

Summary of Safety and Effectiveness

1. General Information

1.1. Device Generic Name: Immunoassay for IgM antibodies to hepatitis B core antigen

1.2. Device Trade Name: ETI-CORE-IGMK PLUS

1.3. Applicant's Name and Address:

DiaSorin
Via Crescentino
Saluggia (VC) 13040, Italy

1.4. U.S. Representative:

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1.5. PMA Number: P990044

1.6. Date of Panel Recommendation: January 20, 2000

1.7. Date of Notice of Approval to the Applicant: March 30, 2001

2. Indications For 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 HBc (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.

3. Device Description

3.1. Principle of Assay

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 to human IgM. An enzyme tracer containing horseradish peroxidase-labeled human IgG antibodies to HBcAg detects any captured IgM anti-HBc. The monoclonal antibody is of the IgG1-k class and is directed to human IgM. In the assay procedure, patient specimens and controls are incubated in antibody-coated microwells. IgM antibodies present in a specimen or control 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 related to the IgM anti-HBc concentration present in the specimen or control. Excess HBcAg and enzyme tracer is 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 of the calibrators.

3.2. Kit Configuration and Components

For detection of IgM antibodies to HBc, the ETI-CORE-IGMK PLUS system is comprised of the following:

Coated Strips

Microwells coated with mouse monoclonal antibodies to human IgM (IgG1-k class; directed to human IgM).

Enzyme Tracer (human)

Horseradish peroxidase-labeled human Fab to HBcAg, buffer, protein stabilizers.
Preservative: 0.2% ProClin 300.

Tracer Diluent

Buffer, protein stabilizers.
Preservative: 0.2% ProClin 300.

Calibrator (Human)

Human serum/plasma non-reactive for all known HBV markers, diluted to 1:4000 with buffer. Preservative: 0.2% ProClin 300.

Negative Control (Human)

Human serum/plasma non-reactive for all known HBV markers, diluted to 1:4000 with buffer. Preservative: 0.2% ProClin 300.

Positive Control

Human serum/plasma reactive for IgM to HBc, diluted to 1:4000 with buffer.
Preservative: 0.2% ProClin 300.

Sample Diluent

Buffer, protein stabilizers, an inert blue dye.
Preservative: 0.2% ProClin 300.
Wash Buffer (concentrate)
Buffer, detergents, preservatives.
Chromogen/Substrate
Tetramethylbenzidine/hydrogen peroxide system
Stop Solution
1N sulfuric acid.
Strip Sealers
Plate Sealers
Pouch Sealer

4. Contraindications

None

5. Warnings and Precautions

For in vitro diagnostic use only.

Warnings and precautions for users of the ETI-CORE-IGMK PLUS assay are stated in the product labeling.

6. Alternative Practices and Procedures

The early assays for IgM antibodies to hepatitis B core antigen (IgM anti-HBc) were based on the principle of immune adherence hemagglutination. These assays were largely supplanted by radioimmunoassay (RIA) and enzyme immunoassay (EIA) methodologies, which were more sensitive, reproducible and practical. IgM antibody-specific molecules were originally detected either by immunosubtraction of IgG in the sample prior to testing or by using anti-human IgM conjugates.

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 antisera and enzyme-labeled anti-HBc conjugate. Because interference from rheumatoid factor or specific IgG antibodies is minimized, the IgM-capture assays usually have better clinical and analytical sensitivity and specificity.

7. Marketing History

The ETI-CORE-IGMK PLUS assay has never been marketed in the US or outside the US.

8. Potential Adverse Effects of the Device on Health

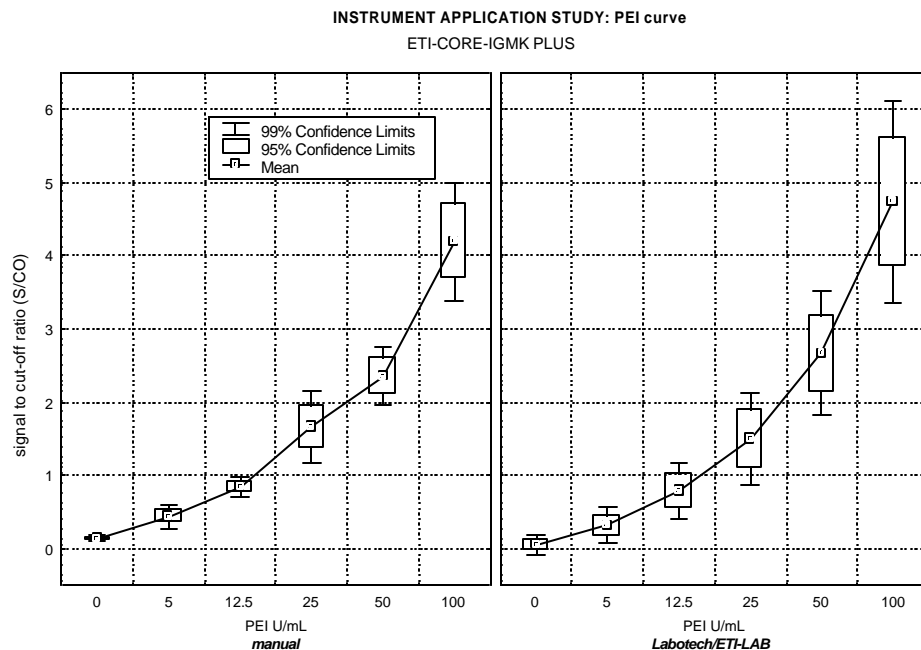
Failure of the product to perform as indicated or human error in use of the product may lead to a false result. A false result cannot be considered a patient or public health concern because the overall hepatitis B marker pattern will indicate the correct disease-state interpretation.

9. Summary of Preclinical Studies

9.1. Comparison of Labotech Instrumentation with the Manual Assay

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 ^a	D/D %CV	Mean	W/R %CV	D/D %CV
S/CO [95% CI] ^b	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 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 (2/22) 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: N/A (0/13) Positive: N/A (0/13)			Negative: 0.11 – 0.14 (13/12) Equivocal: N/A (0/13) Positive: N/A (0/13)		

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

No reproducibility testing with the Labotech instrument was conducted. As part of the conditions of approval agreement, DiaSorin will provide FDA results from a reproducibility study using the Labotech instrument. Until that condition is met, the following statement will be placed in the labeling:

“Assay reproducibility using the Labotech has not been established. If the Labotech is used, the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices.”

9.2. Analytical Sensitivity

The analytical sensitivity of the assay was evaluated using single point serial dilutions of a standard preparation from the Paul-Ehrlich-Institut (PEI), Germany. The analytical sensitivity of the assay (last positive dilution) was determined to be 0.18 PEI U/mL (Mean Signal-to-Cutoff Ratio = 0.61; 95% Confidence Interval = 0.53 to 0.69).

9.3. Potential Cross-Reacting Substances

Serum samples were obtained from patients belonging to a number of different disease categories listed below. Of the 535 potentially interfering samples, 497 (96%) 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 1 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	16	16	0	-
Acute CMV	20	20	0	-
Acute HSV	10	9	1	0% (0/1) ^a
Acute Toxo	18	17	1	100% (1/1)
Acute Parvovirus B19	5	4	1	0% (0/1)
HTLVII/II	50	44	6	33% (2/6) ^b
Syphilis	26	23	3	0% (0/3) ^c
HCV	50	46	4	25% (1/4)
HDV	20	17	3	0% (0/3) ^d
HIV	50	40	10	10% (1/10) ^e
Acute HAV	50	49	1 ^f	-
Past HAV	50	46	4	25% (1/4) ^g
RF +	40	39	1	0% (0/1) ^h
Autoimmune disease	19	19	0	-
SLE	11	11	0	-
Autoimmune hepatitis	5	5	0	-
Myeloma	20	20	0	-
Hyper-γglobulinemia	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
E. coli infection	10	10	0	-
TOTAL	535	497 (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 3/4 samples are total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative IgM anti-HBc are acceptable

^c 1/3 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 1 sample- HBsAg, total anti-HBc & anti-HBe positive indicating acute; false negative Abbott result

^e 3/9 samples are total anti-HBc and anti-HBe positive, 1 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 for additional testing. Sample is total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative results are acceptable

^g 2 of 3 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 2 samples are total anti-HBc, anti-HBs and anti-HBe positive, indicating recovery; both positive and negative results are acceptable

9.4. Interfering Substances

ETI-CORE-IGMK PLUS assay was evaluated for interference by testing the substances identified in the table below. 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 heterophilic anti-mouse antibodies (HAMA). A dilutional panel was used, consisting of 21 samples prepared from a stock pool of high positive human serum. 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 one-site immunoassay, such as 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.

9.5. Stability Studies

9.5.1. Kit Stability

Stability studies were performed on 3 different ETI-CORE-IGMK PLUS kit lots. At specified intervals from time of kit release, performance of the kits was evaluated testing the Calibrator, Negative and Positive Controls, and Quality Control sera panel according to the instructions for use. The kit must meet established acceptance criteria. The stability data demonstrate that the kit performance is acceptable for at least 6 months. On the basis of the stability results, a shelf life of 6 months has been established for the kit.

9.5.2. Working Enzyme Tracer Stability

The Enzyme Tracer was diluted with the Tracer Diluent to obtain the working Enzyme Tracer according to the instructions for use. After 7 days from dilution, the performance of the kit was evaluated, according to the instructions for use, testing various specimens with freshly prepared working Enzyme Tracer and the 7-days old working Enzyme Tracer. The kit must meet established acceptance criteria. The tests on the working Enzyme Tracer demonstrate that the performance of the kit is acceptable when the 7 day-diluted Enzyme Tracer is used. The working Enzyme Tracer can be used for one week if stored at 2-8 °C.

9.5.3. Working Wash Buffer Stability

The Wash Buffer concentrate was diluted with deionized water according to the instruction for use to obtain the working Wash buffer. After 7 days from dilution, the performance of the kit was evaluated by testing various specimens with a freshly prepared working Wash buffer and the 7-days-old working Wash buffer, according to the instructions for use. The kit must meet established acceptance criteria. The tests on the working Wash Buffer demonstrate that the performance of the kit is acceptable when the 7 day-old working Wash Buffer is used. The working Wash Buffer can be used for one week if stored at 2-8 °C.

9.6. Common Reagents Interchangeability Study

Studies were performed to demonstrate that the lots of some components included with ETI-CORE-IGMK PLUS kit and common to all kits of ETI-PLUS line (Wash Buffer, Chromogen/Substrate, Stop Solution), can be exchanged with other lots of the same component produced for the ETI-PLUS line (interchangeability). Three lots of Wash Buffer, Chromogen/Substrate and Stop Solution were combined with one lot of ETI-CORE-IGMK PLUS; the three combinations were then tested with various samples. Regression analysis was applied to the Optical Densities of the samples. The regression analyses for the three studies gave slopes close to 1.0, with low intercepts and excellent correlation values. These results indicate that the use of different lots of Wash Buffer, Chromogen/Substrate and Stop Solution with the same ETI-CORE-IGMK PLUS lot gave equivalent results with samples distributed over the range of reactivity, confirming their interchangeability.

9.7. 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 the 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 showing the reproducibility of the assay to be satisfactory.

Clinical Site Reproducibility Study

ID#		Number of tests per sample	Mean S/CO	Within-run %CV*	Between-runs %CV	Between-lots %CV	Between-days %CV	Between-sites %CV	Total
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.

9.8. Plasma Reproducibility

A plasma reproducibility study using samples near the cutoff was not conducted. The effects of the different anticoagulants in plasma after being diluted 1:4000 is thought to be minimal and the limited data presented by DiaSorin comparing samples with the different plasma matrixes and serum appear to support this assumption. Because the reproducibility performance has not been established with specimens at or near the cutoff the following statement has been placed in the labeling.

“Assay reproducibility using plasma has not been established with samples near the cutoff. If plasma is used, the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices”

9.9. Acute Serial Seroconversion Panels

One hundred twenty-four (124) archived serial samples from 9 individuals were analyzed for the appearance of IgM anti-HBc. 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 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.

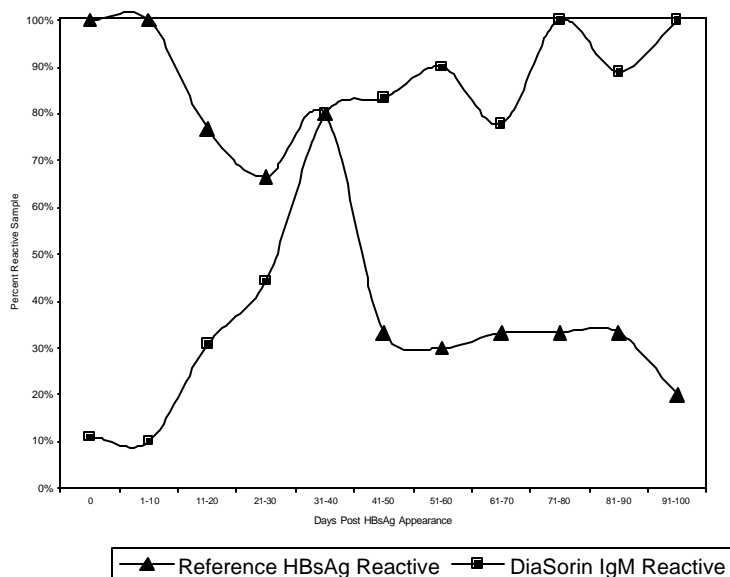
The specimens were collected from individuals undergoing plasmapheresis for further manufacturing purposes. Three individuals were found to be infected with HBV during the

first plasmapheresis and others became infected with HBV during subsequent plasmapheresis. It is unknown how long these three initially HBsAg reactives were infected prior to the first plasmapheresis. All nine individuals underwent sequential plasmapheresis after becoming HBV infected. However, the timing of subsequent plasmapheresis varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by a FDA licensed assay as Day 0. For the remaining specimens, 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 a dashed line for the reference HBsAg percent reactive has been overlaid for reference.

Acute Serial Panel Results

Day Range	Number Specimens	DiaSorin IgM anti-HBc Reactive	% 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	100.0%
81-90	9	8	88.9%
91-100	10	10	100.0%
101-110	6	6	100.0%
111-120	4	4	100.0%
121-130	4	4	100.0%
131-140	3	3	100.0%
141-150	2	2	100.0%
151-160	0	0	NA
161-170	1	1	100.0%
171-180	0	0	NA
181-190	1	1	100.0%
191-200	1	1	100.0%
> 200	1	1	100.0%



9.10. Expected Values Study

The 236 prospective samples used in the expected values study for the detection of IgM anti-HBc by the ETI-CORE-IGMK PLUS assay were from patients who were sent to the laboratory for HBV testing. Of those, 100 (42%) were frozen and 136 (58%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the southeastern US. The group was 69% (162/236) female and 31% (74/236) male; the

ethnicity of the patients was unspecified. The ages ranged from 5 to 88 years old. The prevalence rate for IgM anti-HBc in patients who were sent to the laboratory for HBV testing was 5%.

The Table below summarizes the percent DiaSorin ETI-CORE-IGMK PLUS positive and negative results by gender and age range. There were 6 samples for which gender and age were not reported; they were all positive. There were 6 samples for which age was not reported, 2 were from females and 4 were from males; all were negative. These 12 results were not included in the table.

Expected Values Summary

		ETI-CORE-IGMK PLUS				
		+		-		
Age Range	Gender	N	%	n	%	TOTAL
0-9	F	0	0	2	100	2
	M	0	0	0	0	0
10-19	F	1	6	16	94	17
	M	1	50	1	50	2
20-29	F	1	2	50	98	51
	M	3	23	10	77	13
30-39	F	0	0	49	100	49
	M	1	6	16	94	17
40-49	F	1	5	19	95	20
	M	1	7	13	93	14
50-59	F	1	20	4	80	5
	M	1	13	7	88	8
60-69	F	0	0	3	100	3
	M	0	0	2	100	2
70-79	F	0	0	10	100	10
	M	0	0	5	100	5
80-89	F	0	0	3	100	3
	M	0	0	3	100	3
TOTAL		11	5	213	95	224

9.11. Commercial panels

Single samples from two commercial performance panels (mixed-titer panel and low-titer panel) were tested with the DiaSorin ETI-CORE-IGMK PLUS assay. The mixed titer panel contained 25 samples that had been tested for IgM anti-HBc by the vendor using 8 commercially available assays. The panel consisted of 3 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 7 commercially available assays. The panel consisted of 1 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		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)

10. Summary of Clinical Studies

10.1. Clinical Sample Testing

10.1.1. Prospective Samples

A study of 136 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing. Specimens were collected at a reference laboratory and assayed at the California clinical trial site. The patients were 86% (117/136) female and 14% (19/136) male. The ages ranged from 5 to 77 years old, with 3 specimens not specified. The study (testing) sites were blinded to the previous specimen categorization. All testing was performed using 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 5 unique HBV marker patterns observed in the DiaSorin 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
Recovery	-	-	-	+	+	+	2
Past Infection	-	-	-	+	-	+	4
	-	-	-	+	-	-	4
HBV Vaccine Response	-	-	-	-	-	+	38
Not Previously Infected with HBV	-	-	-	-	-	-	87

Based on the above classifications the DiaSorin 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% confidence intervals with the reference IgM anti-HBc assay.

Prospective Samples Comparison

	-	Reference IgM anti-HBc
Reference Serology Classification	-	DiaSorin IgM anti-HBc
Chronic infection	1	1
Recovery	2	2
Past Infection	8	8
HBV vaccine response	38	38
Not previously infected with HBV	87	87
Grand Total	136	136

Prospective Samples Agreement Rates

Chronic Infection	Positive agreement with reference assay results = NA (0/0)
	95% CI = NA
Recovery	Negative agreement with reference assay results = 100% (1/1)
	95% CI = 2.5 to 100%
HBV Vaccine Response	Positive agreement with reference assay results = NA (0/0)
	95% CI = NA
Past Infection	Negative agreement with reference assay results = 100% (2/2)
	95% CI = 15.8 to 100%
Not Previously Infected	Positive agreement with reference assay results = NA (0/0)
	95% CI = NA
	Negative agreement with reference assay results = 100% (87/87)
	95% CI = 98.5 to 100%

10.1.2. 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 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. 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 6 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 as determined by the methods above. The following table shows this comparison and percent agreement with 95% confidence intervals with the reference IgM anti-HBc results.

Retrospective Samples Comparison

Reference Serology Classification	Reference IgM anti-HBc								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	343	0	0	0	0	0	0	0	343
Uninterpretable	17	0	0	0	0	0	0	0	17
Grand Total	564	18	2	1	53	1	1	10	650

* Indeterminate results

** Equivocal results

Retrospective Agreement Rates

Acute Infection	Positive agreement with reference assay results =	95.7% (45/47)
	95% CI =	85.5 to 99.5%
	Negative agreement with reference assay results =	100% (4/4)
Chronic Infection	95% CI =	39.8 to 100%
	Positive agreement with reference assay results =	100% (5/5)
	95% CI =	47.8 to 100%
Recovery	Negative agreement with reference assay results =	88.7% (118/133)
	95% CI =	82.1 to 93.5%
	Positive agreement with reference assay results =	100% (3/3)
HBV Vaccine Response	95% CI =	29.2 to 100%
	Negative agreement with reference assay results =	89.1% (41/46)
	95% CI =	76.4 to 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)
Not Previously Infected	95% CI =	83.9 to 100%
	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Uninterpretable	Negative agreement with reference assay results =	100% (343/343)
	95% CI =	98.9 to 100%
	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 to 100%

11. Conclusions Drawn from Studies

The study data demonstrates that acceptable performance is obtained with the DiaSorin ETI-CORE-IGMK PLUS assay when testing specimens collected in serum and plasma. The DiaSorin assay shows acceptable within-run, between-run, between-day, site-to-site, and lot-to-lot reproducibility. The quality control procedures described in the package insert are appropriate to assure accurate assay performance. The data from this study provide reasonable assurance that the DiaSorin ETI-CORE-IGMK PLUS assay is safe and effective for its stated purpose when used as instructed in the package insert. The DiaSorin ETI-CORE-IGMK PLUS assay can be stored up to 6 months at 2-8°C

12. Panel Recommendations

The Microbiology Advisory Panel met on January 20, 2000, to consider the safety and effectiveness of the ETI-CORE-IGMK PLUS assay. The panel recommended approval subject to the following conditions.

- Conduct additional studies for the immunity claim by testing individuals immediately after receiving the complete series of three vaccinations with the hepatitis B virus vaccines and three to nine months later.
- Provide more data on acute/chronic infections in high-risk populations such as those individuals that are infected with HIV, sexually transmitted diseases, and those patients that are immunosuppressed.
- Collect more data on patients meeting the standard definition for chronicity, i.e., > 6 months of infection

13. CDRH Decision

CDRH concurred with the Panel's recommendation. DiaSorin Inc. has provided some additional data to address some of the Panel's issues and those issues not fully resolved were addressed with labeling restrictions and the requirement of postapproval studies. The two postapproval studies were:

- Within 6 months of this approval, DiaSorin Inc. should submit a reproducibility study for the Biochem Immunosystems Labotech/ETI-Lab automated instrument.
- To address the concerns made by the Panel regarding the retrospective nature of the clinical studies, within 2 years of this approval, DiaSorin Inc. should submit the results of an additional prospective clinical study. We suggest that this study involve individuals that may be considered representative of an U.S. population, i.e., similar prevalence of HBV disease and serotypes.
- Within 6 months of approval, DiaSorin Inc. should submit a plasma reproducibility study for the ETI-CORE-IGMK PLUS assay.

The applicant's manufacturing facility was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

CDRH issued an approval order on March 30, 2001.

14. Approval Specifications

Directions for use: See Labeling

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the approval order.