510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K060204

B. Purpose for Submission:
New device clearance

C. Measurand:
IgG antibodies to Epstein-Barr Virus early antigen-diffuse EA(D)

D. Type of Test:
Qualitative, CLIA

E. Applicant:
DiaSorin Inc.

F. Proprietary and Established Names:
DiaSorin LIAISON® EA IgG assay

G. Regulatory Information:

a) Regulation section:
21 CFR Part 866.3235, Epstein-Barr Virus Serological Reagents.

b) Classification:
Class I
Product Code:
LSE

c) Panel:
83 Microbiology

H. Intended Use:

a) Intended use(s):
Qualitative detection of specific IgG antibodies to Epstein-Barr virus Early Antigen Diffuse

b) Indication(s) for use:
The LIAISON® EA IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of IgG antibodies to Epstein-Barr virus early antigen-diffuse [EA(D)] in human serum. This assay can be used as an aid in the clinical laboratory diagnosis of Epstein-Barr viral Syndrome such as infectious mononucleosis (IM).

c) Special condition for use statement(s):
LIAISON® Control EA IgG kit is used in conjunction with LIAISON® EA IgG immunoassay for monitoring substantial reagent failure.

The new device is not intended to be sold over the counter and is for prescription use only. Warnings on applicant labeling state “Under United States federal law restricts this device to sale by or on the order of a licensed practitioner or physician”.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal or infant specimens. Assay performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, and other EBV-associated lymphomas.

d) Special instrument Requirements:

Materials required but not provided with the device: LIAISON® EA IgG immunoassay is performed on the LIAISON® Chemiluminescence Analyzer (Model 15970), a fully automated system with continuous loading combining the Chemiluminescence technology with magnetic micro particles as solid phase. The Analyzer was originally cleared on February 12, 2004 (reference K032844).

Other accessories such as LIAISON® Module, LIAISON® Starter Kit, LIAISON® Light Check, LIAISON® Wash System Liquid, LIAISON® Waste Bags, LIAISON® Cleaning Kit are also required to perform the assay.

I. Device Description:

Indirect chemiluminescence immunoassay

J. Substantial Equivalence Information:

a) Predicate device name(s):
DiaSorin ETI-EA-G

b) Predicate K number(s):
K992191

Comparison with predicate:

Characteristic	New Device	Predicate
	Liaison® EA IgG	**ETI-EA-G**
Similarities		
Intended Use	Qualitative detection of specific IgG antibodies to Epstein-Barr virus Early Antigen Diffuse	Qualitative and/or semi-quantitative detection of IgG antibodies to Epstein-Barr virus Early Antigen diffuse [EA(D)]
Indications for Use	Aid in the clinical laboratory diagnosis of Epstein-Barr Viral Syndrome in patients with signs and symptoms of EBV infection such as infectious mononucleosis.	Aid in the diagnosis of primary or reactivated infectious mononucleosis in adult and pediatric populations.
Antigens Used	Epstein-Barr early antigen-diffuse (47-kDa recombinant polypeptide)	Epstein-Barr virus early antigen-diffuse (47-kDa recombinant polypeptide)
Sample Matrix	Serum	Serum
Differences		
Type of Assay	Antibody Capture	Enzyme Linked Immunosorbent

Characteristic	New Device	Predicate
	Liaison® EA IgG	**ETI-EA-G**
	Chemiluminescence Immunoassay (CLIA)	assay (ELISA)
Sample Handling/Processing	Automated	Manual
Detector	Mouse monoclonal antibodies to human IgG conjugated to isoluminol derivative	Goat anti-human IgG conjugated to horseradish peroxidase
Capture Reagent	Magnetic microparticles coated with EA(d) recombinant polypeptide	Microtiter wells coated with Epstein-Barr Early Antigen (D) antigen-(47-kDa recombinant polypeptide)
Controls	Two (negative and positive)	Three (negative, positive and low positive)
Reagent Storage	On-board or in refrigerator	Refrigerator only
Calibration	Two-point verification of stored 10-point master curve	Single point calibrator (Qualitative) or 4-point curve (semi-quantitative)
Equivocal Zone	Yes (± 10% about the cutoff)	No
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (ELISA Plate reader)
Total Incubation Time	21 minutes	150 minutes
Interference Testing	Bilirubin to 20 mg/dl	Bilirubin to 30 mg/dl
	Hemoglobin to 1000 mg/dl	Hemoglobin to 2500 mg/dl
	Triglycerides (Triolein) to 3000 mg/dl	Triglycerides to 1000 mg/dl
		Cholesterol 10 1000 mg/dl

K. Standard/Guidance Document Referenced (if applicable):

L. Test Principle:

The method for the qualitative determination of specific IgG to EBV viral antigens early antigen-diffuse [EA(D)] recombinant polypeptide as an indirect chemiluminescence immunoassay (CLIA). The principal components of the test are magnetic particles (solid phase) coated with EBV synthetic peptides and a conjugate of mouse monoclonal antibody to human IgG or linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, EA(D) antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with EBV IgG antibodies that are already bound to the solid phase. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a

photomultiplier results expressed as U/ml and is indicative of the presence of EBV EA(D) IgG antibodies present in calibrators, samples or controls.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*
   This study was conducted at three external US laboratories and at the applicant address (Stillwater, MN). A nine-member serum coded panel was prepared at DiaSorin, Italy and provided to each site for testing by the applicant device (LIAISON® EA IgG assay). Seven of the nine samples were selected or prepared so as to exhibit a low- to mid-positive Analyte level (i.e. samples demonstrating an expected analyte level approximately two to five times the cutoff). All panel members were divided into aliquots and stored frozen prior to testing. Samples EAS1-3 were tested at sites #1, #3, and #5, while EA1-6 were tested at sites #2, #3, and #5. The same coded panel was tested at all three sites, in three replicates per run for ten runs. LIAISON® EA IgG results were expressed in U/ml. The mean, standard deviation, and coefficient of variation (%CV) of the results were computed by the total and different components of variability for each of the tested specimens for each of the sites and across sites.. The results are summarized in the following tables.

LIAISON® EA IgG

		mean	within run	within run	between run	between run	between site	between site	total	total
ID#	**N**	**U/ml**	**StD**	**%CV**	**StD**	**%CV**	**StD**	**%CV**	**StD**	**%CV**
EAS1	90	47.6	1.78	3.71	4.56	8.65	2.46	5.16	4.78	10.04
EAS2	90	99.1	6.10	6.06	9.24	8.60	4.64	4.68	10.82	10.92
EAS3	90	112.3	7.50	7.07	15.81	13.19	2.84	2.53	17.26	15.36
EA1	90	18.2	0.93	5.17	1.56	5.50	1.37	7.52	1.85	10.15
EA2	90	20.4	0.63	3.17	2.27	5.87	2.31	11.33	2.34	11.45
EA3	90	47.4	2.07	4.60	4.87	5.18	5.09	10.75	5.28	11.15
EA4	90	35.9	1.58	4.60	4.02	6.02	4.08	11.35	4.30	11.99
EA5	90	52.4	2.03	3.88	4.54	3.55	5.01	9.56	4.94	9.43
EA6	90	18.1	0.60	3.38	1.72	4.12	1.86	10.27	1.80	9.91

   The total observed precision (%CV) for the LIAISON® EA IgG assay for low- to high-positive serum samples ranged from 9.4% to 15.4% with a mean of 11.2%. The predicate device, DiaSorin ETI-EA-G ELISA, reported total %CVs ranging from 6.6% to 15.9% for similar samples with a mean of 10.2%. The two assays are relatively equivalent.

   b. *Linearity/assay reportable range:*
   NA

   c. *Traceability, Stability, Expected values (controls, calibrators, or method):*

Controls are provided specifically designed for use in conjunction with the performance of the assay, for more information see the section regarding matrix comparison below.

d. *Detection limit:*

See assay cut-off below

e. *Analytical specificity:*

The cross-reactivity studies for the LIAISON® EA IgG assay were designed to evaluate potential interference from IgG immunoglobulins directed against closely-related members of the herpes virus family (e.g. HSV-1, HSV-2, VZV, CMV), from other organisms that may cause symptoms similar to EBV (Toxoplasma gondii, rubella virus) and from other conditions that may result from atypical immune system activity (rheumatoid factor (RF)). Samples for these studies were selected using commercially available devices.

Organism / condition	Number of Samples	Positive or Equivocal LIAISON® EA IgG Result
EBV VCA IgG	21	0/21
EBV VCA IgG/EBNA IgG	25	1/25
CMV IgG	25	2/25
VZV IgG	22	1/22
HSV-1 IgG	23	0/23
HSV-2 IgG	21	3/21
Toxoplasma gondii IgG	20	2/20
Rubella virus IgG	24	0/24
Hepatitis B virus (anti-HBs)	15	1/15
HIV	11	2/11
RF	2	0/2
ANA	8	0/8
HAMA	5	0/5
Total	222	11/222

Eleven specimens out of 222 total specimens tested from the cross-reaction panel returned positive or equivocal results in the LIAISON® EA IgG assay. Seven of the eleven discordant samples were equivocal by LIAISON® EA IgG. Equivocal results by LIAISON® EA IgG are included in the calculations of non-agreement since it was not possible to acquire follow-up samples collected one to two weeks later as recommended.

f. Assay cut-off:
The cutoff for the LIAISON® EA IgG assay was determined during European clinical trials in which 519 samples were run at two different sites. The samples consisted of either single samples or serial panel from different selected populations (subjects never infected by EBV, patients affected by primary EBV infection, subjects with past EBV infection, patients with suspected EBV reactivation and transplant recipients). The samples were tested in parallel with the LIAISON® EA IgG assay and a commercially available ELISA assay as the comparison method. Consensus with the serological data such as the combined EBV patients was applied to define the expected results: 208 samples were expected to be EA IgG positive and 291 samples were expected to be EA IgG negative, while 20 specimens were equivocal either by the comparison method or the original classification and therefore were not included in the data analysis.
The analysis of the data determined that the best sensitivity and specificity balance was obtained using a cutoff at 10 U/ml. Therefore, in the LIAISON® EA IgG assay, a sample is defined as positive if the level is equal or greater than 10 U/ml, and defined as negative of the level is lower than 10 U/ml.
The selected cutoff was validated in the US clinical trials. To account for observed assay imprecision, an equivocal zone was established about the cut-off. A nominal equivocal zone of ± 10% (dose) was assigned to this assay (for more detail on the establishment of this zone, see the response to question 4 on page 2 of 13 within the second deficiency response). Typical total imprecision (%CV) across the range of the assay is approximately 10%. Based upon the observed separation of the infected and non-infected patient responses, it is not likely that a significant percentage of samples will be affected by the equivocal zone. A total of 70 selected archive samples and 823 unselected prospectively collected samples were tested in the US trials. The resulting data clearly indicated separation for positive and negative results provided by the selected cutoff.

2. Comparison studies:

a. Method comparison with predicate device:
DiaSorin LIAISON® EA IgG assays were compared to the DiaSorin ETI-EA-G assays.

b. Matrix comparison:

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3. Clinical studies:

a. Clinical sensitivity:
See Performance Characteristics below

b. Clinical specificity:
See Performance Characteristics below

c. Other clinical supportive data (when a and b are not applicable):

Clinical Studies

The objective was to obtain sufficient data from a well-controlled clinical trial to support the intended use statement and product claims. Specific objectives were:

i. Evaluate the substantial equivalence of the LIAISON® EA IgG assay to an FDA cleared method (DiaSorin ETI-EA-G; K992191).
ii. Describe the ability of the LIAISON® EA IgG assay to discriminate between samples from subjects not previously infected by EBV and those that have had either previous or current contact with EBV.
iii. Verify the precision of the assay using coded panels supplied by the DiaSorin.
iv. Verify the quality control procedures of the assay using the LIAISON® EA IgG Control Sera.

Four external US laboratories and DiaSorin, Stillwater, MN performed testing. The testing was conducted on retrospective and prospective samples that were collected and tested as described in the following table.

	SITE #1, #2, and #5	SITE #3	SITE #4
Type of Samples Tested	Coded Reproducibility Panel	US Prospective Samples (460) Repository Samples with serological data Coded Reproducibility Panel	US Prospective Samples (363)
Population	N/A	Individuals sent to the lab for EBV testing EBV IgM reactive patients	Individuals sent to the lab for heterophile antibody or VCA IgM testing
# Samples Tested	N/A	530	363
Comparison Assay	N/A	DiaSorin ETI-EA-G (ELISA)	DiaSorin ETI-EA-G (ELISA)
Notes	N/A	A reference laboratory prospectively collected the EBV routine samples and supplied the relevant data. The samples were tested at Site #3.	A reference laboratory prospectively collected the EBV routine samples and supplied the relevant data. The samples were tested at Site #4.

The samples were tested with the LIAISON® EA IgG and the comparison assay at the trail sites, per manufacturers instruction for use. The technicians performing the testing were blinded to the samples' previous results. The resulting data was sent to the clinical site monitor for analysis by DiaSorin.

Study Population: Testing was performed on repository and prospective samples as follows:

i. 460 residual specimens prospectively collected from non-selected subjects that were sent to the laboratory for EBV testing. These were excess serum drawn from patients for whom EBV testing was requested, with sufficient volume to perform all testing required. Collection followed an IRB-approved protocol, with a waiver of informed consent, as there was no link to patient identity. The samples were stored frozen at –20°C prior to testing.
ii. 363 residual specimens prospectively collected from non-selected subjects that were sent to the laboratory for heterophile antibody (Monospot) of VCA IgM Testing. These were excess serum drawn from patients for whom EBV testing was requested, with sufficient volume to perform all testing required. Collection followed an IRB-approved protocol, with a waiver of informed consent, as there was no link to patient identity. The samples were stored frozen at –20°C prior to testing.
iii. 70 single samples from patients with serologic results suggesting an acute EBV infection. The sample selection was based on a specific EBV serological profile: VCA IgG positive, VCA IgM positive.

Study Analysis: LIAISON® EA IgG results were expressed in U/ml. Samples returning values < 9.0 U/ml were classified as negative, samples between 9.0 and 10.9 U/ml as equivocal and those greater than ≥ 11.0 U/ml as positive. The DiaSorin ETI-EA-G kit is an indirect enzyme-linked immunosorbent assay in which the results were expressed in AU and the threshold for negative samples is 20 AU. There is no equivocal zone in the predicate device. The performance of the LIAISON® EA IgG assay was determined by the percent agreement amount negative samples, percent agreement among positive samples, and overall percent agreement with the reference method in specific populations. The relevant 95% confidence limits were computed by applying the extract method. Results are detailed below:

Retrospective Samples: 70 frozen archived (retrospective) single samples with a serological pattern consistent with acute EBV infection (based on IgG and IgM anti-VCA positive). The group was 50% female. Approximately 31% (22) were below 18 years of age and the other 69% (48) ranged between 19 and 42 years of age. Comparison is identified below.

	VCA IgG	VCA IgM	EBNA-1 IgG	Total
EBV seronegative	–	–	–	0
Primary infection	+	+	–	9
Convalescent	+	+	+	61
Past infection	+	–	+	0
Indeterminate				0
VCA IgG only	+	–	–	0
VCA IgM only	–	+	–	0
EBNA IgG only	–	–	+	0
All				70

Based on these serological classifications, the LIAISON® EA IgG results for the retrospective samples were compared with those obtained with the reference assays.

	Positive % Agreement	95% CI
Primary Infection	100.0% (9/9)	21.2-100%
Convalescent	100% (61/61)	95.2-100%
Total Positive Agreement	100% (70/70)	86.0 – 100.0%

Prospective Samples: Using the results for the prospective samples in three reference assays (VCA IgG, EBNA-1 IgG, VCA IgM ELISA), the samples were grouped into serological categories. "Indeterminate" refers to serological patterns that are not consistent with the typical EBV categories; EBV seronegative, acute or past infection. The profiles and number of occurrences are presented in the following table.

Using the results for the prospective samples in three reference assays (VCA IgG, EBNA-1 IgG and VCA IgM ELISA), the samples were grouped into serological categories. The profiles and number of occurrences are presented in the following table:

Prospective samples: Subjects Sent to the Laboratory for EBV Testing

	VCA IgG	VCA IgM	EBNA-1 IgG	Total
EBV seronegative	–	–	–	63
Primary infection	+	+	–	29
Past infection	+	–	+	576
Indeterminate				82
				67
				5
				10
				73

	n	VCA IgG	VCA IgM	EBNA-1 IgG	LIAISON® EA IgG		
					Pos	Neg	Eqv
EBV seronegative	63	–	–	–	1	62	
Primary infection	29	+	+	–	25	3	1
Convalescent	73	+	+	+	44	26	3
Past infection	576	+	–	+	224	328	24
Indeterminate*							
VCA IgG only	67	+	–	–	21	45	1
VCA IgM only	5	–	+	–	1	4	
EBNA IgG only	10	–	–	+	1	9	
Total	823				317	477	29

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	n	VCA IgG	VCA IgM	EBNA-1 IgG	LIAISON® EA IgG		
					Pos	Neg	Eqv
EBV seronegative	63	–	–	–	1	62	
Primary infection	29	+	+	–	25	3	1
Convalescent	73	+	+	+	44	26	3
Past infection	576	+	–	+	224	328	24
Indeterminate*							
VCA IgG only	67	+	–	–	21	45	1
VCA IgM only	5	–	+	–	1	4	
EBNA IgG only	10	–	–	+	1	9	
Total	823				317	477	29

95% Confidence Levels based on the above serological classifications are as follows:

EBV seronegative	**Agreement**	**95% CI**
Negative Agreement	62/63 = 98.4%	95.1 – 100%
Primary Infection		
Positive Agreement	25/29 = 86.2%	71.2 – 95.2%
Convalescent*		
Positive Agreement	44/73 = 60.3%	49.9 – 69.9%
Past Infection		
Negative Agreement	328/576 = 56.9%	53.4 – 60.4%
Indeterminate**		
Positive Agreement	22/25 = 88%	71.8 – 96.6%
Negative Agreement	56/57 = 98.2%	95.0 – 99.9%

* Early Antigen antibodies may or may not be present in convalescence.

** Indeterminate Positive and Negative agreements were established based on a comparison to a Reference EA(d) IgG
ELISA assay and include VCA IgG only, VCA IgM only and EBNA IgG only samples as indicated above.

4. Clinical cut-off:
   See assay cut-off previously described in this documen
5. Expected values/Reference range:
   The LIAISON® EA IgG assay was tested with prospectively collected samples from subjects sent to the laboratory for EBV testing (n=823) to evaluate the prevalence of IgG antibodies to EA(D) in these populations. The subjects sent to the laboratory for EBV testing were 61.7% female (508), 28.1% male (231) and 10.2% unknown (84) and represented the mid-Atlantic and Northeastern US.

The distribution of results for IgG antibodies to EA(D) in this population as determined by the LIAISON® EA IgG Assay is summarized in the following table.

	N	Negative	Equivocal	Positive	Prevalence
Total	823	477	29	317	38.5%
Gender					
Female	508	288	19	201	39.6%
Male	231	137	7	87	37.7%
Unknown	84	52	3	29	34.5%
Age (years)					
≤ 18	173	114	8	51	29.5%
<10	29	20	1	8	27.6%
10 – 19	183	119	8	56	30.6%
20 – 29	190	117	7	66	34.7%
30 – 39	95	55	1	39	41.1%
40 – 49	78	32	3	43	45.3%
50 – 59	59	30	4	25	42.4%
60 – 69	32	14	1	17	53.1%
≥ 70	27	17	0	10	37.0%
Unknown	130	73	4	53	40.8%

N. Proposed labeling:

The labeling is sufficient and it satisfies the requirement of 21 CFR Part 809.10.

WARNINGS and PRECAUTIONS:

1. For *in vitro* diagnostic use.
2. The human blood source material used to produce the components provided in this kit is derived from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 when tested by an FDA-approved method 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 Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue (9, 10, 11).
3. Some reagents contain sodium azide as a preservative. Because sodium azide may form explosive lead or copper azide in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide.
4. Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

5. Do not pipette solutions by mouth.
6. Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.
7. Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.
8. All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each Country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least 30 minutes. Any materials to be reused must be autoclaved using an *overkill* approach (USP 24, 2000, p. 2143). A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
9. Specimens with elevated lipids (3000 mg/dL) may give erroneous results.
10. Assay interference due to circulating antibodies against Hepatitis A and Hepatitis C viruses has not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents.
11. Assay cross-reactivity has been noted with some specimens containing antibody to Human Immunodeficiency Virus (HIV). Reactive results must contain a caution statement regarding possible cross-reactivity with HIV. HIV disease must be excluded before confirmation of diagnosis.

O. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.