatitis B surface antigen, hepatitis C virus (HCV), and human immunodeficiency viruses (HIV-1 and HIV-2)
   1.2 Human source material used in the preparation of the Positive Control (C1) has been treated to reduce the potential for HBV infectivity. It is negative for hepatitis B surface antigen (HBsAg), and positive for anti-HBc and anti-HBs. It has been tested and found nonreactive for antibodies to hepatitis C virus (HCV) and human immunodeficiency viruses (HIV-1 and HIV-2).

2. Following is a list of potential chemical hazards contained in some kit components (See section 4: REAGENTS). Material Safety Data Sheets (MSDS) are available on request
   2.1 ProClin® 300 (0.1%) or ProClin® 950 (0.16%), biocidal preservatives that are irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   2.2 0.005% Gentamicin Sulfate, a biocidal preservative, is a known reproductive toxin, photosensitizer and sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   2.3 < 0.001% Thimerosal, an Organo-Mercury biocidal preservative that targets the central nervous system, is a reproductive toxicant and significant sensitizer. Avoid release to the environment, due to danger of cumulative effects. Spent mercury-containing solutions with a concentration greater than 0.2 ppm must be disposed of as U.S. Federal RCRA hazardous waste (D009), however, dispose of all wastes in accordance with local, regional, and national regulations. Note: mercury (Hg) makes up 49.55% of the Thimerosal molecule, thus a component with 0.001% Thimerosal contains ~0.0005% (~5 ppm) mercury w/v.
   2.4 The 1 N Sulfuric Acid (H_2SO_4) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases and reducing agents; do not pour water into this component. Waste from this material is considered hazardous acidic waste. However, if permitted by local, regional, and national regulations, it might be neutralized to pH 6-9 for non-hazardous disposal.

3. Biological spills: Human source material spills should be treated as potentially infectious. Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus], or a phenolic, etc.) and wiped dry.[25-27]

   Spills containing acid should be appropriately absorbed (wiped up) or neutralized, wiped dry and then the area should be decontaminated with one of the chemical disinfectants; materials used to absorb the spill may require biohazardous waste disposal.

   NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

4. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

6 - PRECAUTIONS FOR USERS

1. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
2. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
3. Do not pipette by mouth.
4. The MONOLISA™ Anti-HBc EIA is intended for the detection of total antibodies to hepatitis B core antigen. The tabs at the end of the plate are labeled with the product code "GG".
5. Do not use any kit components beyond their stated expiration date.
6. Any lot number of the following reagents may be used with this assay provided they have the correct catalog number and are not used beyond their labeled expiration date:
   Chromogen (R9) – Catalog # 26182
   Substrate Buffer (R8) – Catalog # 26181
   Wash Solution Concentrate (R2) – Catalog # 25261
   Stopping Solution (R10) – Catalog # 25260
   Do not mix any other reagents from different lot numbers.
7. Do not use the Chromogen (R6), the Chromogen Diluent (R4) and/or the Buffered Substrate (R7a) color development solutions found in the Bio-Rad rLAV EIA and Bio-Rad HIV-2 EIA test kits.
8. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.
9. Use a clean, disposable container for the conjugate. Exposure of the conjugate to human serum or sodium azide will result in its inactivation.
10. Avoid exposing Chromogen or Working TMB Solution to strong light during storage or incubation. Do not allow the Working TMB Solution to come into contact with any oxidizing agents.
11. Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow Stopping Solution to come into contact with metals.
12. Use clean, polypropylene containers to prepare and store the Working TMB Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware.
13. For the manual pipetting of controls and specimens, use individual pipette tips to eliminate carryover of samples.
14. Handle the Negative and Positive Controls and the Cutoff Calibrator in the same manner as patient specimens.
15. Use only adequately calibrated equipment with this assay.
16. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.
17. The MONOLISA™ Anti-HBc EIA Procedure and the Interpretation of Results must be followed when testing serum or plasma specimens for the presence of antibodies to hepatitis B virus core antigen. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps. Inadequate adherence to package insert instructions may result in erroneous results.
18. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of procedural error.
19. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error that should

be evaluated. That result is invalid and that specimen must be re-run. If repeated results are <0.000, the performance of the instrumentation should be investigated.

20. Factors that can affect the validity of results include failure to add the specimen to the well, inadequate washing of microplate wells, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.

7 - REAGENT PREPARATION AND STORAGE

Working TMB Solution (R8 + R9)

Bring Chromogen (R9) and Substrate Buffer (R8) to room temperature. Invert the Chromogen and Substrate Buffer to mix before using. Prepare a 1:11 dilution for each strip to be tested by mixing 100 µL of Chromogen to each 1 mL of Substrate Buffer in a clean, polypropylene container. Note Chromogen lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix TMB Working Solution gently prior to use. Working TMB Solution should be kept in the dark at room temperature and used within 8 hours of preparation.

Chromogen should be colorless. Any other color indicates that the reagent is compromised and should not be used. Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Extra Chromogen is provided. Use the following table as a guide:

Preparation of Working TMB Solution by Number of Strips Used

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*	24**
Amount of Chromogen (µL)	100	200	300	400	500	600	700	800	900	1000	1100	1200	2400
Amount of Substrate Buffer (mL)	1	2	3	4	5	6	7	8	9	10	11	12	24

* 1 Complete Plate ** 2 Complete Plates

Wash Solution (R2)

Prepare Wash Solution (R2) by adding one part Wash Solution Concentrate (30X) to 29 parts of deionized or distilled water (e.g., 120 mL of Wash Solution Concentrate to 3480 mL of deionized water). Clinical laboratory reagent water Type I or Type II is acceptable. The diluted Wash Solution can be stored ambient for up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Discard if no suds are evident in the Wash Solution. Prepare a sufficient quantity of Wash Solution to complete a full run.

8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum or plasma may be used in the test. The following tube types and anticoagulants, including those in both glass and plastic tubes, have all been evaluated and found to be acceptable: SST, potassium EDTA, sodium citrate, ACD, and sodium and lithium heparin. Specimens that are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. The volume of anticoagulant in Na citrate tubes causes a specimen dilutional effect. Individuals with borderline results obtained from specimens collected in Na citrate should be retested using serum specimens. Specimens with observable particulate matter should be clarified by centrifugation prior to testing.

Serum/plasma should remain at 22°C for no longer than eight hours. If assays are not completed within eight hours, serum/plasma should be refrigerated at 2 to 8°C. Specimens may be stored at 2-8°C for 48 hours. For long-term storage, the specimens should be frozen (at -20°C or lower). Specimens should not be used if they have incurred more than 5 freeze-thaw cycles. Mix specimens thoroughly after thawing.

Note: If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents. *Specimens should be kept frozen (-20°C or lower) for shipment.*

9 - MONOLISA™ Anti-HBc EIA PROCEDURE

Materials Provided
See REAGENTS section on page 4.

Materials required but not provided

1. Precision pipettes to deliver volumes from 10 µL to 220 µL, 1 mL, 5 mL, and 10 mL (accurate within ± 10%). A multichannel pipettor capable of delivering 100 µL is optional.
2. Pipette tips.
3. Appropriately sized graduated cylinders.
4. Dry-heat incubator capable of maintaining 37 ± 2°C.
5. Bio-Rad microwell plate or strip washer, or equivalent. The washer must be capable of dispensing 375 µL per well, cycling 5 times, and soaking for 0-60 seconds between each wash.
6. Bio-Rad microwell plate or strip reader or equivalent. The spectrophotometer should have the following specifications at wavelength 450 nm:

   Bandwidth: 10 nm HBW (Half Band Width) or equivalent
   Absorbance Range: 0 to 2 AU (Absorbance Units)
   Repeatability: ± (0.5% + 0.005) AU
   Linearity or Accuracy: 1% from 0 to 2.0 AU

   The instrument should contain a reference filter for reading at 615 to 630 nm. An instrument without a reference filter can be used; however, areas in the bottoms of the wells that are opaque, scratched or irregular may cause absorbance readings that are falsely elevated.
7. Household bleach (5% to 8% sodium hypochlorite) may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite). Alternative disinfectants include: 70% ethanol or 0.5% Wescodyne™ Plus (West Chemical Products, Inc.).
8. Paper towels or absorbent pads for blotting.
9. Labeled null strips, for testing partial plates.
10. Clean, polypropylene containers of appropriate size for the preparation of TMB (do not use polystyrene).
11. Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.
12. Gloves.
13. Laboratory timer.
14. EIA reagent reservoirs (optional).

Preliminary Statements

1. The expected run time for this procedure is approximately 3 hours from initiation of the first incubation step. Each run of this assay must proceed to completion without interruption after it has been started.
2. Controls and Calibrators to be included on each plate of this assay: Positive Control (run singly), Negative Control (run singly), and Cutoff Calibrator (run in triplicate). The cutoff for patient samples is determined by the Cutoff Calibrator replicates on each individual plate.
3. The procedure specifies the addition of 100 µL volumes of Conjugate Solution, Working TMB Solution, and Stopping Solution while performing the assay.
4. Do not splash controls, specimens, or reagents between microwells of the plate.
5. Cover plates for each incubation step using plate sealers provided or other appropriate means to minimize evaporation.

6. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working TMB Solution).
7. Adhere to the recommended time constraints for the use of the Working TMB Solution (8 hours, ambient) and Wash Solution (4 weeks, ambient).
8. Avoid the formation of air bubbles in each microwell.

EIA Procedure

The MONOLISA™ Anti-HBc EIA performance is dependent upon incubation times and temperatures. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures should be carefully monitored using calibrated thermometers, or equivalent.

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.
2. **Bring all of the reagents to room temperature before beginning the assay procedure.**
3. Prepare Working TMB Solution and Working Wash Solution. Mix gently, by inversion.
4. Remove strips not needed for the assay and replace them with labeled Null Strips, as necessary.
5. If sample identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.
6. **Dilute specimens, controls and calibrator in the Specimen Diluent:**

   Specimens, controls and the calibrator may be prediluted 1:11 in the Specimen Diluent prior to addition to the well (for example, dilute 25 µL of specimen in 250 µL of Specimen Diluent, mix gently to avoid foaming, and then transfer **220 µL** to the well). Alternatively, specimens, Controls and Calibrators may be diluted in-well by adding 200 µL of Specimen Diluent to each well first, followed by 20 µL of specimen or control within 30 minutes, then mix gently to avoid foaming.

   NOTE: It is possible to verify the presence of samples in the wells by spectrophotometric reading at 615 - 630 nm (single wavelength). Refer to section 10: Spectrophotometric Verification of Sample and Reagent Pipetting.

   One well of Positive Control, one well of Negative Control and three wells of Cutoff Calibrator should be assayed on each plate or partial plate of specimens.
7. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 ± 5 minutes at 37 ± 2 °C.**
8. At the end of the incubation period, carefully remove the plate sealer and aspirate the fluid from each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 375 µL/well/wash). **Soak each well for 0 to 60 seconds between each wash cycle.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on clean, absorbent paper towels. ***NOTE:*** *Grasp the plate holder firmly at the center of the long sides before inverting to blot.*
9. **Add 100 µL of the Conjugate Solution to each well containing a specimen, calibrator, or control.** Avoid bumping plates containing conjugate solution to prevent contamination of the plate sealer and/or top edges of the wells. ***NOTE:*** *The conjugate is colored green.*

   It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 630 nm (single wavelength). Refer to section 10: Spectrophotometric Verification of Sample and Reagent Pipetting.
10. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 ± 5 minutes at 37± 2 °C.**
11. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid in each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times**

with the Wash Solution (at least 375 µL/well/wash). **Soak each well for 0 to 60 seconds between each wash cycle.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on a clean, absorbent paper towel. ***NOTE:*** *Grasp the plate holder firmly at the center of the long sides before inverting to blot.*

12. **Add 100 µL of the Working TMB Solution to each well containing a specimen, calibrator, or control. Incubate plates in the dark for 30 ± 5 minutes at room temperature (18 to 30°C).** Use of a plate sealer or cover is optional.
13. **Add 100 µL of Stopping Solution to each well** to terminate the reaction. Use the same sequence and rate of distribution as for the substrate solution addition. **Tap the plate gently, or use other means to assure complete mixing. Complete mixing is required for acceptable results.**
14. Carefully wipe the plate bottom and ensure that all strips have been pressed firmly into place before reading. **Read absorbance within 30 minutes after adding the Stopping Solution,** using the **450 nm filter with 615 nm to 630 nm** as the reference. (Blank on air.)

Decontamination

Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.

10 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING (OPTIONAL)

Verification of Sample Pipetting

After the sample addition to the Specimen Diluent, the purple diluent turns blue. However, the visual color change is subtle and best verified by spectrophotometric reading at 615 - 630 nm (single wavelength). Note that air bubbles in the well may affect the accuracy of the reading, and should be avoided. Specimens with low levels of protein (< 4 g/dL) may require visual verification.

Method 1: Without the Use of a Pipetting Control Well

The presence of sample in the well can be verified by reading at 615 - 630 nm. Compare the optical densities measured for each well, after the addition of Specimen Diluent (R3) only, and then again after addition of the sample.

- The absorbance values of the wells containing Specimen Diluent (R3) only must be greater than 0.500.
- Each well containing sample plus Diluent must show an absorbance difference of greater than 0.150 compared to the reading with Diluent only. A difference of less than 0.150 indicates inadequate sample addition.

Method 2: Using a Pipetting Control Well

The presence of sample in the wells can be verified by reading at 615 - 630 nm with the use of a Pipetting Control Well containing the Specimen Diluent (R3) only. Read the plate after adding both Specimen Diluent and sample, and compare the optical density obtained for each sample with that obtained for the Pipetting Control Well.

- The absorbance value of the Pipetting Control Well containing Specimen Diluent (R3) only must be greater than 0.500.
- The absorbance difference between well with sample and the Pipetting Control Well must be greater than 0.150 compared to the reading with Diluent only. A difference less than 0.150 indicates inadequate sample addition.

Verification of Conjugate Dispense

The Conjugate (R4) is green in color.
The presence of Conjugate (R4) in the well can be verified by spectrophotometric reading at 615 -

630 nm (single wavelength):

- The OD value of each well must be greater than or equal to 0.100. A value lower than this indicates poor dispense of the Conjugate Solution.

11 - QUALITY CONTROL –VALIDATION OF RESULTS

Each plate should contain a Positive Control, a Negative Control, and three Cutoff Calibrators. The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. In addition, the quality control supplied in the MONOLISA™ Anti-HBc EIA is in a serum matrix and may not adequately control the assay for plasma specimens. The user should include alternate control material for plasma matrices.

The test is invalid and must be repeated if the absorbance readings of the controls and the calibrator do not meet specifications. If the test is invalid, patient results cannot be reported. Quality control testing must be performed in conformance with local, state, and/or federal regulations, or accreditation requirements, and your laboratory's standard Quality Control procedures. It is recommended that the user refer to CLSI C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices.

Assay Validation

A run is valid if the following criteria are met:

- The absorbance values of the individual Cutoff Calibrators (CCi) are greater than 0.000 AU and less than or equal to 0.100 AU ($0.000 < CCi \leq 0.100$). One Cutoff Calibrator may be discarded if the other two values are within range. If two or more Cutoff Calibrators are out of limit, the assay must be repeated.
- The absorbance value of the Positive Control must be greater than or equal to 1.000 AU ($PC \geq 1.000$).
- The absorbance value of the Negative Control must be greater than 0.000 AU and less than the assay cutoff ($0.000 < NC < cutoff$).

If any one of the above criteria is not met, the assay is invalid and must be repeated.

12 - INTERPRETATION OF RESULTS

Mean Cutoff Calibrator Absorbance Value ($\overline{CCx}$)

Determine the mean absorbance for the Cutoff Calibrator by dividing the sum of the absorbance values by the numbers of acceptable wells. The individual absorbance values of the Cutoff Calibrator must be greater than 0.000 and less than or equal to 0.100. One Cutoff Calibrator absorbance value may be discarded if it is outside the acceptable range. The mean absorbance for the Cutoff Calibrator should then be calculated from the two remaining absorbance values.

Cutoff Calibrator Sample Number	Absorbance	Total Absorbance	= 0.121	= 0.040 ($\overline{CCx}$)
1	0.045	3	3	
2	0.040			
3	0.036			
	0.121			

Cutoff Value

The mean absorbance of the Cutoff Calibrator plus 0.250 is the Cutoff Value for the assay. This cutoff corresponds approximately to 0.5 PEI units/ml.

Cutoff Value: $(\overline{CCx}) + 0.250$

An example of the calculation of the Cutoff Value is shown below:

$$(CC\bar{x}) + 0.250 = 0.040 + 0.250 = 0.290$$

Borderline: Specimens with antibody levels of 90-110% of the Cutoff Value should be interpreted as borderline, as the specific immune status for those patients can't be determined without other clinical information or subsequent testing. The borderline interpretation zone is calculated based on Cutoff Value, which is the mean of the Cutoff Calibrator + 0.250.

Borderline: 0.9 X Cutoff Value ≤ Borderline ≤ 1.1 X Cutoff Value

For specimens that are borderline the subject can be re-drawn in 2-3 weeks for additional testing. In conjunction with these results, the immune status of subjects should be evaluated based on their clinical status, related risk factors, and other diagnostic test results.

Reactive: Specimens with absorbance values greater than the borderline zone are considered reactive.

Reactive: >1.1 X Cutoff Value

Nonreactive: Specimens with absorbance values less than the borderline zone are considered nonreactive. The absorbance value of a specimen must be compared to the borderline zone determined for the microwell plate on which it is assayed.

Nonreactive: < 0.9 X Cutoff Value

Example:

Positive Control OD Value	**1.554**		Valid
Negative Control OD value	0.040		Valid
Cutoff Calibrator OD value	0.045 0.040 0.036		Valid
Cut Off	0.290		
Borderline O.D. zone	0.261 – 0.319	*Cutoff Calibrator* ($CAL\bar{x}$) ± 10%	
Specimen OD values	**2.156** 0.260 0.029 0.319	*Interpretation:*	Reactive Nonreactive Nonreactive Borderline

Specimens with absorbance values that are less than 0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

Value	**Result**	**Interpretation**
< 0.9 X Cutoff Value	Nonreactive	Negative for anti-HBc antibodies
0.9 X Cutoff Value to 1.1 X Cutoff Value	Borderline	The specific immune status can't be determined without other clinical information or subsequent testing
>1.1 X Cutoff Value	Reactive	Positive for anti-HBc antibodies

13 - LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
2. A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus.
3. Results obtained with the MONOLISA™ Anti-HBc EIA assay may not be used interchangeably with values obtained with different manufacturers' Anti-HBc assay methods.
4. Results from immunosuppressed patients should be interpreted with caution.
5. A reactive anti-HBc result does not exclude co-infection by another hepatitis virus.

6. The performance of the MONOLISA™ Anti-HBc EIA has not been established with cord blood, neonatal specimens, cadaver specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.
7. Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or those who have been treated with animal serum products. Results which are inconsistent with clinical observations indicate the need for additional testing.

14 - 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 (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 1.

Table 1
Expected Values by Gender and Age - MONOLISA™ Anti-HBc EIA

		MONOLISA™ Anti-HBc EIA Result						Total
		Reactive		Borderline		Non-reactive		
Age Range	Gender	N	%	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.

15 - PERFORMANCE CHARACTERISTICS

A multi-center clinical trial was conducted to evaluate the performance of the MONOLISA™ Anti-HBc 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.

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 2 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 2
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
Recovered or Immune due to natural infection	-	-	-	+	-	+

FDA Characterization based on single point specimen	HBsAg	HBeAg	Anti-HBc IgM	Total HBc	Anti-HBe	Anti-HBs
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 3.

Table 3
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**

[1] BRD = Borderline
[2] 12 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 4.

4D

Table 4
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.

MONOLISA™ Anti-HBc EIA	Positive Agreement	Negative Agreement
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 5.

Table 5
HBV Seroconversion Panel Results

Panel ID	Day since 1st 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 6.

Table 6
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 7.

Table 7
Chronic HBV Sample Results
MONOLISA™ Anti-HBc EIA versus a Reference Anti-HBc EIA 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%.

Potentially Cross-reactive Medical Conditions

The specificity of the MONOLISA™ Anti-HBc EIA assay was evaluated by testing 415 serum samples from 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. A summary of the results is shown in Table 8.

Table 8
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 Facter (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.

Potentially 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

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.

	Distribution of % Difference to Serum		
Collection Tube Type	**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)

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 9 & 10.

Table 9
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%(Li Heparin)	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%(Li Heparin)	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%(Li Heparin)	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 10
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.

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 11.

Table 11
MONOLISA™ Anti-HBc EIA 20-Day Precision Results in O.D.

Panel Member	N	Mean O.D.	Within Run SD	Within Run %CV	Between Day SD	Between Day %CV	Between Run SD	Between Run %CV	Total SD	Total %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

16 - REFERENCES

1. Lai CL, Ratziu V, Yuen M-F, Poynard T: Viral hepatitis B. **Lancet 362:** 2089-2094, 2003.
2. Maddrey WC: Hepatitis B: an important public health issue. **J Med Virol 61:** 362-366, 2000.
3. Delmonico FL, Snydman DR: Organ donor screening for infectious diseases. **Transplantation 65:** 603-610, 1998.
4. Centers for Disease Control: Recommendations for preventing transmission of infections among chronic hemodialysis patients. **MMWR 50 (No. RR-5):** 1-43, 2001.

4L

5. Tiollais P, Pourcel C, Dejean A: The hepatitis B virus. **Nature 317:** 489-495, 1985.

6. Lee WM: Hepatitis B infection. **New Engl J Med 337:** 1733-1745, 1997.

7. Hoofnagle JH, Gerety RJ, Barker LF: Antibody to Hepatitis-B-virus core in man. **Lancet 2:** 869-873, 1973.

8. Szmuness W, Hoofnagle JH, Stevens CE, Prince A.M: Antibody against the hepatitis type B core antigen. **Am J Epidemiol 104:** 256-262, 1976.

9. Neurath AR, Szmuness W, Stevens CE, Strick N, Harley EJ: Radioimmunoassay and some properties of human antibodies to hepatitis B core antigen. **J Gen Virology 38:** 549-559, 1978.

10. Mushahwar IK, Dienstag JL, Polesky HF, McGrath LC, Decker RH, Overby LR: Interpretation of various serological profiles of hepatitis B virus infection. **Am J Clin Pathol 76:** 773-777, 1981.

11. Gerlich WL, Luer W and Thomssen R: Diagnosis of acute and inapparent hepatitis B virus infections by measurement of IgM antibody to hepatitis B core antigen. **J Infect Dis 142:** 95-101, 1980.

12. Dormeyer HH, Arnold W, Schonborn H, Hess G, Knolle J, Meyer zum Buschenfelde KH: Follow-up of anti-HBc titers in healthy HBsAg carriers and patients with chronic inflammatory liver diseases. **Digestion 22:** 289-293, 1981.

13. Lemon SM, Gates NL, Simms TE, and Bancroft WH: IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. **J Infect Dis 143:** 803-809, 1981.

14. Slade BA, Vroon DH: Anti-HBc to screen for susceptibility to hepatitis B. **Lancet 1:** 1246-1247, 1984.

15. Gerlich WH, Uy A, Lambrecht F, and Thomssen R: Cutoff levels of immunoglobulin M antibody against viral core antigen for differentiation of acute, chronic and past Hepatitis B virus infections. **J Clin Microbiol 24:** 288-293, 1986.

16. Nakajima E, Tsuji T, Kachi K, Kagawa K, Okanoue T, and Takino T, Yamada A, Imanishi J: Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) as a marker of interferon therapy in patients with persistent hepatitis B virus infection. **Biken J 30:** 17-23, 1987.

17. Lemon SM: What is the role of testing for IgM antibody to core antigen of Hepatitis B virus? **Mayo Clin Proc 63:** 201-204, 1988.

18. Hu K-Q: Occult hepatitis B infection and its clinical implications. **J Viral Hepatitis 9:**243-257. 2002.

19. Bos ES, van der Doelen AA, van Rooy N, Schuurs AHWM: 3,3',5,5' - tetramethylbenzidine as an ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. **J Immunoassay 2:** 187-204, 1981.

20. Garner RC, Walpole AL, Rose FL: Testing of some benzidine analogues for microsomal activation to bacterial mutagens. **Cancer Letters 1:** 39-42, 1975.

21. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.

22. US Department of Health and Human Services. **Biosafety in Microbiological and Biomedical Laboratories**. 4th ed. Washington, DC: US Government Printing Office, 93-8395. May 1999.

23. World Health Organization. **Laboratory Biosafety Manual**. 3rd ed. Geneva: World Health Organization, 2004.

24. Clinical and Laboratory Standards Institute. **Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline**—Third Edition. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

25. Bond WW, Favero MS, Petersen NJ, Ebert JW: Inactivation of hepatitis B virus by intermediate-

to-high-level disinfectant chemicals. **J Clin Microbiol 18:** 535-538, 1983.

26. U.S. Environmental Protection Agency, Office of Solid Waste: **EPA Guide for Infectious Waste Management,** Washington D.C., 1986. (USG PO 530-SW-86-014).

27. Sehulster LM, Hollinger FB, Dreesman GR, Melnick JL: Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. **Appl and Envi Microbiol 42:** 762-767, 1981.

BIO-RAD *Bio-Rad Laboratories*

Redmond, WA 98052, U.S.A.
For Customer Orders or Technical Service call:
1-800-2BIORAD (1-800-224-6723)

April 2007
506089