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

A. 510(k) Number:
k040120

B. Purpose for Submission:
New device clearance

C. Measurand:
Epstein Barr Virus IgG to Viral Capsid Antigen, Epstein Barr Virus IgM to Viral Capsid Antigen, and Epstein Barr Virus IgG to Nuclear Antigen

D. Type of Test:
Qualitative, CLIA

E. Applicant:
DiaSorin Inc.

F. Proprietary and Established Names:
DiaSorin LIAISON® VCA IgG
DiaSorin LIAISON® VCA IgM
DiaSorin LIAISON® EBNA IgG

G. Regulatory Information:

a) Regulation section:
Epstein–Barr Virus, serological reagents (21 CFR 866.3235).

b) Classification:
Class I
Product Code:
LSE
LLM

c) Panel:
83 Microbiology

H. Intended Use:

a) Intended use(s):
The LIAISON® VCA IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of specific IgG antibodies to Epstein-Barr virus (EBV) viral capsid antigen (VCA) p18 synthetic peptide in human serum. When performed in conjunction with other EBV markers, this assay can be used as an aid in the clinical laboratory diagnosis of Epstein-Barr Viral Syndrome in patients with signs and symptoms of EBV infection such as infectious mononucleosis.

The LIAISON® EBV IgM assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of specific IgM antibodies to Epstein-Barr virus (EBV) viral capsid antigen (VCA) p18 synthetic peptide in human serum. When performed in conjunction with other EBV markers, this assay can be used as an aid in the

clinical laboratory diagnosis of Epstein-Barr Viral Syndrome in patients with signs and symptoms of EBV infection such as infectious mononucleosis.

The LIAISON® EBNA IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of specific IgG antibodies to Epstein-Barr virus (EBV) nuclear antigen synthetic peptide (EBNA-1) in human serum. When performed in conjunction with other EBV markers, this assay can be used as an aid in the clinical laboratory diagnosis of Epstein-Barr Viral Syndrome in patients with signs and symptoms of EBV infection such as infectious mononucleosis.

b) Indication(s) for use:
The LIAISON® VCA IgG, the LIAISON® VCA IgM, and the LIAISON® EBNA IgG assay is for the laboratory diagnosis of EBV-associated infectious mononucleosis in individuals with signs and symptoms consistent with infectious mononucleosis.

c) Special condition for use statement(s):
The device is for prescription use only

d) Special instrument Requirements:
NA

I. Device Description:

Indirect chemiluminescence immunoassay

J. Substantial Equivalence Information:

a) Predicate device name(s):
DiaSorin ETI-VCA-G Kit
DiaSorin ETI-EBV-M reverse Kit
DiaSorin ETI-EBNA-G Kit

b) Predicate K number(s):
K946159
K946157
K946158

Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	DiaSorin LIAISON® VCA IgG, LIAISON® VCA IgM, and the LIAISON® EBNA IgG assay (K041020)	DiaSorin ETI-VCA-G Kit DiaSorin ETI EBV-M Reverse Kit, DiaSorin ETI-EBNA-G Kit
Same target population.	Test persons who have symptoms of infectious mononucleosis	Test persons who have symptoms of infectious mononucleosis

Differences		
Item	**Device**	**Predicate**
Different Methodology	Indirect chemiluminescence immunoassay	IgG Indirect ELISA
Different Indications for Use	Qualitative	Qualitative/Semiquantitative
Capture Reagent	Magnetic particles coated with antigen	Microtiter plate wells coated with antigen
Detector antibody Species	Mouse	Goat
Equivocal Zone	Yes	No

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

L. Test Principle:

The method for the qualitative determination of specific IgG or IgM to EBV viral antigens (VCA, EBNA) is 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 IgM linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, VCA or EBNA antibodies, if present, bind to the solid phase. During the second incubation, the antibody conjugate reacts with EBV IgG or IgM antibodies that are bound to the solid phase. After each incubation step, unbound material is removed with wash cycles. 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 as relative light units (RLU) and is indicative of the presence or absence of EBV VCA or EBNA IgG, or EBV VCA IgM antibodies.

M. Performance Characteristics (if/when applicable):

Analytical performance:

a) *Precision/Reproducibility:*

An assay reproducibility study was conducted at two external US laboratories and at DiaSorin. A coded panel comprised of 9 frozen repository serum samples was prepared by DiaSorin and provided to each site for testing by the LIAISON® VCA IgG, VCA IgM and by the LIAISON® EBNA assay. The panel members were prepared to represent low to mid positive analyte levels. All panel members were divided into aliquots and stored

frozen prior to testing. The same coded panel was tested at all three sites, in three replicates per run for ten runs. The results are summarized in the following tables.

LIAISON® VCA IgG

ID#	N	mean U/mL	within run S.D.	within run %CV	between run S.D.	between run %CV	between site S.D.	between site %CV	overall S.D.	overall %CV
VGS1	90	266.6	9.86	3.68	23.56	7.23	12.98	4.87	25.19	9.45
VGS2	90	52.9	1.89	3.83	3.75	5.43	2.81	5.32	4.35	8.22
VGS3	90	145.5	7.55	5.78	11.69	7.41	4.71	3.23	15.37	10.56
VG1	90	31.6	0.71	2.31	2.94	6.36	2.64	8.35	2.99	9.45
VG2	90	52.2	1.09	2.06	2.88	5.43	0.97	1.82	3.07	5.89
VG3	90	61.0	1.17	1.92	3.75	5.64	2.05	3.35	3.86	6.32
VG4	90	69.2	1.58	2.31	4.56	5.38	3.28	4.74	4.79	6.92
VG5	90	58.0	1.21	2.11	3.07	4.08	2.47	4.26	3.27	5.63
VG6	90	49.6	1.23	2.46	3.05	5.76	1.46	2.94	3.25	6.55

LIAISON® VCA IgM

ID#	N	mean U/mL	within run S.D.	within run %CV	between run S.D.	between run %CV	between site S.D.	between site %CV	overall S.D.	overall %CV
EMS1	90	67.2	2.22	3.30	3.36	4.26	2.21	3.28	3.92	5.83
EMS2	89	89.7	4.00	4.55	6.90	5.16	6.26	6.98	7.79	8.70
EMS3	90	<10.0	*69.6**	*7.15**	*83.6**	*6.50**	*69.5**	*7.35**	*115.7**	*12.24**
EM1	90	36.4	2.79	7.88	4.93	5.50	5.43	14.92	5.52	15.16
EM2	90	37.2	2.23	6.09	5.43	6.17	5.91	15.90	5.77	15.52
EM3	90	79.5	6.22	7.79	12.04	5.63	13.16	16.55	13.25	16.67
EM4	89	65.9	4.34	6.60	8.95	5.35	9.95	15.10	9.77	14.83
EM5	89	37.1	2.65	7.27	4.25	6.92	4.14	11.16	4.88	13.18
EM6	90	64.6	4.26	6.65	6.07	6.58	5.36	8.30	7.18	11.11

**EMS3 dose was below the reading range of the assay. Precision calculations are based on signal (RLU) for this sample.*

LIAISON® EBNA IgG

ID#	N	mean U/mL	within run S.D.	within run %CV	between run S.D.	between run %CV	between site S.D.	between site %CV	overall S.D.	overall %CV
EBS1	90	28.5	0.73	2.57	1.04	2.70	0.83	2.90	1.24	4.35
EBS2	90	62.2	4.81	6.94	8.84	11.78	3.70	5.95	15.02	24.15
EBS3	90	290.4	12.18	4.27	26.81	6.02	24.49	8.43	29.04	10.00
EB1	90	72.2	1.81	2.48	3.32	2.82	3.12	4.32	3.72	5.15
EB2	90	71.0	2.12	3.01	5.45	3.86	5.74	8.09	5.73	8.08
EB3	90	52.6	1.71	3.27	3.06	4.30	2.53	4.81	3.45	6.55
EB4	90	48.6	1.51	3.17	3.30	4.72	2.93	6.04	3.57	7.34
EB5	90	59.9	1.76	2.98	3.10	4.35	2.18	3.63	3.53	5.88
EB6	90	51.6	1.69	3.33	2.61	4.61	1.46	2.83	3.06	5.94

a. ***Linearity/assay reportable range:***
NA

b. Traceability, Stability, Expected values (controls, calibrators, or method):
NA

c. ***Detection limit:***
NA

d. Analytical specificity:

The cross-reactivity studies for the LIAISON® EBV assays were designed to evaluate potential interference from IgG immunoglobulins directed against closely-related members of the herpes virus family (HSV-1, HSV-2, VZV, CMV, HHV6), 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 LIAISON® VCA IgG Result
CMV IgG	16	(1/16)
VZV IgG	7	(0/7)
HSV-1 IgG	18	(2/18)
HSV-2 IgG	3	(0/3)
HHV6 IgG	3	(0/3)

Toxoplasma gondii IgG	8	(1/8)
Rubella virus IgG	30	(0/30)
RF	4	(0/4)
Total	89	(4/89)

Four specimens out of 89 total specimens tested from the disease panel were positive. Due to the limited availability of certain samples, the possibility of cross-reactivity cannot be excluded. The user is advised to perform other EBV serology assays to confirm EBV-associated infectious mononucleosis.

Organism / condition	Number of Samples	Positive LIAISON® EBV IgM Result
CMV IgM	29	(2/29)
VZV IgM	16	(1/16)
HSV-1 IgM	2	(0/2)
HSV-2 IgM	7	(0/7)
Hepatitis A virus IgM	10	(0/10)
Hepatitis B virus (core) IgM	23	(0/23)
Toxoplasma gondii IgM	12	(0/12)
Rubella virus IgM	11	(0/11)
Rubeola virus IgM	3	(0/3)
Mumps virus IgM	2	(1/2)
RF	6	(0/6)
ANA Ig	10	(0/10)
Total	131	(4/131)

Four specimens out of 131 total specimens tested from the disease panel were positive. Due to the limited availability of certain samples, the possibility of cross-reactivity cannot be excluded. Other EBV serology assays should be performed to confirm EBV-associated infectious mononucleosis.

Organism / condition	Number of Samples	Positive LIAISON® EBNA IgG Result
CMV IgG	19	(0/19)
VZV IgG	7	(0/7)
HSV-1 IgG	19	(1/19)

HSV-2 IgG	3	(0/3)
HHV6 IgG	1	(0/1)
Toxoplasma gondii IgG	7	(0/7)
Rubella virus IgG	30	(1/30)
ANA	1	(0/1)
RF	4	(0/4)
Total	91	(2/91)

Two specimens out of 91 total specimens tested from the disease panel were positive. Due to the limited availability of certain samples, the possibility of cross-reactivity cannot be excluded. The user is advised to perform other EBV serology assays to confirm EBV-associated infectious mononucleosis.

a. Assay cut-off:

The cutoff for the LIAISON® VCA IgG assay was determined during European clinical trials in which 1133 samples were tested at four separate sites. The LIAISON® EBV IgM assay was determined using 1332 samples and the LIAISON® EBNA IgG assay cutoff was determined using 1166 samples. The samples were drawn from several different populations, including seronegative subjects, subjects with primary EBV infection, past EBV infection, suspected chronic EBV infection and reactivated EBV infection and from an apparently healthy adult population.

b. Comparison studies:

a. Method comparison with predicate device:

DiaSorin LIAISON® EBV assays were compared to the DiaSorin ETI-EBV assays.

Matrix comparison:

NA

c. Clinical studies:

a. ***Clinical sensitivity:***

NA

b. Clinical specificity:

NA

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

PERFORMANCE CHARACTERISTICS

A total of 893 samples was tested – 823 prospectively and 70 retrospectively collected. The prospective samples represented 823 samples from subjects sent to the laboratory for EBV testing. The retrospective samples represented 70 samples from patients positive for

VCA IgM. The testing was performed at three sites – a hospital, a donor laboratory, and at DiaSorin. All samples were tested with the DiaSorin LIAISON® EBV Assays and the DiaSorin ETI-EBV ELISAs.

Retrospective Samples: VCA IgM-positive Samples

Using the results for the retrospective samples in three reference assays (VCA IgG, EBNA-1 IgG and VCA IgM ELISA), the samples were grouped into serological categories. Indeterminate is defined as a serological category which does not fall into the typical EBV marker pattern for EBV seronegative, acute or past infection. The profiles and number of occurrences are presented in the following table:

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

Based on these serological classifications, the LIAISON® VCA IgG results for the retrospective samples were compared with those obtained with the reference assay (VCA IgG ELISA).

	Percent Agreement	95% confidence interval
EBV	N/A	N/A
Acute infection	100.0% (9/9)	71.7 – 100.0%
Past infection	N/A	N/A
Indeterminate	100.0% (61/61)	95.2 – 100.0%
Overall	100.0% (70/70)	95.8 – 100.0%

Based on these serological classifications, the LIAISON® EBV IgM results for the retrospective samples were compared with those obtained with the reference assay (VCA IgM ELISA).

	Percent Agreement	95% confidence interval
EBV	N/A	N/A
Acute infection	77.8% (7/9)	40.0 – 97.2%
Past infection	N/A	N/A
Indeterminate	98.4% (60/61)	91.2 – 100.0%
Overall	95.7% (67/70)	88.0 – 99.1%

Based on these serological classifications, the LIAISON® EBNA IgG results for the retrospective samples were compared with those obtained with the reference assay (EBNA-1 IgG ELISA).

	Percent Agreement	95% confidence interval
EBV	N/A	N/A
Acute infection	55.6% (5/9)	21.2 – 86.3%
Past infection	N/A	N/A
Indeterminate	100.0% (61/61)	95.2 – 100.0%
Overall	94.3% (66/70)	86.0 – 98.4%

Prospective samples: Subjects Sent to the Laboratory for EBV Testing

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. Indeterminate is defined as a serological category which does not fall into the typical EBV marker pattern for EBV seronegative, acute or past infection. Four samples tested in the LIAISON® VCA IgG assay had insufficient volume for the entire test profile and are omitted from this analysis. The profiles and number of occurrences are presented in the following table:

	VCA IgG	VCA IgM	EBNA-1 IgG	Total
EBV seronegative	–	–	–	62
Acute infection	+	+	–	29
Past infection	+	–	+	573
Indeterminate				
VCA IgG only	+	–	–	67
VCA IgM only	–	+	–	5
EBNA IgG only	–	–	+	10
Convalescent	+	+	+	73

Based on these serological classifications, the LIAISON® VCA IgG results for the prospective samples were compared with those obtained with the reference assay (VCA IgG ELISA).

	Percent Agreement		95% confidence interval
EBV	98.4%	(61/62)	91.3 – 100.0%
Acute infection	82.8%	(24/29)	64.2 – 94.2%
Past infection	98.3%	(563/573)	96.8 – 99.2%
Indeterminate	89.7%	(139/155)	83.8 – 94.0%
Overall	96.1%	(787/819)	94.5 – 97.3%

Based on these serological classifications, the LIAISON® EBV IgM results for the prospective samples were compared with those obtained with the reference assay (VCA IgM ELISA).

	Percent Agreement		95% confidence interval
EBV	93.5%	(58/62)	84.3 – 98.2%
Acute infection	100.0%	(29/29)	90.2 – 100.0%
Past infection	95.3%	(546/573)	93.2 – 96.9%
Indeterminate	63.9%	(99/155)	55.8 – 71.4%
Overall	89.4%	(732/819)	87.1 – 91.4%

Based on these serological classifications, the LIAISON® EBNA IgG results for the prospective samples were compared with those obtained with the reference assay (EBNA-1 IgG ELISA).

	Percent Agreement		95% confidence interval
EBV	100.0%	(62/62)	95.3 – 100.0%
Acute infection	100.0%	(29/29)	90.2 – 100.0%
Past infection	98.1%	(562/573)	96.6 – 99.0%
Indeterminate	81.9%	(127/155)	75.0 – 87.7%
Overall	95.2%	(780/819)	93.6 – 96.6%

d. Clinical cut-off:

NA

e. Expected values/Reference range:

The LIAISON® VCA 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 VCA 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 VCA in this population as determined by the LIAISON® VCA IgG Assay is summarized in the following table.

	N	Negative	Equivocal	Positive	Prevalence
Total	823	97	5	721	87.6%
Gender					
Female	508	56	2	450	88.6%
Male	231	32	3	196	84.8%
Unknown	84	9	0	75	89.3%
Age (years)					
≤ 18	173	36	2	135	78.0%
< 10	29	9	0	20	69.0%
10 – 19	183	30	2	151	82.5%
20 – 29	190	28	2	160	84.2%
30 – 39	95	5	1	89	93.7%

40 – 49	78	2	0	76	97.4%
50 – 59	59	2	0	57	96.6%
60 – 69	32	0	0	32	100.0%
≥ 70	27	3	0	24	88.9%
Unknown	130	18	0	112	86.2%

The LIAISON® EBV IgM Assay was tested with prospectively collected samples from subjects sent to the laboratory for EBV testing (n=819) to evaluate the prevalence of IgM antibodies to VCA in these populations. The subjects sent to the laboratory for EBV testing were 61.8% female (506), 28.0% male (229) and 10.3% unknown (84) and represented the mid-Atlantic and Northeastern US.

The distribution of results for IgM antibodies to VCA in this population as determined by the LIAISON® EBV IgM Assay is summarized in the following table.

	N	Negative	Equivocal	Positive	Prevalence
Total	819	719	14	86	10.5%
Gender					
Female	506	453	8	45	8.9%
Male	229	194	3	32	14.0%
Unknown	84	72	3	9	10.7%
Age (years)					
≤ 18	172	138	2	32	18.6%
< 10	29	23	0	6	20.7%
10 – 19	182	148	2	32	17.6%
20 – 29	190	159	6	25	13.2%
30 – 39	95	90	2	3	3.2%
40 – 49	77	71	2	4	5.2%
50 – 59	59	56	1	2	3.4%
60 – 69	32	32	0	0	0.0%
≥ 70	25	24	0	1	4.0%
Unknown	130	116	1	13	10.0%

The LIAISON® EBNA 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 EBNA 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 EBNA in this population as determined by the LIAISON® EBNA IgG Assay is summarized in the following table.

	N	Negative	Equivocal	Positive	Prevalence
Total	823	177	5	641	77.9%
Gender					
Female	508	96	1	411	80.9%
Male	231	64	3	164	70.0%
Unknown	84	17	1	66	78.6%
Age (years)					
≤ 18	173	63	2	108	62.4%
< 10	29	11	1	17	58.6%
10 – 19	183	65	1	117	63.9%
20 – 29	190	41	0	149	78.4%
30 – 39	95	8	1	86	90.5%
40 – 49	78	5	1	72	92.3%
50 – 59	59	7	1	51	86.4%
60 – 69	32	5	0	27	84.4%
≥ 70	27	6	0	21	77.8%
Unknown	130	29	0	101	77.7%

N. Proposed labeling:

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

WARNINGS: Assay interference due to circulating antibodies against HIV and Hepatitis A, Hepatitis B and Hepatitis C viruses has not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents

The recommended LIAISON® EBNA IgG quality control material contains a 5% serum matrix. It may not adequately control the DiaSorin LIAISON® EBNA IgG assay for serum specimens. The user must provide quality control material for serum specimens. Alternative materials for the control of serum specimens include commercial quality control materials or your laboratory's own pooled serum specimens. Choose control levels that check assay performance at all clinically relevant points (e.g., assay cutoff). The recommendation is to run a positive and

negative control close (± 50%) to the assay's decision point. It is the responsibility of the user to validate the use of alternative control materials with this assay and to establish appropriate control ranges

O. Conclusion:

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