ETI-CORE-IGMK PLUS

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

Instruction Manual

Catalog No.: P001928

Manufactured By:

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Saluggia, Italy

Distributed By:

DiaSorin Inc.
Stillwater, MN, USA

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

Pay attention to changes!

1. INTENDED USE

ETI-CORE-IGMK PLUS is an *in vitro* enzyme immunoassay (EIA) intended for use in the qualitative determination of IgM antibody to hepatitis B core antigen (IgM anti-HBc) in human serum or plasma (EDTA, citrate or heparin). The ETI-CORE-IGMK PLUS is intended for manual use and with the Biochem Immunosystems Labotech/ETI-LAB automated instrument.

The presence of IgM anti-HBc, in the presence of total antibody to HBcAg (anti-HBc), is indicative of a laboratory diagnosis for acute infection. The absence of IgM anti-HBc, in the presence of total anti-HBc, is indicative of a laboratory diagnosis for recovery from HBV infection. Further HBV serological marker testing is required to define the specific disease state. The ETI-CORE-IGMK PLUS assay's performance has not been established for the monitoring of HBV disease or therapy. This assay has not been FDA-approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established when the ETI-CORE-IGMK PLUS IgM anti-HBc assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. **Users are responsible for establishing their own performance characteristics**.

The performance characteristics of this assay have not been established for newborn testing.

2. SUMMARY AND EXPLANATION OF THE TEST

Historically, the use of antibodies to hepatitis B core antigen (anti-HBc) to diagnose acute hepatitis B virus (HBV) infection was based on the demonstration of a fourfold or greater rise in total anti-HBc titer in paired sera collected four weeks apart. Early anti-HBc assays were based on the principle of immune adherence hemagglutination; these were largely supplanted by radioimmunoassay (RIA) and enzyme immunoassay (EIA) methodologies, which were more sensitive, reproducible, and practical. Immunoglobulin M (IgM) antibody-specific molecules were originally detected either by immunosubtraction of immunoglobulin G (IgG) in the sample prior to testing or by using anti-human IgM conjugates. The latter procedure, however, can be affected by the presence of rheumatoid factor or IgG in the sample. These procedures have been replaced by the IgM-capture immunosorbent assays (RIA and ELISA). In these assays, anti-human IgM (μ chain-specific), bound to the solid phase, is first used to capture IgM in the sample. Detection of specific IgM is then accomplished by the addition of assay-specific antigen and enzyme-labeled anti-HBc conjugate. Because interference from rheumatoid factor or specific IgG antibodies is minimized, the IgM-capture assays usually have better analytical sensitivity and specificity.

In acute hepatitis B infection, anti-HBc is detectable in the serum shortly before clinical symptoms appear (1) and slightly after the occurrence of hepatitis B surface antigen (HBsAg). In cases in which hepatitis B infection resolves, anti-HBc is also detectable during the window period following loss of HBsAg and prior to the development of antibody to HBsAg (anti-HBs) (1). In cases of asymptomatic or subclinical hepatitis B, anti-HBc detectability follows the same pattern as in acute symptomatic infection. IgG anti-HBc antibody will develop shortly after the onset of hepatitis B infection and will persist over time in all patients who have previously been infected with hepatitis B, regardless of the outcome of their infection. However, during the prodromal, acute and early convalescent phases of hepatitis B infection, anti-HBc exists primarily as IgM antibody. IgM antibody diminishes and disappears over time (usually in approximately six months). Thus, IgM anti-HBc is present in high titers during the acute phase of hepatitis B infection and therefore can be helpful when used in conjunction with HBsAg testing to differentiate between primary acute HBV infection and early convalescence (2-4). When determining the stage of disease

caused by HBV, an IgM anti-HBc test result enables clinicians to discriminate between HBsAg-positive patients whose acute hepatitis is due to HBV infection and those whose acute hepatitis is due to other causes (5). A positive test result for IgM anti-HBc is also of value in diagnosing fulminant HBV infections in patients negative for HBsAg (5).

In patients with chronic hepatitis B infection or an asymptomatic chronic carrier state (6), HBsAg appears during the incubation phase of the disease and may persist for years and possibly for life (1, 7). Total anti-HBc also appears during this early phase, rises in titer, and persists over time; the highest titers of total anti-HBc are found in the chronic HBsAg carrier state (1, 7, 8). Thus, in chronic infection, total anti-HBc will be detectable in association with other hepatitis B serological markers. Because low titers of IgM anti-HBc can be either absent or present in chronic HBV carriers, IgM anti-HBc assessment is not definitive in determining the stage of chronic disease, i.e. chronic active or chronic persistent hepatitis B (3, 5). However, when present, these low titers of IgM anti-HBc are more likely to be found in patients with chronic HBV-induced liver disease as evidenced by intrahepatic HBcAg expression and liver enzyme elevations (elevated ALT and AST levels), due to HBV reactivation and induced hepatocyte damage (3, 4).

3. PRINCIPLE OF THE PROCEDURE

ETI-CORE-IGMK PLUS uses a monoclonal antibody to human IgM as the basis for this enzyme immunoassay. The assay is an antibody-capture, non-competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibody (IgG_1, κ class) to human IgM. An enzyme tracer containing horseradish peroxidase-labeled human antibodies to HBcAg detects any captured IgM anti-HBc via recombinant HBcAg.

In the assay procedure, patient specimens and controls are incubated in antibody-coated microwells. If IgM antibodies are present in a specimen or control, they bind to the antibody. Excess sample is removed by a wash step, and a solution of recombinant hepatitis B core antigen (HBcAg) and the enzyme tracer are then added to the microwells and allowed to incubate. The presence of IgM anti-HBc enables the HBcAg and the enzyme tracer to bind to the solid phase: enzyme activity is therefore indicative of IgM anti-HBc present in the specimen or control. Excess HBcAg and enzyme tracer are removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains IgM anti-HBc, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color (650 nm). The blue color turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain IgM anti-HBc, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is indicative of the presence of IgM anti-HBc. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean absorbance of the calibrator.

4. REAGENTS AND OTHER MATERIALS PROVIDED

Catalog Number	Product Description	Quantity/Volume
P001928	**ETI-CORE-IGMK PLUS**	96 tests
	Coated Strips Microwells coated with mouse monoclonal antibody directed to human IgM (IgG_1, κ class).	Twelve 8-well strips (contained in one pouch)
	Enzyme Tracer (Human) Horseradish peroxidase-labeled human Fab to HBcAg, buffer, protein stabilizers. Preservative: 0.2% ProClin 300.	0.5 mL
	Tracer Diluent Recombinant HBcAg (22 kd, expressed in *E. coli*), buffer, human serum/plasma, protein stabilizers. Preservative: 0.2% ProClin 300.	14.7 mL
	Calibrator (Human) Human serum/plasma non-reactive for IgM anti-HBc, diluted 1:4000 with buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	3.3 mL
	Negative Control (Human) Human serum/plasma non-reactive for IgM anti-HBc, diluted 1:4000 with buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	3.3 mL
	Positive Control (Human) Human serum/plasma reactive for IgM anti-HBc, diluted 1:4000 with buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	2.5 mL
	Sample Diluent Buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	Two 60-mL bottles
	Wash Buffer (concentrate)* Buffer, detergents, preservatives.	40 mL
	Chromogen/Substrate* Tetramethylbenzidine/hydrogen peroxide system.	16 mL
	Stop Solution* 0.4N sulfuric acid. Caution: irritant.	30 mL
	Strip Sealers	24
	Plate Sealers	2
	Pouch Sealer	1

* *All lots of wash buffer concentrate, chromogen/substrate and stop solution are interchangeable between assay kits.*

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- The human blood source material used to prepare this product (i.e., enzyme tracer, calibrator, negative control) derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 (AIDS) when tested by licensed screening tests, and found to be non-reactive for syphilis when tested by a serological test. The positive control is prepared from human blood source material reactive for HBsAg and non-reactive for antibodies to HCV, HIV-1 and HIV-2 when tested by licensed screening tests, and found to be non-reactive for syphilis when tested by a serological test. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 2007, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections (9, 10, 11).
- All specimens, reagents, and controls should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures:
  - Do not pipette by mouth.
  - Do not eat, drink, smoke, or apply cosmetics in areas where specimens are handled.
  - Wear protective clothing such as lab coats, protective glasses, and disposable gloves when handling specimens and assay reagents. Wash hands thoroughly afterwards.
  - Perform all work with infectious materials in a designated area.
- Dispose of all specimens and used assay materials as if capable of transmitting disease:
  - Decontaminate liquid wastes, including those containing neutralized acid, either:
    (a) by autoclaving for 60 minutes at 121°C; or
    (b) by treating with 1:10 or 1:100 dilution of household bleach (sodium hypochlorite concentration approximately 5%). The wastes should remain in contact with the sodium hypochlorite solution for 30 minutes for effective decontamination, after which they can be disposed of in the sink (9, 11). Do not autoclave solutions containing sodium hypochlorite.
  - Autoclave non-ignitable solids for 60 minutes at 121°C.
  - Incinerate disposable ignitable materials.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Sample storage time at 2-8°C varies according to the specific DiaSorin hepatitis B assay. Some samples may not be suitable for use in all assays depending on their storage time at 2-8°C.
- Refer to the Specimen Collection and Preparation section of each DiaSorin assay's Instructions for Use to determine the appropriate sample storage time at 2-8°C, particularly when the sample will be tested by more than one DiaSorin hepatitis B assay.
- Prior to testing any samples, determine if the samples are appropriately stored for the assay(s) you are using. Testing a sample with more than one DiaSorin hepatitis B assay may not be feasible if the sample's storage time does not match the individual assays' recommendations.
- Use only dispensing equipment that has been calibrated to deliver accurate volumes, per the laboratory's standard procedures.
- **WARNING - Chromogen/substrate and the stop solution contain ingredients that can irritate skin and cause eye damage. Handle them with care. Avoid getting them in eyes or on skin or clothing. In case of contact with skin or eyes, immediately flush the affected area with water for 15 minutes. For eyes, obtain medical attention.**

Reagents containing ProClin 300 may cause allergic reactions. Avoid prolonged contact with skin. Wash thoroughly after handling.

6. REAGENT PREPARATION

- Bring reagents to room temperature (20-25°C).
- The coated strips, calibrator, negative and positive controls, sample diluent, chromogen/substrate and stop solution are provided ready to use.
  Note *- Use clean, plastic containers or acid-washed glassware for preparing the following solutions. A clean, dedicated dispenser is recommended for the working enzyme tracer to avoid contamination.*
- **Working enzyme tracer**. Bring reagents to room temperature (20-25°C). To prepare the working enzyme tracer, dilute the enzyme tracer 1:50 with tracer diluent (see chart below). After dilution, the working enzyme tracer can be used for one week if stored at 2-8°C.
  Caution *- Verify that the total volume prepared is sufficient for the number of tests included in the run. Use a clean container for each dilution and label the container with the reagent name, lot number of kit, lot number of reagent, plus the date of preparation and date of expiration of the working enzyme tracer.*

Number of Strips	Enzyme Tracer (µL)	Tracer Diluent (µL)	Total Volume (mL)
2	48	2352	2.4
4	80	3920	4.0
6	112	5488	5.6
8	144	7056	7.2
10	176	8624	8.8
12	208	10192	10.4

Note *- Sufficient reagents are provided to allow for six runs per kit.*

- **Wash solution**. To prepare the working wash buffer, dilute the wash buffer concentrate (40 mL) to 1000 mL (1.0 L) with distilled or deionized water. If crystallization has occurred at 2-8°C, warm the wash buffer concentrate to 37°C and mix well before diluting. Water used for wash buffer dilution should be stored in a clean, non-metallic container to prevent contamination with peroxidase-inactivating substances. Record on the storage vial the expiration date and date of preparation of the working wash buffer. The working wash buffer can be stored for one week at 2-8°C.
  Smaller volume users may prepare less than 1 L of working wash buffer at a time if desired. If diluting only a portion of the wash buffer concentrate, check concentrate for crystallization. If crystallization has occurred during storage, warm the wash buffer concentrate to 37°C and mix well to eliminate crystals before removing aliquot for dilution.
  Note *- All lots of wash buffer concentrate are interchangeable between assay kits.*
- Working wash buffer containers should be thoroughly cleaned with 70% ethanol and thoroughly rinsed with distilled or deionized water before preparation of the next batch of working wash buffer.

7. REAGENT STORAGE AND HANDLING INSTRUCTIONS

- Store the test components in the refrigerator at 2-8°C away from intense light. Allow them to reach room temperature (20-25°C) before use. Return the test components to the refrigerator after use.
- Do not expose the test components to intense light, direct sunlight, or temperatures above 25°C. Do not freeze the kit.
- When stored as directed, test components will remain stable until expiration dates printed on their labels.
- Keep unused coated strips sealed in their pouches until time for use. Allow the pouch to reach room temperature (20-25°C) before opening it. Return any unused strips to the pouch as soon as possible; seal the pouch with the pouch sealer and refrigerate pouch at 2-8°C.

- After dilution, the working enzyme tracer can be stored for one week at 2-8°C.
- After dilution, the working wash buffer can be stored for one week at 2-8°C.

8. REAGENT INSTABILITY OR DETERIORATION

- The chromogen/substrate may have a slightly blue tinge. If the chromogen/substrate turns a darker blue, it may have become contaminated and should be discarded.
- Any reagent that contains visible particulate matter should be discarded.

9. SPECIMEN COLLECTION AND PREPARATION

- **This assay is not designed to test body fluids other than human serum or plasma**.
- Specimens containing precipitate may give inconsistent test results. Do not test specimens containing particulate material, or grossly hemolyzed or lipemic specimens.
- The testing of heat-inactivated samples is not recommended.
- There is a specimen dilutional effect with citrated plasma due to the liquid nature of this anticoagulant. Borderline or high-negative results obtained from citrated specimens should be retested using serum as the matrix.
- Each assay requires 10 µL human serum or plasma. EDTA, citrate or heparin anticoagulants have been tested and may be used with this assay. Follow manufacturers' instructions carefully when using gel separator containers and plasma collection containers with anticoagulants.
- Samples that are to be used fresh may be stored for up to two hours at 2-8°C in the presence of clots. Serum separated from the clot may be stored at 2-8°C for no more than three days, but then must be frozen and stored deep-frozen (at –20°C or below) in sterile containers until use. If sample is stored frozen, mix thawed sample well before testing. Samples that have been frozen and thawed up to three times give acceptable results. Freeze-thaw recommendations were not assessed with samples stored at 2-8°C for three days.
- For shipping, serum should be separated from the clot and shipped at 2-8°C for no more than three days, or frozen at –20°C or below and shipped on dry ice. If specimens are shipped frozen, temperature level during entire shipment should be no greater (warmer) than –20°C. Pack specimens in compliance with government regulations covering the transportation of etiologic agents (12).
- All patient samples must be diluted 1:4000 with sample diluent before assaying. To avoid cross-contamination, use a clean micropipette tip to dispense each sample. To dilute samples using a pipetter-diluter, follow the appropriate manufacturer's instructions for use. Alternatively, samples may be diluted manually. Manual dilution is time-consuming and should be performed for all samples before beginning the assay procedure, thus reducing the time each sample is exposed to the coated microwell. Use the following procedure to dilute samples manually:
  - For each sample to be diluted, set up two polystyrene or glass test tubes in a rack. Add 990 µL sample diluent to the first row of test tubes and 390 µL sample diluent to the second row of test tubes.
  - If sample was stored frozen, mix thawed sample well (vortex) before proceeding. Add 10 µL of a sample to the first tube and vortex. The sample is now diluted 1:100.
  - Transfer 10 µL of the *diluted* sample from the first test tube to the second test tube and vortex. The sample is now diluted 1:4000.
  - Repeat the above two steps for all patient samples. The diluted samples are now ready to be assayed. Begin the assay procedure immediately (see Section 10, Manual Assay Procedure). Diluted samples cannot be stored.

10. MANUAL ASSAY PROCEDURE

Materials Provided

ETI-CORE-IGMK PLUS

- Coated Strips
- Enzyme Tracer
- Tracer Diluent
- Calibrator (Human)
- Negative Control (Human)
- Positive Control (Human)
- Sample Diluent
- Wash Buffer (Concentrate)
- Chromogen/Substrate
- Stop Solution
- Strip Sealers
- Plate Sealers
- Pouch Sealer.

Materials Required But Not Provided

Microwell plate washer - The following instrument specifications are recommended for the kit performance:

Volume dispensed: 350-370 µL
Number of wash cycles: 5
Soak time: 30 seconds
Aspirate the last aliquot of dispensed liquid: yes.

***Note** - The volume of each microwell is approximately 400 µL. Make sure the volume of working wash buffer dispensed into each well does not cause the wells to overflow. If the wells overflow, set the washer to dispense less working wash buffer.*

Microwell plate reader - The following instrument specifications are recommended for the kit performance:

Wavelength: dual wavelength, 450 nm and 600-650 nm
Bandwidth: ≤ 10 nm
Absorbance range: 0 absorbance units to ≥ 2.5 absorbance units
Repeatability: better than or equal to 0.005 absorbance units, or 1%, whichever is greater
Linearity or accuracy: better than or equal to 0.010 absorbance units, or 2%, whichever is greater
Drift: less than 0.005 absorbance units per hour.

Incubator, 37°C ± 1°C.

***Note** - Gravity convection incubators are recommended. Forced-air incubators may cause edge effects. Do not use water baths as incubators.*

Micropipettes with disposable clean tips (10 µL, 100 µL and 1000 µL).

***Note** - Suggested specifications for micropipetters (based on gravimetric testing) are:*
10 µL: accuracy ± 3%, precision 2%
100 µL: accuracy ± 2%, precision 1%
1000 µL: accuracy ± 2%, precision 1%.

Miscellaneous clean glass or plastic containers
Hazardous waste disposal materials
Disposable gloves
Distilled or deionized water
Pipetter-diluter (optional)
Multichannel pipetter (optional)
Pipette tips for multichannel pipetter (if multichannel pipetter is used)
Disposable reagent reservoirs (if multichannel pipetter is used)
Printer compatible with microwell reader.

Automated Procedure Using Biochem Immunosystems Labotech/ETI-LAB Instrument. See the Labotech (ETI-LAB) instrument Instruction Manual.

Assay Procedure

Perform all assay steps in the order given and without any delays between the steps. A cutoff value is calculated for each plate based on the absorbance values of the calibrators run on that plate. A maximum of one plate should be set up (completed through the first incubation step) at a time. If multiple plates are being run as a batch, each plate must be treated as a single entity; i.e., the calibrators, controls and patient specimens for the plate must be added and the

incubation time started before moving on to the next plate. Proper instrument maintenance is critical for good assay performance. Follow the manufacturer's instructions for performing instrument warm-up, quality control, calibration and maintenance procedures on all equipment used in this assay.

Note - Steps 2-12 must be completed within five hours. Calibrator, positive and negative controls must be run with each plate of patient specimens.

1. Prepare assay reagents and patient samples. Allow all test components to reach room temperature (20-25°C). Prepare working wash buffer and working enzyme tracer according to the directions given in Section 6, Reagent Preparation. Dilute patient samples 1:4000 according to the directions given in Section 9, Specimen Collection and Preparation. *Do not dilute calibrators and controls, which are ready to use*. Refer to the chart in Section 6 to ensure preparation of sufficient reagent volumes for the number of tests included in the run.

2. Prepare coated plate. Prepare enough microwells for the calibrators, controls and patient samples to be tested. Allow one blank well containing only chromogen/substrate and stop solution in well A1. Allow one well for each patient sample. The calibrator must be tested in triplicate and the negative and positive controls tested in singlet. Calibrators are to be placed in wells B1, C1 and D1; negative control is to be placed in well E1; positive control is to be placed in well F1 (for details, refer to the recommended plate map at the end of this section). Test calibrator and controls as you would patient specimens. Coated strips may be separated. Avoid handling the bottoms of the microwells because scratches or marks could affect the reading of test results. Store unused strips in their original pouch, seal the pouch carefully, and refrigerate at 2-8°C.

3. Add samples and controls. Add **100 µL** of each sample (diluted 1:4000), calibrator or control to its respective microwell. To avoid cross-contamination, use a clean micropipette tip to dispense each calibrator, control or specimen. Record the microwell position of each calibrator, control or patient specimen on a laboratory data sheet.

4. Incubate. Cover the microwells with a plate or the strip sealer provided with this kit. Use a roller to affix the sealer or press firmly by hand around microwell and plate edges to ensure that the sealer is firmly attached over the entire strip or plate. Tap the coated plate gently to release any air bubbles trapped in the liquid making sure samples do not splash onto the sealer. Ensure that all microwells are filled equally. Incubate the microwells **for 2 hours ± 10 minutes at 37°C ± 1°C**.

5. Wash coated plate. Remove and discard the sealer. Aspirate the liquid from the microwells and wash each well five times as follows: Deliver 350-370 µL of working wash buffer to each microwell, let the wells soak for 30 seconds, and then aspirate the working wash buffer completely from each microwell. Microwell plate washers vary by manufacturer. Make sure the volume of working wash buffer dispensed into each well completely fills the well but does not cause the well to overflow.

6. Remove excess working wash buffer. Ensure that all microwells are aspirated completely before proceeding. With some washers it may be necessary to invert the microplate and tap it forcefully on a paper towel to effectively remove residual working wash buffer.

7. Add working enzyme tracer. Immediately add **100 µL** working enzyme tracer to each well (except for the blank well).

8. Incubate. Cover the microwells with a plate or the strip sealer provided with this kit. Ensure that sealer is applied correctly (see Step 4). Tap the coated plate gently to release any air bubbles trapped in the liquid. Ensure that all microwells are filled equally. Incubate the microwells **for 60 ± 5 minutes at 37°C ± 1°C**.

***Warning** - Timing of this incubation step is critical.*

9. Wash coated plate. Remove and discard the sealer. Aspirate the working enzyme tracer from the microwells and wash them as described in Steps 5 and 6.

10. Add chromogen/substrate. Immediately add **100 µL** chromogen/substrate to all microwells, including the blank well.
Note *- The chromogen/substrate may have a slightly blue tinge. However, if it turns a darker blue, it may have become contaminated and should be discarded.*

11. Incubate. Incubate the microwells **for 30 ± 2 minutes at room temperature** (20-25°C). Avoid exposing the microwells to direct or intense light. Do not exceed the time limits of this incubation.

12. Add stop solution. Add **100 µL** stop solution to each microwell in the same order as chromogen/substrate was added.

13. Read results. Within one hour after addition of stop solution, read the absorbance values of the calibrators, negative control, positive control, and samples with the microwell reader set at 450/630 nm in the bichromatic mode. If time before reading exceeds one hour, the tests must be discarded and specimens retested. Check for and remove air bubbles before reading results. Record the absorbance value for each calibrator, control and sample.
Note *- Blank the instrument on the blank well. The absorbance of the blank well containing only chromogen/substrate and stop solution (see Step 2 in Section 10, Manual Assay Procedure) is evaluated as described in the QC section. The value for the blank well should be recorded and subtracted from each calibrator, control and sample value before calculating mean values and cutoff, and before interpreting results.*

14. Perform assay quality control procedures. Before evaluating results, perform quality control procedures (see Section 11, Quality Control).

15. Perform equipment quality control and maintenance procedures. Proper instrument maintenance including calibration is critical for good assay performance. Follow the manufacturer's instructions for performing quality control and maintenance procedures on all equipment used in this assay.

Recommended Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	CAL1	S4										
C	CAL2	S5										
D	CAL3	S6										
E	NC	S7										
F	PC	S8										
G	S1	etc.										S89
H	S2											S90

11. QUALITY CONTROL

The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff.

Quality material furnished is prediluted. This material does not control all procedural aspects, e.g., specimen dilution. The user is advised to include appropriate control material to monitor the specimen dilution process.

The quality control material furnished is in a serum matrix. It may not adequately control the assay for plasma specimens. The user should provide alternate control material for testing of plasma matrices.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Use the following steps to validate quality control. References 13 and 14 provide guidance on quality control recommendations. Record the results on the QC Verification Worksheet provided for the assay.

Compute the mean absorbance value for the calibrator.

Always evaluate mean calibrator value and negative and positive control values for each plate when running more than one plate in a batch. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.

1. Evaluate the absorbance value of the substrate blank.

Blank the instrument on the blank well containing only chromogen/substrate and stop solution (see Step 13 in Section 10, Manual Assay Procedure). The absorbance value for the blank well must be between 0.000 and 0.150 for the assay to be valid. If the absorbance value of the substrate blank is less than 0.000 or greater than 0.150, the run must be repeated.

Note - Subtract the substrate blank absorbance value from each absorbance value before performing the following evaluations.

2. Evaluate the mean calibrator absorbance value (Cal $\bar{x}$).

Each calibrator absorbance value (after subtraction of the blank) must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{Cal} < 0.120$$

If one of the calibrator absorbance values does not meet this criterion, it should be discarded and the mean value recalculated using the remaining two values. If more than one calibrator absorbance values do not meet this criterion, the run is invalid and must be repeated.

Example 1: Calculation of mean of calibrators

Calibrator well	Absorbance	Minus blank absorbance	Final calibrator absorbance
B1	0.054	0.030	0.024
C1	0.051	0.030	0.021
D1	0.052	0.030	0.022
Total absorbance			0.067

$$\text{Mean of calibrators (Cal } \bar{x}) = \frac{\text{Total absorbance}}{3} = \frac{0.067}{3} = 0.022.$$

The mean calibrator absorbance value must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{Cal } \bar{x} < 0.120$$

If the mean calibrator absorbance value does not meet this criterion, the run is invalid and must be repeated.

3. Evaluate the negative control absorbance value (NC).

After subtracting the substrate blank absorbance, the negative control absorbance value must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{NC} < 0.120$$

If the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

4. Evaluate the positive control absorbance value (PC).

After subtracting the substrate blank absorbance, the positive control absorbance value must be greater than 0.500 and less than 2.500.

$$0.500 < \text{PC} < 2.500$$

If the positive control absorbance value does not meet this criterion, the run is invalid and must be repeated.

5. Evaluate the difference between the positive control absorbance value and the negative control absorbance value.

The difference between the positive control absorbance value and the negative control absorbance value must be greater than 0.450.

$$PC - NC > 0.450$$

If the difference between the positive control absorbance value and the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

Example 2: Calculation of difference between PC and NC

Positive control absorbance (PC)	= 1.237
Negative control absorbance (NC)	= 0.020
Difference (PC – NC) = 1.237 – 0.020	= 1.217

12. QUALITY CONTROL PROBLEM SOLVING

It is important to follow the assay procedure precisely. If calibrator or control values are not within acceptable limits (see Section 11, Quality Control) or results differ markedly from those expected, check these assay variables:

- Check incubator, incubation times, and temperatures.
- A properly functioning washer is critical to the assay. Ensure that the washer is filling and aspirating all wells, that no probes are plugged, and that the probes are placed correctly in the microwells. No fluid should be left in the wells at the end of the wash step.
- Be sure that wells do not dry out between washing and addition of the next reagent. Add reagent within a few minutes of removal of the plate from the washer. If a probe (or probes) on the washer becomes plugged during washing, identify the affected microwell(s) but continue with the assay procedure. Retest the affected specimen(s). To unplug probes, refer to the washer operator's manual.
- Check that all reagents and specimens are at room temperature (20-25°C) before starting the assay.
- Check that all reagents are within the expiration date, that appropriate assay kit components and ancillaries are used, and that there are no visible signs of contamination such as cloudiness or precipitates.
- Avoid cross-contamination of reagents and wells. If multichannel pipette tips have been contaminated, replace the tips.

13. INTERPRETATION OF RESULTS

The presence or absence of IgM anti-HBc is determined by comparing the absorbance values of patient samples with a cutoff value. The cutoff value is determined for each plate based on the absorbance values of the calibrators run on that plate. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.

Calculation of Cutoff Value

The cutoff value is determined by adding 0.200 to the mean absorbance of the calibrator values after subtraction of the substrate blank.

$$CUTOFF = Cal\ \bar{x} + 0.200$$

Example 3: Calculation of cutoff value

Calibrator mean absorbance	0.022
Constant	+ 0.200
Cutoff value for this run	0.222

The cutoff was established by testing 348 samples (174 volunteer blood donors and 174 hospitalized patients) with three lots of ETI-CORE-IGMK PLUS. The results were examined as the difference (delta) between single sample absorbance and calibrator absorbance. In the apparently healthy adult (volunteer donor) population, 99% had delta values less than 0.028; in the hospitalized patient population, 99% had delta values less than 0.168.

Interpretation of Results (Manual or Labotech/ETI-LAB assay)

Absorbance Values	Result	Interpretation
Absorbance < 90% x Cutoff	Negative	IgM anti-HBc not detected by ETI-CORE-IGMK PLUS. This result should not be used alone but in conjunction with other hepatitis B serological markers to determine disease state.
Absorbance within 90-110% of Cutoff	Equivocal	Presence of IgM anti-HBc indeterminate by ETI-CORE-IGMK PLUS. Specimen should be retested using ETI-CORE-IGMK PLUS kit to establish presence or absence of antibody. If a specimen is found repeatedly equivocal, the pattern of other hepatitis B serological markers should be used to identify status of disease, or another sample should be collected and tested at a later date.
Absorbance > 110% x Cutoff	Positive	IgM anti-HBc detected by ETI-CORE-IGMK PLUS. This result should not be used alone but in conjunction with other hepatitis B serological markers to determine disease state.

Note *- The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antibody present. Due to the expression of the recombinant HBcAg in E. coli, samples containing antibodies to E. coli may cause false positive results.*

Example 4: Interpretation of results

Cutoff = 0.222.
Equivocal Zone = 0.200-0.244.
Sample No. 1 absorbance = 0.058.
Sample No. 2 absorbance = 1.546.

Sample No. 1 should be considered negative for IgM anti-HBc; sample No. 2 should be considered positive for IgM anti-HBc.

14. LIMITATIONS OF THE PROCEDURE

- Results obtained from immunosuppressed patients should be interpreted with caution.
- This assay is not designed to test body fluids other than human serum or plasma.
- Any laboratory test result should be interpreted in conjunction with the patient's clinical presentation and the results of other diagnostic tests. A negative result on a given laboratory assay does not by itself rule out the possibility of infection.
- The prevalence of the analyte will affect the assay's predictive value.
- Assay performance characteristics have not been established when the ETI-CORE-IGMK PLUS IgM anti-HBc assay is used in conjunction with the other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Performance characteristics have not been established for any other automated instrument than the Biochem Immunosystems Labotech/ETI-LAB automated instrument. If another automated instrument is used, the user is responsible for establishing their own assay performance characteristics.
- Some false positive results have been observed with the assay in patients with hepatitis C virus, hepatitis D virus, HIV, or HTLV-I/II infection.
- Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may produce false negative results when tested with a direct, non-competitive immunoassay containing a monoclonal antibody such as the ETI-CORE-IGMK PLUS assay. Specimens from these individuals should not be tested with this assay.

- Although it has not been shown in this assay, individuals with antibodies to *E. coli* may produce false positive results due to the expression of the recombinant HBcAg in *E. coli*.
- The analytical sensitivity of the ETI-CORE-IGMK PLUS assay has been determined to be approximately 25 PEI U/mL.

15. EXPECTED VALUES

The 436 prospective samples used in the expected values study for the ETI-CORE-IGMK PLUS assay were from patients who were sent to the laboratory for HBV testing. Of those samples, 100 (23%) were frozen and 336 (77%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the Southeastern US. The group was 71% (309/436) female, 28% (121/436) male and 1% (6/436) unspecified; the ethnicity of the patients was unspecified. The ages ranged from 1 to 89 years old, with six samples unspecified. The percent ETI-CORE-IGMK PLUS positive results observed in these samples was 3%.

The table below summarizes the percent ETI-CORE-IGMK PLUS positive and negative results by gender and age range. There were six samples for which gender and age were not reported; they were all positive. There were six other samples for which age was not reported, two were from females and four were from males; all were negative. There were an additional four samples that were QNS for testing. These 16 results were not included in the table.

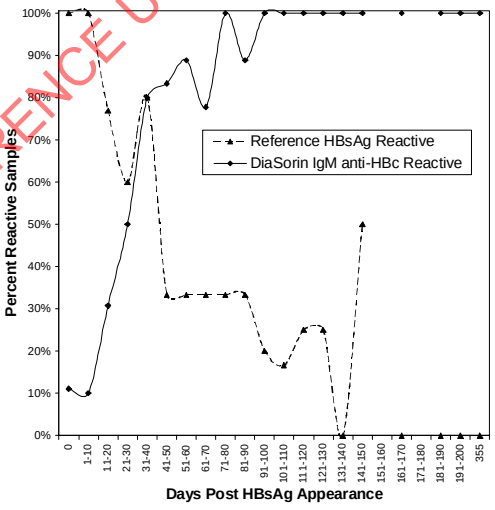
		ETI-CORE-IGMK PLUS				
		+		–		
Age Range	Gender	n	%	n	%	Total
0-9	F	0	0%	2	100%	2
	M	0	0%	1	100%	1
10-19	F	1	4%	27	96%	28
	M	1	33%	2	67%	3
20-29	F	1	1%	111	99%	112
	M	3	14%	18	86%	21
30-39	F	0	0%	89	100%	89
	M	1	3%	36	97%	37
40-49	F	2	6%	33	94%	35
	M	1	4%	24	96%	25
50-59	F	1	8%	12	92%	13
	M	1	8%	12	92%	13
60-69	F	0	0%	5	100%	5
	M	0	0%	5	100%	5
70-79	F	0	0%	14	100%	14
	M	0	0%	5	100%	5
80-89	F	0	0%	6	100%	6
	M	0	0%	6	100%	6
Total		12	3%	408	97%	420

Acute Serial Panels. One hundred twenty-four (124) archived serial samples from nine individuals were analyzed. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequent appearance of IgM anti-HBc, total anti-HBc, anti-HBe, and anti-HBs. One individual had detectable HBsAg, but did not have detectable HBeAg in any specimen. However, this individual did seroconvert for anti-HBe.

The specimens were collected from individuals undergoing plasmapheresis for further manufacturing purposes. Three individuals were found to be infected with HBV during the first plasmapheresis and others became infected with HBV during subsequent plasmaphereses. It is unknown how long these three initially HBsAg-reactives were infected prior to the first plasmapheresis. All nine individuals underwent sequential plasmaphereses after becoming HBV-infected. However, the timing of subsequent plasmaphereses varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by an FDA-licensed assay as day 0. Draw days ranged from day 0 (HBsAg first detected) through day 355 post-day 0. Since all panels did not contain the same draw day, sample results were grouped within day intervals (e.g., days 0, 1-10, 11-20, etc., representing days since first detection of HBsAg).

The results are summarized in the following table and graph. All specimens were reactive for IgM anti-HBc by the DiaSorin assay after day 90. In the graph below the pattern for the reference HBsAg percent reactive has been overlaid for reference.

Day Range	Number of Specimens	DiaSorin IgM anti-HBc Reactive	Percent Positive
0	9	1	11.1
1-10	10	1	10.0
11-20	13	4	30.8
21-30	9	4	44.4
31-40	10	8	80.0
41-50	6	5	83.3
51-60	10	9	90.0
61-70	9	7	77.8
71-80	6	6	100
81-90	9	8	88.9
91-100	10	10	100
101-110	6	6	100
111-120	4	4	100
121-130	4	4	100
131-140	3	3	100
141-150	2	2	100
151-160	0	0	NA
161-170	1	1	100
171-180	0	0	NA
181-190	1	1	100
191-200	1	1	100
355	1	1	100



16. PERFORMANCE DATA

Clinical Samples

Prospective Samples. A study of 836 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing or represented a high-risk population. Specimens were collected at a reference laboratory or an STD clinic and assayed at the California clinical trial site. The patients were 61% (510/836) female and 39% (326/836) male. The ages ranged from 1 to 89 years old, with three specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-CORE-IGMK PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure, including confirmation by neutralization of repeatably reactive HBsAg samples.

Results by Specimen Classification. After study completion, all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were eleven unique HBV marker patterns observed in the ETI-CORE-IGMK PLUS prospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Chronic Infection	+	–	–	+	+	–	1
	+	–	–	+	–	–	1
Recovery	–	–	+	+	–	–	2
	–	–	+	+	–	+	2
	–	–	–	+	+	+	19
	–	–	–	+	+	–	9
Past Infection	–	–	–	+	–	+	43
	–	–	–	+	–	–	42
HBV Vaccine Response	–	–	–	–	–	+	185
Not Previously Infected with HBV	–	–	–	–	–	–	529
Unknown	–	–	–	–	–	I	3

I = Indeterminate result.

Based on the above classifications, the ETI-CORE-IGMK PLUS IgM anti-HBc results for the prospective samples were compared to a reference assay's IgM anti-HBc results. The following table shows this comparison and percent agreement with 95% exact confidence intervals with the reference IgM anti-HBc assay results. Four samples were QNS for IgM anti-HBc testing and were not included in the table (two Past Infection, one Recovery, one HBV Vaccine Response).

Prospective Samples Comparison

Reference Serology Classification	Reference IgM anti-HBc Assay					Total
	–			+		
	ETI-CORE-IGMK PLUS			ETI-CORE-IGMK PLUS		
	–	+	E	–	+	
Chronic Infection	2	0	0	0	0	2
Recovery	26	1	0	0	4	31
Past Infection	82	0	1	0	0	83
HBV Vaccine Response	184	0	0	0	0	184
Not Previously Infected with HBV	529	0	0	0	0	529
Unknown	3	0	0	0	0	3
Total	826	1	1	0	4	832

E = Equivocal results.

Prospective Samples

Chronic Infection
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (2/2)
95% CI = 15.8-100%

Recovery
Positive agreement with reference assay results = 100% (4/4)
95% CI = 39.8-100%
Negative agreement with reference assay results = 96.3% (26/27)
95% CI = 81.0-99.9%

Past Infection
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 98.8% (82/83)
95% CI = 93.5-99.97%

HBV Vaccine Response
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (184/184)
95% CI = 98.0-100%

Not Previously Infected with HBV
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (529/529)
95% CI = 99.3-100%

Unknown
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (3/3)
95% CI = 29.2-100%

Retrospective Samples. Retrospective studies were carried out at three clinical laboratories in the United States (California, Missouri, and Minnesota) and at DiaSorin (Italy) to assess the performance of the ETI-CORE-IGMK PLUS assay in detecting IgM anti-HBc. The study set included 650 frozen repository samples (the majority of which were purchased from commercial vendors) from the following populations:

- patients with chronic hepatitis B infection (HBsAg-positive for greater than six months) - 111 frozen repository samples;
- patients with serologically diagnosed hepatitis B infection (acute, chronic, asymptomatic, convalescent, etc.) - 82 frozen repository samples;
- patients sent to the laboratory for hepatitis B testing - 100 frozen repository samples;
- a general hospital patient population - 357 frozen repository samples.

The specimens represented Midwestern (2%), Southeastern (25%), Western (13%), and Northeastern US (2%), outside of the US (1%), and unspecified (57%). The group was 44% (287/650) female, 42% (270/650) male, and 14% (93/650) unspecified. Approximately 13% (84/650) were Caucasian, 4% (27/650) were African American, < 1% (5/650) were Hispanic, < 1% (3/650) were Asian, and 82% (531/650) were unspecified. The ages ranged from 5 to 98 years old, with 131 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-CORE-IGMK PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure with the exception of the HBsAg assay at two of the three sites. At these sites, the majority of specimens that were initially HBsAg-positive were repeated in duplicate; however, the repeatedly reactive specimens were not confirmed by the licensed HBsAg confirmation assay at the two sites. Therefore, true HBsAg result was determined in one of three ways: 1) confirmed by reference assay neutralization during clinical trials, 2) based on a statement by the attending physician that HBsAg was positive for greater than six months, or 3) information provided by the vendor regarding confirmatory testing performed at their location or by the material source facility.

Results by Specimen Classification. After study completion, all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 35 unique HBV marker patterns observed in the ETI-CORE-IGMK PLUS retrospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Acute Infection	+	+	+ or I	+	–	–	52
	+	–	+ or I	+	+	–	4
	+	–	–	–	–	–	2
	+	+	–	–	–	–	2
Chronic Infection	+	–	–	+	+	–	82
	+	+	–	+	–	–	21
	+	–	–	+	– or I	–	23
	+	+	+	+	–	+	4
	+	+	– or I	+	–	+	2
	+	–	–	+	+	+	2
	+	+	–	+	+ or I	+	2
	+	+	+	+	+	+	1
	+	+	–	+	+	–	1
	+	–	–	+	–	+	1
Recovery	–	–	–	+	+ or I	+	40
	–	–	–	+	+	–	6
	–	–	+	+	+	–	2
	–	–	+ or I	+	+	+	2
Past Infection	–	– or I	–	+	–	+	12
	–	–	–	+	–	–	9
HBV Vaccine Response	–	–	–	–	–	+	20
Not Previously Infected with HBV	–	–	–	–	–	–	343
Uninterpretable	–	+ or I	–	–	–	–	13
	–	+	–	+	–	+	2
	–	+	–	+	+	+	1
	–	I	–	+	–	–	1

I = Indeterminate result.

Based on the above classifications, the ETI-CORE-IGMK PLUS IgM anti-HBc results for the retrospective samples were compared to a reference assay's IgM anti-HBc results. The following table shows this comparison and percent agreement with 95% exact confidence intervals with the reference IgM anti-HBc assay results.

Retrospective Samples Comparison

Reference Serology Classification	Reference IgM anti-HBc Assay								TOTAL
	–			+			I		
	ETI-CORE-IGMK PLUS			ETI-CORE-IGMK PLUS			ETI-CORE-IGMK PLUS		
	–	+	E	–	+	E	–	+	
Acute Infection	4	0	0	1	45	1	1	8	60
Chronic Infection	118	13	2	0	5	0	0	1	139
Recovery	41	5	0	0	3	0	0	1	50
Past Infection	21	0	0	0	0	0	0	0	21
HBV Vaccine Response	20	0	0	0	0	0	0	0	20
Not Previously Infected with HBV	343	0	0	0	0	0	0	0	343
Uninterpretable	17	0	0	0	0	0	0	0	17
Total	564	18	2	1	53	1	1	10	650

I = Indeterminate results. E = Equivocal results.

Retrospective Samples

Acute Infection Positive agreement with reference assay results = 95.7% (45/47)
95% CI = 85.5-99.5%
Negative agreement with reference assay results = 100% (4/4)
95% CI = 39.8-100%

Chronic Infection Positive agreement with reference assay results = 100% (5/5)
95% CI = 47.8-100%
Negative agreement with reference assay results = 88.7% (118/133)
95% CI = 82.1-93.5%

Recovery Positive agreement with reference assay results = 100% (3/3)
95% CI = 29.2-100%
Negative agreement with reference assay results = 89.1% (41/46)
95% CI = 76.4-96.4%

Past Infection Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (21/21)
95% CI = 83.9-100%

HBV Vaccine Response Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (20/20)
95% CI = 83.2-100%

Not Previously Infected with HBV Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (343/343)
95% CI = 98.9-100%

Uninterpretable Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (17/17)
95% CI = 80.5-100%

Commercial panels. Single samples from two commercial performance panels (mixed-titer panel and low-titer panel) were tested with the ETI-CORE-IGMK PLUS assay. The mixed-titer panel contained 25 samples that had been tested for IgM anti-HBc by the vendor using eight commercially available assays. The panel consisted of three negative samples and 22 positive samples ranging from low positive (mean sample absorbance-to-cutoff ratio ranges [S/CO] = 1.6-2.4) to high positive (mean S/CO ranges = 6.9-8.7). The low-titer panel contained 15 samples that had been tested for IgM anti-HBc by the vendor using seven commercially available assays. The panel consisted of one negative sample and 14 low-positive samples (mean S/CO ranges = 1.3-5.6).

The table below presents the percent agreement between the ETI-CORE-IGMK PLUS results and the vendor's IgM anti-HBc results. The data in the table represent the number of specimens in each group.

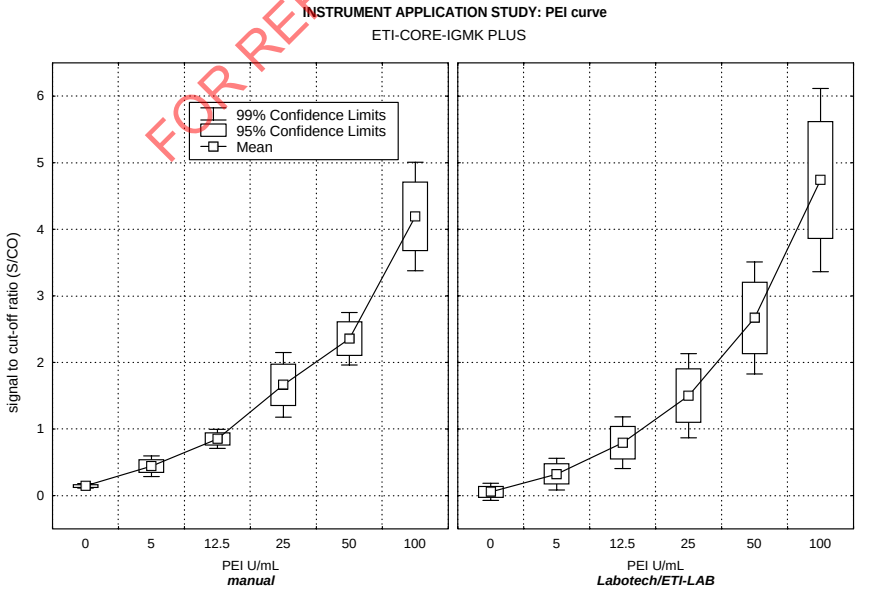
Group	Reference IgM anti-HBc Assay		TOTAL
	–	+	
	ETI-CORE-IGMK PLUS –	ETI-CORE-IGMK PLUS +	
Low-Titer Panel	1	14	15
Mixed-Titer Panel	2	23	25
Total	3	37	40

Positive agreement with reference assay results = 100% (37/37)
Negative agreement with reference assay results = 100% (3/3)

Instrument Comparison of Biochem Immunosystems Labotech/ETI-LAB to the Manual Method

An instrument application study was conducted at DiaSorin, Saluggia (Italy), to evaluate the performance of the ETI-CORE-IGMK PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB, an automated microplate processing instrument, compared to the manual analysis. The Paul-Ehrlich-Institut (PEI) Standard, 12 serum samples near the ETI-CORE-IGMK PLUS cutoff and samples from the clinical trials (27 suspected hepatitis B patients and 13 apparently healthy adults) were tested in parallel manually and on the Labotech.

Serial dilutions of the PEI Standard were prepared in fetal calf serum to obtain a panel ranging from high concentration to below the analytical sensitivity of the assay. The diluted Standard samples were tested in duplicate, one run per day for three days both manually and on the Labotech. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The 95% confidence intervals were established for the S/CO values of each point of the Standard-referenced curve and therefore the analytical endpoint sensitivity was defined (first dilution with S/CO > 1.1). A graph summarizing these results is presented below.



The 12 samples near the cutoff were tested in triplicate, one run per day for three days both manually and on the Labotech. The samples from the clinical trials were tested in singlet in one run on one day, both manually and on Labotech. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. A summary of the data is presented in the following table.

	Manual			Labotech/ETI-LAB		
Analytical Endpoint Sensitivity (25 PEI U/mL)	Mean	W/R* %CV	D/D* %CV	Mean	W/R %CV	D/D %CV
S/CO [95% CI]*	1.66 [1.35-1.97]	8.2	18.3	1.50 [1.10-1.90]	16.4	23.3
12 Cutoff Samples S/CO Range of mean S/CO	1.01 0.71-1.40	10.9	16.6	0.98 0.68-1.26	13.2	23.1
Clinical Samples						
Suspected Hepatitis B Range of S/CO	Negative: 0.16-0.64 (10/27) Equivocal: 1.03-1.05 (2/27) Positive: 1.14-11.32 (15/27)			Negative: 0.11-0.86 (10/27) Equivocal: 1.03 (1/27) Positive: 1.29-10.47 (16/27)		
Healthy Adults Range of S/CO	Negative: 0.09-0.16 (13/13) Equivocal: NA (0/13) Positive: NA (0/13)			Negative: 0.11-0.14 (13/13) Equivocal: NA (0/13) Positive: NA (0/13)		

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day.

Reproducibility

Manual Assay. Intra-assay, inter-assay, inter-lot, and inter-site variability studies were carried out on the ETI-CORE-IGMK PLUS kit to test the variability within runs, between runs, between days, between kit lots, and between test sites. Variability was measured on a panel of ten sera that included negative, borderline, and positive samples. Three ETI-CORE-IGMK PLUS kit lots were tested at three independent test sites. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The results of that study are tabulated below.

Clinical Site Reproducibility Study

ID#	Class.	# of Tests per Sample	Mean S/CO	Within-run %CV	Between-run %CV	Between-lot %CV	Between-day %CV	Between-site %CV	Total %CV
S01	High	108	5.55	8.71	5.92	3.83	4.59	8.34	11.21
S02	High	108	3.80	4.42	6.64	3.82	5.06	6.13	8.82
S03	Low	108	2.25	4.12	6.67	5.56	3.78	11.47	9.96
S04	B-line	108	1.18	8.24	11.27	6.43	2.26	11.49	16.45
S05	B-line	108	0.78	7.01	6.26	6.72	7.69	16.41	13.00
S06	Neg	108	0.53	4.07	6.76	5.42	3.88	9.13	9.92
S07	Low	108	2.27	5.60	5.20	3.91	10.73	17.70	13.39
S08	B-line	108	1.23	5.46	6.53	4.26	12.75	10.17	15.96
S09	B-line	108	1.44	8.89	7.32	5.36	6.36	12.30	14.90
S10	Neg	108	0.16	7.12	13.89	10.97	4.37	5.33	18.04

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.

Instrument Reproducibility. An instrument reproducibility study was conducted at two external sites (Georgia, Massachusetts) and at DiaSorin (Italy) to evaluate the performance of the ETI-CORE-IGMK PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB (an automated microplate processing instrument). Twelve samples were used in the study: eight low-positive and four high-negative samples. The samples were prepared by spiking high-positive sera into each of 12 negative serum samples to reach the various analyte lev-

els. All samples were tested in triplicate in each run, two runs per day for five days. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The mean, standard deviation and coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each instrument and across instruments. A summary of the data is presented in the following table.

Sample ID#	# of Tests	Mean S/CO	Within-run %CV	Between-run %CV	Between-day %CV	Between-instrument %CV	Total %CV
LP1	90	1.41	10.3	8.0	9.1	10.8	12.9
LP2	90	1.18	6.1	7.1	7.6	11.4	15.2
LP3	90	1.44	14.9	5.0	10.9	11.9	20.7
LP4	90	1.63	6.9	2.6	7.9	9.3	14.9
LP5	90	1.61	7.0	6.1	7.6	16.1	14.1
LP6	90	1.64	11.0	10.3	6.4	15.9	16.1
LP7	90	1.37	8.2	6.3	7.2	11.4	14.1
LP8	90	1.59	11.8	3.5	10.9	15.6	18.0
HN1	90	0.58	9.6	8.3	6.5	14.3	16.2
HN2	90	0.64	12.8	5.3	5.0	12.0	18.6
HN3	90	0.62	9.3	5.1	8.3	14.0	15.9
HN4	90	0.67	7.4	6.6	9.4	15.4	17.1
Low pos. mean		1.49	9.2	6.2	7.8	11.7	15.0
High neg. mean		0.63	9.4	5.9	7.4	14.1	16.7

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
LP = Low Positive; HN = High Negative.

Plasma reproducibility on the Labotech has not been established. If plasma is used on the Labotech, the user should establish appropriate assay reproducibility in accordance with CLSI EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices.

Plasma Reproducibility. A plasma reproducibility study was conducted at DiaSorin, Saluggia (Italy), to evaluate the performance of the manual ETI-CORE-IGMK PLUS assay on serum versus a variety of plasma types. The plasma types evaluated were citrate, heparin and EDTA. Twelve sample sets of matched serum/multiple plasma were used in the study: eight low-positive and four high-negative sample sets. A sample set was prepared by spiking the same high-positive sample into each of the matrices (serum and plasmas). All matched serum/multiple plasma sample sets were tested in triplicate in each run, one run per day for five days, all tested in a manual mode. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. In this assay, the S/CO is inversely related to reactivity. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. The 95% confidence intervals were established for the S/CO values of all serum samples and each plasma type. A summary of the data is presented in the following table.

Low-Positive Samples (n = 8)				
	Serum	Citrate	Heparin	EDTA
Mean S/CO	1.83	1.71	1.81	1.68
95% CI*	1.70-1.95	1.62-1.80	1.68-1.93	1.58-1.78
W/R* %CV	9.9%	8.6%	4.9%	13.2%
D/D* %CV	16.4%	13.0%	9.6%	10.9%
Total %CV	18.7%	14.7%	10.5%	16.6%
Between matrix %CV: 10.9%				
Across matrix total %CV: 18.5%				

High-Negative Samples (n = 4)				
	Serum	Citrate	Heparin	EDTA
Mean S/CO	0.85	0.67	0.77	0.83
95% CI*	0.80-0.90	0.63-0.72	0.73-0.81	0.78-0.88
W/R* %CV	8.3%	5.5%	10.6%	7.2%
D/D* %CV	19.5%	15.0%	16.7%	23.5%
Total %CV	19.8%	15.1%	18.5%	23.1%
Between matrix %CV: 13.5%				
Across matrix total %CV: 24.0%				

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day.

Analytical Sensitivity

The analytical sensitivity of the assay (the smallest quantity of analyte that can be distinguished from background) was evaluated using single-point serial dilutions of a standard preparation from the Paul-Ehrlich-Institut (PEI). The analytical sensitivity of the assay (last positive dilution) was determined to be 25 PEI U/mL (Mean Signal-to-Cutoff Ratio = 1.66; 95% Confidence Interval = 1.35-1.97).

Cross-Reactivity

Of the 525 potentially interfering samples, 487 (93%) were negative and 38 (7%) were positive by ETI-CORE-IGMK PLUS. Among the 38 positive samples, seven were positive by reference testing or review of hepatitis B marker patterns for those samples and one indicated a false negative result for the reference testing. Fourteen samples had marker patterns that indicated recovery, both positive and negative IgM anti-HBc are acceptable. Disease was determined by serological testing; there is no guarantee that the associated IgM antibody was present in the tested material. Interference testing with the described specimens was not performed.

Cross-Reactivity Study Results

Group	n	ETI-CORE-IGMK PLUS Negative Samples	ETI-CORE-IGMK PLUS Positive Samples	% Confirmed Positive By Additional Testing
Acute EBV Infection	16	16	0	–
Acute CMV Infection	20	20	0	–
Acute HSV Infection	10	9	1	0% (0/1)[a]
Acute Toxoplasmosis	18	17	1	100% (1/1)
Acute Parvovirus B19 Infection	5	4	1	0% (0/1)
HTLV-I/II Infection	50	44	6	33% (2/6)[b]
Syphilis	26	23	3	0% (0/3)[c]
HCV Infection	50	46	4	25% (1/4)
HDV Infection	20	17	3	0% (0/3)[d]
HIV Infection	50	40	10	10% (1/10)[e]
Acute HAV Infection	50	49	1[f]	–
Past HAV Infection	50	46	4	25% (1/4)[g]
Rheumatoid Factor (RF) +	40	39	1	0% (0/1)[h]
Autoimmune Disease	19	19	0	–
Systemic Lupus Erythematosus (SLE)	11	11	0	–
Autoimmune Hepatitis	5	5	0	–
Myeloma	20	20	0	–
Hypergammaglobulinemia	20	20	0	–
Influenza Vaccine	5	5	0	–
Elevated Liver Enzymes	10	10	0	–
Non-viral Liver Disease	30	27	3	33% (1/3)[i]
TOTAL	525	487 (93%)	38 (7%)	19% (7/37)

[a] Total anti-HBc and anti-HBe positive, indicating recovery; both positive and negative IgM anti-HBc are acceptable.
[b] Three of four samples are total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative IgM anti-HBc are acceptable.
[c] One of three samples is total anti-HBc, anti-HBs and anti-HBe positive; another sample is total anti-HBc and anti-HBe positive, indicating recovery for both samples; both positive and negative IgM anti-HBc are acceptable.
[d] One sample is HBsAg, total anti-HBc and anti-HBe positive, indicating acute infection; false negative reference assay result.
[e] Three of nine samples are total anti-HBc and anti-HBe positive, one sample is total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery for those samples; both positive and negative results are acceptable.
[f] QNS (quantity not sufficient) for additional testing. Sample is total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative results are acceptable.
[g] Two of three samples are total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative results are acceptable.
[h] DiaSorin negative on repeat testing.
[i] Two samples are total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative results are acceptable.

Substances That Do Not Interfere

As recommended by CLSI Protocol EP7 (15), the ETI-CORE-IGMK PLUS assay was evaluated for interference by testing the following substances. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with high-titer IgM anti-HBc samples to obtain a result near the cutoff. None of the compounds at the levels indicated were found to interfere with the clinical interpretation of the assay in serum. No interference was found with bilirubin in plasma (EDTA, heparin or citrate); testing for interference with hemoglobin and triolein was not performed in plasma.

Compound	Concentration	
Bilirubin	0.35 mmol/L	20 mg/dL
Hemoglobin	0.06 mmol/L	100 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

The ETI-CORE-IGMK PLUS assay was also evaluated for possible interference from anti-mouse antibodies (HAMA). A dilutional panel was used, consisting of 21 samples prepared from a stock pool of high-positive human sera. The HAMA concentrations in the samples ranged from 0 to 2975.5 ng/mL, as determined by a HAMA ELISA. In a direct, non-competitive immunoassay such as the ETI-CORE-IGMK PLUS, interference would manifest as false negative results. No interference was seen in that all 21 dilutions were negative by the ETI-CORE-IGMK PLUS assay, including the HAMA negative panel member.

17. ABBREVIATED TEST PROCEDURE

1 - DILUTE ALL SPECIMENS 1:4000 WITH SAMPLE DILUENT.

2 - DISPENSE 100 µL CALIBRATOR, CONTROLS AND DILUTED SAMPLES INTO WELLS, LEAVING AN EMPTY WELL FOR THE BLANK.

3 - INCUBATE FOR TWO HOURS AT 37°C.

4 - ASPIRATE THE LIQUID. WASH THE WELLS REPEATEDLY WITH WORKING WASH BUFFER.

5 - DISPENSE 100 µL ENZYME TRACER TO EACH WELL.

6 - INCUBATE FOR 60 MINUTES AT 37°C.

7 - ASPIRATE THE LIQUID. WASH THE WELLS REPEATEDLY WITH WORKING WASH BUFFER.

8 - DISPENSE 100 µL CHROMOGEN/SUBSTRATE TO EACH WELL.

9 - INCUBATE FOR 30 MINUTES AT ROOM TEMPERATURE.

10 - DISPENSE 100 µL STOP SOLUTION TO EACH WELL.

11 - READ THE ABSORBANCE VALUES WITH A PHOTOMETER AT 450/630 nm WITHIN 60 MINUTES.

18. REFERENCES

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SYMBOLS USED WITH IVD DEVICES

Consult instructions for use.

In vitro diagnostic.

Biohazard.

Lot No.

Use by:

Catalogue number.

Temperature limitation.

Caution, consult accompanying documents.

Manufacturer.

Number of tests.

Manufactured By:

DiaSorin

DiaSorin S.p.A.
Saluggia, Italy

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