

Summary of Safety and Effectiveness

1. General Information

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

1.2. Device Trade Name: ETI-AB-COREK PLUS

1.3. Applicant's Name and Address:

DiaSorin
Via Crescentino
Saluggia (VC) 13040, Italy

1.4. U.S. Representative:

Sienna Partners, LLC
P.O. Box 103
Baldwin, MD 21013

1.5. PMA Number: P990045

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

The detection of total anti-HBc is indicative of a laboratory diagnosis for exposure to hepatitis B virus (HBV) infection. Further HBV serological marker testing is required to define the specific disease-state. The ETI-AB-COREK 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-AB-COREK PLUS uses a monoclonal antibody directed to the immunodominant domain of hepatitis B core antigen as the basis for this enzyme immunoassay. The assay is a competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibody (IgG2b-k class) to HBcAg. An enzyme tracer containing horseradish

peroxidase-labeled human antibodies to HBcAg detects any captured total anti-HBc from the patient's specimen.

In the assay procedure, patient specimens or controls and recombinant HBcAg contained in the neutralizing solution are incubated in antibody-coated microwells. If total anti-HBc is present in a specimen or control, it competes with the antibody coated on the microwell for the recombinant HBcAg. Excess sample and recombinant HBcAg are removed by a wash step, and the enzyme tracer is then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. The quantity of enzyme tracer that binds to the solid phase via recombinant HBcAg and the consequent enzyme activity are inversely related to the anti-HBc concentration present in the specimen or control. Excess 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 does not contain total 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 contains total 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 inversely indicative of the concentration of total 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 total antibodies to HBc, the ETI-AB-COREK PLUS system is comprised of the following:

Coated Strips

Microwells coated with mouse monoclonal antibodies to HBcAg (IgG2b-k class; directed to immunodominant domain of hepatitis B core antigen).

Enzyme Tracer

Horseradish peroxidase-labeled human antibodies 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.
Preservative: 0.2% ProClin 300.

Negative Control (Human)

Human serum/plasma non-reactive for all known HBV markers.
Preservative: 0.2% ProClin 300.

Positive Control

Human IgG anti-HBc, protein stabilizers.
Preservative: 0.2% ProClin 300.

Neutralizing Solution

Recombinant HBcAg (22Kd, expressed in E-coli), buffer, protein stabilizers.
Preservative: 0.2% ProClin 300.

Incubation Buffer

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-AB-COREK PLUS assay are stated in the product labeling.

6. Alternative Practices and Procedures

Several methods are available for detecting antibodies to HBcAg in human serum or plasma. Methods commonly used include immunodiffusion, electrophoresis, counter immunoelectrophoresis, reverse passive hemagglutination, radioimmunoassay, and enzyme immunoassay.

7. Marketing History

The ETI-AB-COREK PLUS assay has never been marketed in the US or outside the US.

8. Potential Adverse Effects of the Device on Health

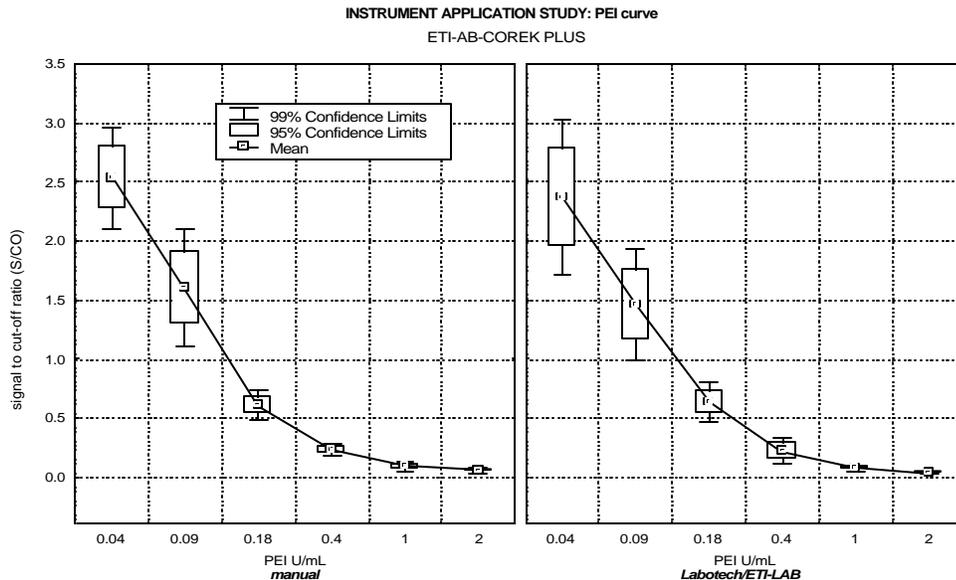
Failure of the product to perform as indicated or human error in use of the product may lead to a false result. A false result cannot be considered a patient or public health concern because the overall hepatitis B marker pattern will indicate the correct disease-state interpretation.

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-AB-COREK PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB, an automated microplate processing instrument, against the manual analysis. The Paul-Ehrlich-Institut (PEI) Standard, 12 serum samples near the ETI-AB-COREK PLUS cutoff and samples from the clinical trials (29 suspected hepatitis B patients and 11 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. In this assay, the S/CO is inversely related to reactivity. 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 < 0.9). 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. The data demonstrate that the ETI-AB-COREK PLUS assay can be performed manually and with the Labotech/ETI-LAB automated instrument. A summary of the data is presented in the following table.

	Manual			Labotech/ETI-LAB		
Analytical Endpoint Sensitivity (0.18 PEI U/mL)	Mean	W/R %CV ^a	D/D %CV	Mean	W/R %CV	D/D %CV
S/CO [95% CI ^b]	0.61 [0.53 - 0.69]	4.8	13.2	0.64 [0.53 - 0.74]	15.2	10.8
12 Cutoff Samples	0.95	8.3	5.6	1.08	19.1	15.5
Range of S/CO	0.60 – 1.57			0.75 – 1.66		
Clinical Samples:						
Suspected Hepatitis B Range of S/CO	Negative: 1.23 (1/29) Equivocal: N/A (0/29) Positive: 0.00 – 0.08 (28/29)			Negative: 1.42 (1/29) Equivocal: N/A (0/29) Positive: 0.00 – 0.15 (28/29)		
Healthy Adults Range of S/CO	Negative: 1.36 – 2.73 (11/11) Equivocal: N/A (0/11) Positive: N/A (0/11)			Negative: 1.38 – 2.98 (11/11) Equivocal: N/A (0/11) Positive: N/A (0/11)		

^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.

9.3. Potential Cross-Reacting Substances

Serum samples were obtained from patients belonging to a number of different disease categories listed below. Of the 525 potential interfering samples, 392 (75%) were negative and 133 (25%) were positive by ETI-AB-COREK PLUS. Among the 133 positive samples, 1 was QNS for reference testing and could not be confirmed by other serological markers, 127 were positive by reference testing or review of hepatitis B marker patterns for those samples. Some equivocal samples were not repeated due to volume constraints. Disease was determined by serological testing, there is no guarantee that the associated antibody was present in the tested material. Interference testing with the described specimens was not performed. The following table shows the summary of cross-reactivity tests:

Cross-Reactivity Study Results

GROUP	n	ETI-AB-COREK PLUS Negative or Equivocal Samples	ETI-AB-COREK PLUS Positive Samples	% Confirmed Positive By Additional Testing
Acute EBV infection	16	16	0	–
Acute CMV infection	20	19	1	100% (1/1)
Acute HSV infection	10	9	1	100% (1/1)
Acute Toxoplasmosis	18	16	2	100% (2/2)
Acute Parvovirus B19 infection	5	5	0	–
HTLV-I/II infection	50	39	11	100% (11/11)
Syphilis	26	23	3	100% (3/3)
HCV infection	50	35	15	87% (13/15)
HDV infection	20	2	18	100% (18/18)
HIV infection	50	32 ^a	18	100% (18/18)
Acute HAV infection	50	44 ^b	6	100% (6/6) ^c
Past HAV infection	50	24	26	96% (25/26) ^d
Rheumatoid infection (RF) +	40	36	4	100% (4/4)
Autoimmune disease including SLE	30	28	2	100% (2/2)
Autoimmune hepatitis	5	5	0	–
Myeloma	20	15	5	80% (4/5) ^e
Hypergammaglobulinemia	20	19	1	100% (1/1)
Influenza vaccine	5	5	0	–
Elevated liver enzymes	10	6	4	50% (2/4) ^f
Non-viral liver disease	30	14	16	100% (16/16) ^g
TOTAL	525	392 (75%)	133 (25%)	95% (127/133)

^a 1 sample was equivocal by DiaSorin, not repeated. All other markers were negative, indicating not previously infected; an equivocal result is acceptable.

^b 1 sample was equivocal by DiaSorin, not repeated. The sample was HBsAg and total anti-HBc positive and negative for other markers by reference method, indicating recovery; DiaSorin false negative result.

^c 3 QNS for reference method testing but reference method anti-HBs and anti-HBe positive, indicating recovery, true positive total anti-HBc.

^d 1 QNS for reference method testing, but negative for all other serological markers, indicating recovered.

^e anti-HBs positive and all other serological markers negative by reference method, indicating recovery; both positive and negative total anti-HBc results are acceptable.

^f 1 sample was QNS for reference method testing, but all other serological markers were negative, indicating past infection. 1 sample was anti-HBe positive by reference method testing, indicating recovery; false negative anti-HBc by reference method.

^g 1 samples was anti-HBc negative by reference testing but positive for anti-HBs and anti-HBe, indicating recovery, true positive total anti-HBc.

9.4. Interfering Substances

The ETI-AB-COREK PLUS assay was evaluated for interference by testing the following substances identified in the table below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with high-titer 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-AB-COREK PLUS assay was also evaluated for possible interference from human anti-mouse antibodies (HAMA). A dilutional panel was used, consisting of 21 samples prepared from a stock pool of HAMA 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 an indirect competitive assay such as the ETI-AB-COREK PLUS, interference would manifest as false positive results. No interference was seen in that all 21 dilutional samples including the sample with no HAMA were positive by the ETI-AB-COREK PLUS assay and confirmed by a reference method.

9.5. Stability Studies

9.5.1. Kit Stability

Stability studies were performed on 3 different ETI-AB-COREK 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 7 months. Based on the stability results, a shelf life of 7 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-AB-COREK 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-AB-COREK 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-AB-COREK 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-AB-COREK 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-AB-COREK 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. In this assay, the S/CO is inversely related to reactivity. 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	102	0.01	47.14	68.67	66.82	42.63	39.68	126.94
S02	High	102	0.43	13.49	20.13	33.83	16.47	28.23	48.64
S03	Low	98 ^a	0.51	9.94	20.80	40.13	13.29	24.98	50.19
S04	Low	102	0.66	10.09	19.59	30.47	14.31	19.78	46.00
S05	Neg	102	1.87	9.22	12.99	11.41	7.03	1.13	20.72
S06	High	102	0.46	9.32	19.25	23.22	10.89	23.04	32.90
S07	High	102	0.35	9.61	14.76	27.48	11.44	21.52	37.90
S08	Equiv	102	0.80	12.53	18.23	24.24	7.84	10.48	28.98
S09	Equiv	102	1.09	11.63	8.37	11.96	6.74	18.85	21.52
S10	Neg	102	3.23	5.80	9.98	9.36	3.44	5.81	16.62

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

^a Site 1 experienced dispensing errors for Sample #3 (S03) during Day 1, Lot 3, Run 2 and Day 2, Lot 3, Run 2. Four samples were lost.

9.8. Plasma Reproducibility

A plasma reproducibility study was conducted at DiaSorin, Saluggia Italy, to evaluate the performance of the manual ETI-AB-COREK PLUS assay on serum versus a variety of plasma types. The plasma types evaluated were citrate, heparin and EDTA. Sample sets of matched serum/multiple plasma were used in the study. A sample set was prepared by spiking the same high-positive sample into each of the matrices (serum and plasmas) resulting in a total of four specimens per set around the cutoff. Several high-positive samples were used in the preparation of the 12 different near-cutoff sample sets. Six matched serum/multiple plasma samples sets were tested in triplicate in each run; thus, there were two runs per day for three 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. The study supports the use of plasma specimens in the ETI-AB-COREK PLUS assay. A summary of the data is presented in the following table.

	Serum	Citrate	Heparin	EDTA
Mean S/CO	0.97	1.05	1.17	1.06
95% CI*	[0.96-0.99]	[1.04-1.06]	[1.16-1.18]	[1.05-1.07]
W/R %CV**	8.0%	7.7%	6.4%	6.4%
D/D %CV*	6.1%	7.3%	6.2%	8.0%
Total %CV	9.3%	10.2%	8.3%	10.1%
Between matrix %CV: 9.1%				
Across matrix total %CV: 12.6%				

* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day

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

9.9. Acute Serial Seroconversion Panels

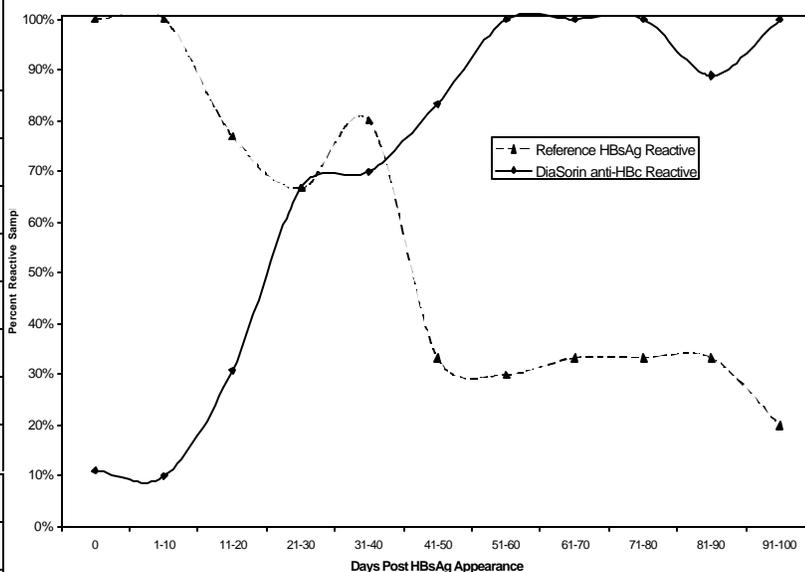
One hundred twenty-four (124) archived serial samples from nine individuals were tested for the appearance of anti-HBc. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequent appearance of IgM anti-HBc, total anti-HBc, anti-HBe, 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 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 total 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 Total anti-HBc positive	Percent Positive
0	9	1	11.1%
1-10	10	1	10.0%
11-20	13	4	30.8%
21-30	9	6	66.7%
31-40	10	7	70.0%
41-50	6	5	83.3%
51-60	10	10	100.0%
61-70	9	9	100.0%
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 ETI-AB-COREK PLUS assay to detect anti-HBc 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 anti-HBc in patients who were sent to the laboratory for HBV testing was 24%.

The table below summarizes the percent DiaSorin ETI-AB-COREK 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

		DiaSorin ETI-AB-COREK PLUS						
		+		-		E*		TOTAL
Age Range	Gender	N	%	n	%	n	%	
0-9	F	1	50	1	50	0	0	2
	M	0	0	0	0	0	0	0
10-19	F	4	24	13	76	0	0	17
	M	1	50	1	50	0	0	2
20-29	F	6	12	44	86	1	2	51
	M	5	38	8	62	0	0	13
30-39	F	5	10	44	90	0	0	49
	M	6	35	10	59	1	6	17
40-49	F	9	45	11	55	0	0	20
	M	7	50	7	50	0	0	14
50-59	F	1	20	4	80	0	0	5
	M	5	63	3	38	0	0	8
60-69	F	0	0	3	100	0	0	3
	M	0	0	2	100	0	0	2
70-79	F	2	20	8	80	0	0	10
	M	3	60	2	40	0	0	5
80-89	F	0	0	3	100	0	0	3
	M	0	0	3	100	0	0	3
TOTAL		55	24	167	75	2	1	224

E* = equivocal

9.11. High Risk Population

Single repository samples belonging to high-risk populations (66 hemodialyzed patients, 148 hemophiliacs, 150 IV drug users) were tested with the DiaSorin ETI-AB-COREK PLUS assay to determine frequency of positive results in that population. The group was 12% (42/364) female, 69% (252/364) male, and 19% (70/364) unspecified, with ages ranging from 19 to 87 years old. No geographical locations were specified. The table below summarizes the ETI-AB-COREK PLUS results. The data in the table represent the number of specimens in each category.

High Risk Population

Population	Frequency of Positive Results (# Positive/Total # Samples)
IV drug users	98/150 = 65.3%
Hemophiliacs	46/148 = 31.1%
Hemodialysis patients	8/66 = 12.1%
TOTAL	152/364 = 41.8%

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-AB-COREK 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 labeling, 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-AB-COREK 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 anti-HBc results for the prospective samples were compared to a reference assay's anti-HBc results. The following table shows this comparison and percent agreement with 95% confidence intervals with the reference anti-HBc assay.

Prospective Samples Comparison

Reference Serology Classification	Reference anti-HBc				TOTAL
	-		+		
	ETI-AB-COREK PLUS		ETI-AB-COREK PLUS		
	-	+	-	+	
Chronic infection	0	0	0	1	1
Recovery	0	0	0	2	2
Past infection	0	0	2	6	8
HBV vaccine response	37	1	0	0	38
Not previously infected	87	0	0	0	87
Grand Total	124	1	2	9	136

Prospective Samples Agreement Rates

Chronic Infection	Positive agreement with reference assay results = 100% (1/1) 95% CI = 2.5 to 100%
	Negative agreement with reference assay results = NA (0/0) 95% CI = NA
Recovery	Positive agreement with reference assay results = 100% (2/2) 95% CI = 15.8 to 100%
	Negative agreement with reference assay results = NA (0/0) 95% CI = NA
HBV Vaccine Response	Positive agreement with reference assay results = NA (0/0) 95% CI = NA
	Negative agreement with reference assay results = 97.4% (37/38) 95% CI = 86.2 to 99.9%
Past Infection	Positive agreement with reference assay results = 75.0% (6/8) 95% CI = 34.9 to 96.8%
	Negative agreement with reference assay results = NA (0/0) 95% CI = NA
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 = 95.8 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-AB-COREK PLUS assay in detecting 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 (diagnosed by a physician as having HBsAg positive for greater than 6 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-AB-COREK 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 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-AB-COREK 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
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-AB-COREK PLUS results for the retrospective samples were compared to a reference assay's 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 anti- HBc results.

Retrospective Samples Comparison

Reference Serology Classification	Reference anti-HBc						TOTAL
	-			+			
	ETI-AB-COREK PLUS			ETI-AB-COREK PLUS			
	-	+	E*	-	+	E	
Acute infection	3	0	1	0	56	0	60
Chronic infection	0	0	0	2	137	0	139
Recovery	0	0	0	0	50	0	50
Past infection	0	0	0	3	17	1	21
HBV vaccine response	15	4	1	0	0	0	20
Not previously infected	333	9	1	0	0	0	343
Uninterpretable	12	1	0	0	4	0	17
Grand Total	363	14	3	5	264	1	650

* Equivocal result

Retrospective Samples Agreement Rate

Acute Infection	Positive agreement with reference assay results = 100% (56/56)
	95% CI = 93.6 to 100%
Chronic Infection	Negative agreement with reference assay results = 75.0% (3/4)
	95% CI = 19.4 to 100%
Recovery	Positive agreement with reference assay results = 98.6% (137/139)
	95% CI = 94.9 to 99.8%
HBV Vaccine Response	Negative agreement with reference assay results = NA (0/0)
	95% CI = NA
Past Infection	Positive agreement with reference assay results = 100% (50/50)
	95% CI = 92.9 to 100%
Not Previously Infected	Negative agreement with reference assay results = NA (0/0)
	95% CI = NA
Uninterpretable	Positive agreement with reference assay results = NA (0/0)
	95% CI = NA
Acute Infection	Negative agreement with reference assay results = 75% (15/20)
	95% CI = 50.9 to 91.3%
Chronic Infection	Positive agreement with reference assay results = 81% (17/21)
	95% CI = 58.1 to 94.6%
Recovery	Negative agreement with reference assay results = NA (0/0)
	95% CI = NA
HBV Vaccine Response	Positive agreement with reference assay results = NA (0/0)
	95% CI = NA
Past Infection	Negative agreement with reference assay results = 97.1% (333/343)
	95% CI = 94.7 to 98.6%
Not Previously Infected	Positive agreement with reference assay results = 100% (4/4)
	95% CI = 39.8 to 100%
Uninterpretable	Negative agreement with reference assay results = 92.3% (12/13)
	95% CI = 64.0 to 99.8%

11. Conclusions Drawn from Studies

The study data demonstrates that acceptable performance is obtained with the DiaSorin ETI-AB-COREK 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-AB-COREK PLUS assay is safe and effective for its stated purpose when used as instructed in the package insert. The DiaSorin ETI-AB-COREK PLUS assay can be stored up to 7 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-AB-COREK 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.

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.