

MONOLISA™ Anti-HBc IgM EIA**26174**

Enzyme Immunoassay (EIA) for the Detection of IgM
Antibody to Hepatitis B Core Antigen (anti-HBc IgM) in
Human Serum or Plasma

For *In Vitro* Diagnostic Use

MONOLISA™ Anti-HBc IgM EIA

192 Tests

For Reference Use Only

Lexicon

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

For Reference Use Only

CONTENTS

- 1 - NAME AND INTENDED USE**
- 2 - SUMMARY AND EXPLANATION OF THE TEST**
- 3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE**
- 4 - REAGENTS**
- 5 - WARNINGS**
- 6 - PRECAUTIONS FOR USERS**
- 7 - REAGENT PREPARATION AND STORAGE**
- 8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE**
- 9 - MONOLISA™ ANTI-HBc IgM EIA PROCEDURE**
- 10- SPECTROPHOTOMETRIC VERIFICATION OF REAGENT PIPETTING**
- 11- QUALITY CONTROL - VALIDATION OF RESULTS**
- 12- INTERPRETATION OF RESULTS**
- 13- LIMITATIONS**
- 14- PERFORMANCE CHARACTERISTICS**
- 15- REFERENCES**

1 - NAME AND INTENDED USE

The MONOLISA™ Anti-HBc IgM EIA is an enzyme immunoassay intended for use in the qualitative detection of IgM antibodies to hepatitis B core antigen (anti-HBc IgM) in human serum or 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 chronically 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 per-mucosal 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 impor-

tant 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), followed by anti-HBe and finally anti-HBs, which marks the immune stage.⁷⁻¹⁰ Anti-HBc IgM antibodies are the initial response to infection and remain detectable for several weeks to months, 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.¹¹⁻¹⁶

Patients with chronic hepatitis B virus infection usually show high levels of anti-HBc and low or no detectable levels of anti-HBc IgM. The presence of anti-HBc IgM is an indication of active replication of HBV, and as such, it is a useful marker of patient prognosis and the efficacy of therapeutic intervention.¹⁷⁻¹⁹

3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The MONOLISA™ Anti-HBc IgM EIA is an enzyme immunoassay (IgM antibody capture format) for the detection of IgM antibodies to hepatitis B core antigen. In the assay procedure, patient specimens and controls are incubated with anti-human IgM antibodies coated on the microwells. If IgM antibodies to HBc are present in a specimen or control, they bind to the antibody. Excess sample is removed by a wash step. The conjugate is then added to the microwells and allowed to incubate. The conjugate binds to any antibody-IgM antibody complexes (specific for HBc) that are present in the microwells. Excess conjugate is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HBc IgM, the bound enzyme (HRP) causes the colorless tetramethyl-

benzidine (TMB) in the chromogen solution to change to blue. The blue color turns yellow after the addition of a stopping solution. If a sample does not contain anti-HBc IgM, the chromogen/substrate solution in the well remains colorless during the substrate incubation, and after addition of the stopping solution. The color intensity, measured spectrophotometrically, is proportional to the amount of anti-HBc IgM present in the specimen. Absorbance value readings for patient specimens are compared to a cutoff value.

4 - REAGENTS

MONOLISA™ Anti-HBc IgM EIA Product Description Product No. 26174 (192 test kit)

Component	Contents	Preparation
R1• Anti-HBc IgM Microwell Strip Plates (2)	<ul style="list-style-type: none"> • Microwell strips in holder, coated with goat anti-human IgM • Tabs are labeled "HH" • ProClin® (trace) 	Use as supplied. Return unused strips to the pouch. Do not remove desiccant.
R2• Wash Solution Concentrate (30X) 1 bottle (120 mL)	<ul style="list-style-type: none"> • Sodium Chloride • Tween 20 	Dilute 1:30 with deionized water. Clinical laboratory reagent water Type I or Type II is acceptable.
R3• Specimen Diluent 4 bottles (30 mL)	<ul style="list-style-type: none"> • Bovine serum albumin • Buffer with protein stabilizers • ProClin® 300, 0.5% • Red dye 	Use as supplied.
C0• Anti-HBc IgM Negative Control 1 vial (0.8 mL)	<ul style="list-style-type: none"> • 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 IgM Positive Control 1 vial (1.6 mL)	<ul style="list-style-type: none"> • Prediluted human serum; prepared from infectious material positive for anti-HBc IgM and HBsAg, and negative for HIV and HCV antibodies • ProClin® 300, 0.5% • Red dye 	Use as supplied.
C2• Anti-HBc IgM Cutoff Calibrator 1 vial (1.5 mL)	<ul style="list-style-type: none"> • 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.

Component	Contents	Preparation
R4• Anti-HBc IgM Conjugate 4 vials (6 mL)	<ul style="list-style-type: none"> • HBc recombinant conjugated to HRP, Lyophilized • ProClin® 300, 0.1% 	Rehydrate in Conjugate Diluent as described.
R5• Anti-HBc IgM Conjugate Diluent 1 bottle (24 mL)	<ul style="list-style-type: none"> • Buffer with protein stabilizers • ProClin® 300, 0.5% • < 0.001% Thimerosal • Green dye 	Ready to use as described under Working Conjugate Solution.
R8• Substrate Buffer 1 bottle (120 mL)	<ul style="list-style-type: none"> • Hydrogen Peroxide • Citric Acid/Sodium Acetate buffer • Dimethylsulfoxide (DMSO) 	Use as supplied.
R9• Chromogen (11X) 1 bottle (12 mL)	<ul style="list-style-type: none"> • Tetramethylbenzidine (TMB)* 	Dilute with Substrate Buffer as described.
R10• Stopping Solution 1 bottle (120 mL)	<ul style="list-style-type: none"> • 1N H₂SO₄ (Sulfuric Acid) 	Use as supplied.
Plate Sealers	<ul style="list-style-type: none"> • Clear plastic sealers 	Use as supplied.

*NOTE: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.^{20,21}

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.²² Biosafety Level 2²³ or other appropriate biosafety practices^{24,25} should be used for materials that contain or are suspected of containing infectious agents. The following human blood derivatives are found in this kit:
 - a. 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).

- b. Human source material used in the preparation of the Positive Control (C1) has been treated to reduce the potential for HBV infectivity. It is positive for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen. 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
- a. ProClin® 300 (0.5% or 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.
 - b. 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.
 - c. The 1.0 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 Iso-propanol, an iodophor [such as 0.5% Wescodyne™ Plus], or a phenolic, etc.) and wiped dry.²⁶⁻²⁸

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 IgM EIA is intended for the detection of **IgM antibodies** to hepatitis B core antigen. The tabs at the end of the microwell strips are labeled with the product code “HH”.

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 GS rLAV HIV-1 EIA and Bio-Rad GS 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 IgM EIA Procedure and the Interpretation of Results must be followed when testing serum or plasma specimens for the presence of IgM 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 Conjugate Solution (R4 + R5)

Note: The lyophilized Conjugate is supplied under vacuum. Do not open Conjugate vials until ready to reconstitute.

Bring Conjugate Diluent (R5) to room temperature. Rehydrate the contents of one vial of lyophilized Conjugate (R4) with 6 mL of Conjugate Diluent (R5). Wait 2 minutes, and then mix. Note Conjugate lot number, date and time of preparation, and date and time of expiration of the Working Conjugate Solution. Working Conjugate Solution is stable for 8 hours at room temperature, and for 1 month if stored at 2-8°C. Always mix Working Solution just prior to use.

Return unused Working Conjugate Solution to the refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes. One vial of lyophilized Conjugate diluted with Conjugate Diluent is enough Working Conjugate Solution for one to six strips. When more strips are used, reconstitute additional vials.

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 (μ L)	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, EDTA, sodium and lithium heparin, ACD, and sodium citrate. 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 2-8°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 IgM EIA PROCEDURE

Materials Provided

See REAGENTS section on pages 6-7.

Materials required but not provided

1. Precision pipettes to deliver volumes from 10 μ L to 220 μ L, 1 mL, 5 mL, and 10 mL (accurate within \pm 10%). A multichannel pipettor capable of delivering 100 μ L is optional.
2. Pipette tips.
3. Appropriately sized graduated cylinders.
4. Dry-heat incubator capable of maintaining $37 \pm 2^\circ\text{C}$.
5. Bio-Rad microwell plate or strip washer, or equivalent. The washer must be capable of dispensing 375 μ L per well, cycling 5 times, and soaking for 30-60 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: \pm (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 Negative Control and Cutoff Calibrator are diluted 1:101 when performing the assay. **The Positive Control is supplied at working strength and does not need to be diluted.**
4. The procedure specifies the addition of 100 μ L volumes of Working Conjugate Solution, Working TMB Solution, and Stopping Solution while performing the assay.
5. Do not splash controls, specimens, or reagents between microwells of the plate.

6. Cover plates for each incubation step using plate sealers provided or other appropriate means to minimize evaporation.
7. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working TMB Solution).
8. Adhere to the recommended time constraints for the use of the Working TMB Solution (8 hours, ambient) Working Conjugate Solution (8 hours ambient or 1 month at 2-8°C), and Wash Solution (4 weeks, ambient).
9. Avoid the formation of air bubbles in each microwell.

EIA Procedure

The MONOLISA™ Anti-HBc IgM 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. Prepare Working Conjugate Solution by adding 6 mL of Conjugate Diluent to one vial of lyophilized Conjugate. Wait two minutes. Mix.
5. Remove strips not needed for the assay and replace them with labeled Null Strips, as necessary.
6. If sample identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.
7. **Dilute the Negative Control, Cutoff Calibrator and specimens 1:101 in the Specimen Diluent: Specimens, Negative Control, and Cutoff Calibrator must be prediluted**

1:101 in the Specimen Diluent prior to addition to the well (for example, dilute 5 μL of specimen or Negative Control or Cutoff Calibrator in 0.5 mL of Specimen Diluent, mix gently to avoid foaming, and then transfer **100 μL** to the well.) **Positive Control is supplied at working strength and does not need to be diluted.** Add 100 μL of Positive Control to the appropriate well.

One well of Positive Control, one well of Negative Control, and three wells of Cutoff Calibrator must be assayed on each plate or partial plate of specimens.

8. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 \pm 5 minutes at 37 \pm 2°C.**
9. At the end of the incubation period, carefully remove the plate sealer or cover 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 $\mu\text{L}/\text{well}/\text{wash}$). **Soak each well for 30 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.*

10. **Add 100 L of the Working Conjugate Solution to each well containing a specimen, calibrator, or control.** Avoid bumping plates containing Working Conjugate Solution to prevent contamination of the plate sealer and/or top edges of the wells.

NOTE: *The Working Conjugate Solution is colored green.*

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

11. 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.**
12. 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 30 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.*
13. **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 (18-30°C).** Use of a plate sealer or cover is optional.
14. **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.**
15. 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 REAGENT PIPETTING (OPTIONAL)

Verification of Conjugate Dispense

The Working Conjugate Solution (R4 + R5) is green in color. The presence of Working Conjugate Solution 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 Working 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 IgM EIA is in a defibrinated plasma matrix (e.g., serum). The user should include alternate control material for plasma matrices when necessary.

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 NCCLS 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 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 0.400 AU ($PC \geq 0.400$).
- The absorbance value of the Negative Control must be greater than 0.000 AU and less than the assay cutoff ($0.000 < NC < \text{cutoff}$).

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 ($CC_{\bar{x}}$):

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

<u>Sample Number</u>	<u>Absorbance</u>	<u>Total Absorbance</u>	<u>= 0.192</u>	<u>= 0.064 ($CC_{\bar{x}}$)</u>
1	0.061	3	3	
2	0.068			
3	<u>0.063</u>			
	0.192			

Cutoff Value:

The mean absorbance of the Cutoff Calibrator plus 0.150 is the Cutoff Value for the assay

$$\text{Cutoff Value: } (CC_{\bar{x}}) + 0.150$$

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

$$(CC_{\bar{x}}) + 0.150 = 0.064 + 0.150 = 0.214$$

Borderline: Specimens with antibody levels of 90-110% of the Cutoff Value should be interpreted as borderline, as the specific HBc IgM antibody 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 + 0.150.

Borderline: $0.9 \times \text{Cutoff Value} \leq \text{Borderline} \leq 1.1 \times \text{Cutoff Value}$

For specimens that are borderline the subject can be re-collected 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: $> 1.1 \times \text{Cutoff Value}$

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: $< 0.9 \times \text{Cutoff Value}$

Example:

Positive Control OD Value	1.127		Valid
Negative Control OD value	0.045		Valid
Cutoff Calibrator OD value	0.061 0.068 0.063		Valid
Cutoff	0.214 [<i>Cutoff Calibrator mean</i> (0.064) + 0.150]		
Borderline OD zone	0.193 – 0.235 [<i>Cutoff Calibrator (CC\bar{x})</i> \pm 10%]		
Specimen OD values	1.786 0.190 0.029 0.235	<i>Interpretation:</i>	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 IgM 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. These patients may be at the beginning or the end of the acute infection. Testing for anti-HBc IgM at two week intervals will distinguish between early acute or a recovering individual. Rapid rise is associated with an early acute infection. Gradual decrease or a steady level of antibody is normally associated with late acute stage of infection.
> 1.1 X Cutoff Value	Reactive	Positive for anti-HBc IgM antibodies. Detection of anti-HBc IgM does not necessarily imply an acute hepatitis B infection due to the longevity of anti-HBc IgM. The detection of anti-HBc IgM can be useful for the differential diagnosis of Hepatitis B from other forms of viral hepatitis. Any diagnosis should take into consideration the patient's clinical history and symptoms, as well as other laboratory data.

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.

13-LIMITATIONS

1. A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus.
2. Results from immunosuppressed patients should be interpreted with caution.
3. A reactive anti-HBc IgM result does not exclude co-infection by another hepatitis virus.
4. The performance of the MONOLISA™ Anti-HBc IgM 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.

14-PERFORMANCE CHARACTERISTICS

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

Expected Values

A total of 1352 prospective asymptomatic subjects were tested with the MONOLISA™ Anti-HBc IgM EIA. All subjects (100%) were at high risk for viral hepatitis including intravenous drug users (N = 461), homosexual males (N = 143), sex workers (N = 172), prison history (N = 340), high risk sex partners (N = 165), high risk occupation/health care workers (N = 75), hemodialysis (N = 55), hemophiliacs (N = 3), and other (N = 481). Many had more than 1 high-risk behavior or risk factor. One hundred sev-

enty-four (174, 12.9%) of these high-risk subjects also reported having received a full course of injections of an HBV vaccine. Subjects in the asymptomatic prospective population were from the following geographic locations: 459 from Los Angeles, CA, (34.0%); 57 from Santa Ana, CA (4.2%); 72 from Miami, FL (5.3%); 344 from Cocoa, FL (25.4%); 254 from San Francisco, CA (18.8%); and 166 from Seattle, WA (12.3%). The group was Caucasian (36.6%), Black or African American (41.1%), Hispanic or Latino (13.2%), Asian (4.3%), Native Hawaiian or other Pacific Islander (0.7%), and American Indian or Alaska Native (2.3%), with the remaining 1.9% represented by multiple ethnic groups or unknown ethnicity. The subjects were male (70.2%) and female (29.8%) and ranged in age from 18 to 81 years.

The MONOLISA™ Anti-HBc IgM EIA results for the asymptomatic prospective population (N = 1352) are presented in Table 1 by gender and age range.

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

Age Range	Gender	MONOLISA™ Anti-HBc IgM EIA Result						Total
		Reactive		Borderline		Non-reactive		
		N	%	N	%	N	%	N
10-19	F	0	0.0%	0	0.0%	6	100.0%	6
	M	0	0.0%	1	10.0%	9	90.0%	10
20-29	F	0	0.0%	1	1.0%	103	99.0%	104
	M	8	6.6%	1	0.8%	113	92.6%	122
30-39	F	6	5.6%	2	1.9%	100	92.6%	108
	M	4	1.9%	4	1.9%	204	96.2%	212
40-49	F	3	2.8%	3	2.8%	101	94.4%	107
	M	12	3.4%	9	2.6%	327	94.0%	348
50-59	F	0	0.0%	1	1.6%	61	98.4%	62
	M	10	4.8%	6	2.9%	194	92.4%	210
60-69	F	0	0.0%	0	0.0%	11	100.0%	11
	M	2	5.4%	0	0.0%	35	94.6%	37
70-79	F	0	0.0%	0	0.0%	2	100.0%	2
	M	0	0.0%	0	0.0%	5	100.0%	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	0	0.0%	0	0.0%	3	100.0%	3
	M	0	0.0%	0	0.0%	4	100.0%	4
Totals		45	3.3%	28	2.1%	1279	94.6%	1352

Reference Markers

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

MONOLISA™ Anti-HBc IgM EIA was assessed relative to the reference anti-HBc IgM result and to HBV classifications.

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 33 unique reference marker patterns observed in the MONOLISA™ Anti-HBc IgM EIA clinical study across the three clinical sites.

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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	+	+	+	+	-	-
	+	+		+	-	-
	+	+	-	-	-	-
	+	-	+	-	-	+
	+	-	+	-	-	-
	+	-	-	-	-	-
Chronic infection	+	+	+	+	-	+
	+	+	-	+	-	-
	+	+	-	+	-	+
	+	+	-	+	+	+
	+	+	-	+	+	-
	+	-	-	+	+	-
	+	-	-	+	+	+
Early recovery	-	-	+	+	+	+
	-	-	+	+	+	-
	-	-	-	+	-	-
	-	-	-	+	+	-
	-	-	-	+		-
	-	-		+	+	+
HBV vaccine response	-	-	-	-	-	+
HBV vaccine response status indeterminate	-	-	-	-	-	
Not previously infected with HBV	-	-	-	-	-	-
Recovered	-	-	-	+		
	-	-	-	+	-	
Recovered or Immune due to natural infection	-	-	-	+	-	+
Recovery	-	-	-	-	+	+
	-	-	-	+		+
	-	-	-	+	+	
	-	-	-	+	+	+
Uninterpretable	+	-	-	-	+	-
	-	+	-	-	-	+
	-	+	-	-	-	-
	-	-	-	-	+	-

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

Comparison of Results by Specimen Classification

Table 3 compares the MONOLISA™ Anti-HBc IgM EIA results with the previously determined anti-HBc IgM reference assay results for each specimen classification. The data in the table are representative of the number of specimens in each result category.

**Table 3 - FDA HBV Classification of High Risk Prospective Samples
MONOLISA™ Anti-HBc IgM EIA versus FDA Approved Anti-HBc IgM EIA**

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

¹ GRZ = Gray Zone Reactive; ² BRD = Borderline

Percent Agreement

The percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the reference anti-HBc IgM assays for each specimen classification was determined, including the upper and lower 95% Wilson confidence bounds. The percent agreement between the MONOLISA™ Anti-HBc IgM EIA and the anti-HBc IgM reference

assay for the prospective population is presented in Table 4 for each HBV classification.

**Table 4 - Percent Agreement
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA**

HBV Classification	N ¹ =	Positive Percent Agreement ²		95% Confidence Interval	Negative Percent Agreement ³		95% Confidence Interval
Acute Infection	14	(7/7)	100.0%	64.5%, 100%	(6/7)	85.7%	48.6%, 97.4%
Chronic Infection	79	(1/1)	100.0%	20.6%, 100%	(75/78)	96.2%	89.3%, 98.7%
Early recovery	106	(3/4)	75.0%	30%, 95.4%	(91/101)	90.1%	82.7%, 94.5%
HBV vaccine response	309	(0/0)	NA	NA	(300/ 309)	97.1%	94.6%, 98.5%
HBV vaccine response status indeterminate	31	(0/0)	NA	NA	(31/31)	100.0%	89%, 100%
Not previously infected with HBV	606	(0/0)	NA	NA	(596/ 606)	98.3%	97%, 99.1%
Recovered	12	(0/0)	NA	NA	(11/12)	91.7%	64.6%, 98.5%
Recovered or Immune due to natural infection	92	(0/0)	NA	NA	(83/92)	90.2%	82.4%, 94.8%
Recovery	173	(0/0)	NA	NA	(149/ 173)	86.1%	80.2%, 90.5%
Uninterpretable	8	(0/0)	NA	NA	(8/8)	100.0%	67.5%, 100%
Total	1430	(11/12)	91.7%	64.6%, 98.5%	(1350/ 1417)	95.3%	94%, 96.3%

- ¹ N = Total number of samples; refer to Table 3 for correlation of borderline samples. The one specimen that was indeterminate by both assays was not included in the percent agreement calculations. Positive or negative results from the MONOLISA™ Anti-HBc IgM EIA were considered as non-agreements in the calculation of percent positive agreement and percent negative agreement when the corresponding reference assay result was indeterminate/ borderline.
- ² Compares number of samples positive on both assays to sum of all positive samples on the reference assay + samples indeterminate on the reference assay and negative on MONOLISA™ Anti-HBc IgM EIA.
- ³ Compares number of samples negative on both assays to sum of all negative samples on the reference assay + samples indeterminate on the reference assay and positive on MONOLISA™ Anti-HBc IgM EIA.

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

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

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

Seroconversion Panels

The comparative sensitivity of the MONOLISA™ Anti-HBc IgM EIA was determined by testing 6 commercially available Anti-HBV seroconversion panels and comparing the results to those in the associated certificates of analysis. Comparative results for panel members near the point of seroconversion are presented in Table 5.

Table 5 - HBV Seroconversion Panel Results

Panel ID	Day since 1 st bleed	# Members	MONOLISA™ Anti-HBc IgM		Reference anti-HBc IgM test
			S/CO	Result	Result
PHM935A-11	35	18	0.23	NR	NR
PHM935A-12	50		0.22	NR	NR
PHM935A-13	66		1.83	R	NR
PHM935A-14	68		3.02	R	R
RP009-06	31	20	0.45	NR	NR
RP009-07	36		2.20	R	NR
RP009-08	43		5.46	R	R
RP009-09	56		5.76	R	R
RP016-06	25	20	0.27	NR	NR
RP016-07	57		0.67	NR	NR
RP016-08	60		3.90	R	R
RP016-09	74		5.34	R	R
RP017-13	65	30	0.37	NR	NR
RP017-14	71		0.54	NR	NR
RP017-15	76		1.14	R	NR
RP017-16	78		2.26	R	R
RP017-27	179		1.94	R	R
RP017-28	181		1.72	R	NR
RP017-29	186		1.76	R	NR
RP017-30	188	1.51	R	NR	
6278-08	26	11	0.28	NR	NR
6278-09	33		0.55	NR	NR
6278-10	37		0.98	BRD	NR
6278-11	41		3.97	R	R
6281-08	36	12	0.29	NR	NR
6281-09	41		1.30	R	NR
6281-10	43		3.41	R	R
6281-11	50		5.02	R	R

In 4 of the 6 (66%) seroconversion panels, the MONOLISA™ Anti-HBc IgM EIA detected reactive levels of hepatitis B core IgM antibody 1 member before the reference anti-HBc IgM test. In 2 of the 6 (33%) seroconversion panels, the MONOLISA™ Anti-HBc IgM

EIA detected reactive levels of hepatitis B core IgM antibody at the same member as the reference anti-HBc IgM test. MONOLISA™ Anti-HBc IgM EIA appears to detect IgM for a longer period than the reference assay for qualitative determination of IgM antibody to Hepatitis B core antibody.

Clinical Performance with Acute HBV Samples

Retrospective acute HBV samples from 85 individuals were tested with the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All testing was according to the protocol and the manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 6.

**Table 6 - Acute HBV Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA**

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

*All of these samples were reactive on a reference assay for Anti-HBc total antibodies (IgG and IgM)

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

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

Retrospective chronic HBV samples (HBsAg positive for more than 6 months) from 120 individuals were tested with the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All

testing was according to the protocol and the manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 7.

**Table 7 - Chronic HBV Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA**

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

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

Clinical Performance with Pre-HBV Vaccination Samples

Pre-vaccine samples from 38 individuals were tested on one lot of the MONOLISA™ Anti-HBc IgM EIA and a reference anti-HBc IgM EIA. All testing was according to the protocol and the manufacturer's package insert instructions. The results of the MONOLISA™ Anti-HBc IgM EIA are compared to results of the reference anti-HBc IgM method in Table 8.

Table 8 - Pre-Vaccination Sample Results
MONOLISA™ Anti-HBc IgM EIA versus Reference Anti-HBc IgM EIA

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

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

Potentially Cross-reactive Medical Conditions

The specificity of the MONOLISA™ Anti-HBc IgM EIA assay was evaluated by testing 357 characterized serum and plasma samples from 22 potentially cross-reacting sub-groups. Each sample was tested once on the MONOLISA™ Anti-HBc IgM EIA. Any sample that was reactive on the MONOLISA™ Anti-HBc IgM EIA was also tested on a reference Anti-HBc IgM assay. A summary of the results is given in Table 9.

Table 9 - Potentially Cross-Reactive Medical Conditions

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

Of the 357 samples from 22 unrelated medical conditions tested, 342/357 (95.8%) were non-reactive on the MONOLISA™ Anti-HBc IgM EIA. Of the 9 samples that were reactive, 2/9 (22.2%) were also positive on the reference Anti-HBc IgM assay. Of the 7 samples that were negative on the reference Anti-HBc IgM and reactive on the MONOLISA™ Anti-HBc IgM EIA, 2 were from the Anti-HBc positive/Anti-HBc IgM negative group, 1 was CMV IgM

positive, 1 was HAV positive, 1 was HIV-1 positive, 1 was HIV-2 positive and 1 was RF positive. Six (6) samples (1 CMV IgM, 3 Rheumatoid factor, 1 Rubella IgM and 1 Toxoplasmosis positive) were borderline on the MONOLISA™ Anti-HBc IgM EIA.

Potentially Interfering Substances

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

Hemolyzed: 500 mg/dL of hemoglobin
Lipemic: 500 mg/dL of triglycerides
Icteric: 20 mg/dL of bilirubin
Proteinemic: 11 g/dL of protein

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 non-reactive 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	40% (20/50)	50% (25/50)	10% (5/50)
ACD	40% (20/50)	44% (22/50)	16% (8/50)
Potassium EDTA	66% (33/50)	18% (9/50)	16% (8/50)
Lithium Heparin	64% (32/50)	26% (13/50)	10% (5/50)
Sodium Heparin	54% (27/50)	36% (18/50)	10% (5/50)

Reproducibility

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

combined to obtain standard deviation (SD) and percent coefficient of variation (CV) for within run, between run, and total variance, as summarized in Tables 10 & 11. The data were analyzed according to the principles described in CLSI EP5-A2 and ISO/TR 22971:2005.

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Table 10 - MONOLISA™ Anti-HBc IgM EIA Reproducibility Results by Panel Member Signal to Cutoff (S/CO)

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

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

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

³ Total variability of the assay performance includes within run, between run and between lot.

Table 11 - Summary of MONOLISA™ Anti-HBc IgM EIA Reproducibility Results (Positive, Low Positive, and Negative) by Panel Member S/CO

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

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

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

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

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

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

Precision

A precision study was performed with the MONOLISA™ Anti-HBc IgM EIA using panels prepared in serum, EDTA plasma, and sodium heparin. The 10 specimens were tested in triplicate, twice a day, for 20 days, on one lot, and results are summarized in Table 12.

Table 12 - MONOLISA™ Anti-HBc IgM EIA 20-Day Precision Results in S/CO

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

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

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

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

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

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

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