

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K160650

**B. Purpose for Submission:**

Modifications to earlier device cleared under K082050. These changes include modifications to the formulation of LIAISON<sup>®</sup> HAV IgM and LIAISON<sup>®</sup> Control HAV IgM, addition of claimed sample matrices, and longer stability claims.

**C. Measurand:**

IgM Antibody to *Hepatitis A Virus*

**D. Type of Test:**

Qualitative, Chemiluminescence Immunoassay (CLIA)

**E. Applicant:**

Diasorin, Inc.

**F. Proprietary and Established Names:**

LIAISON<sup>®</sup> HAV IgM and LIAISON<sup>®</sup> Control HAV IgM

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.3310 – Hepatitis A virus (HAV) serological assays

2. Classification:

Class II

3. Product code:

LOL: Hepatitis A Test (Antibody and IgM Antibody)  
JJX: Single (specified) analyte controls (assayed and unassayed)

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The LIAISON<sup>®</sup> HAV IgM assay is an *in vitro* chemiluminescent immunoassay intended for the qualitative detection of IgM antibodies to hepatitis A virus (IgM anti-HAV) in human serum and plasma (sodium citrate, potassium EDTA, lithium and sodium heparin and citrate dextrose (ACD)) using the LIAISON<sup>®</sup> Analyzer. Assay results, in conjunction with other serological and clinical information, may be used for testing specimens from individuals who have signs and symptoms consistent with acute hepatitis as an aid in the laboratory diagnosis of acute or recent HAV infection.

This assay is not intended for screening blood or solid or soft tissue donors. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations.

The LIAISON<sup>®</sup> Control HAV IgM (negative and positive) are intended for use as assayed quality control samples to monitor the performance of the LIAISON<sup>®</sup> HAV IgM assay.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

LIAISON<sup>®</sup> Analyzer

**I. Device Description:**

The LIAISON<sup>®</sup> HAV IgM assay for the qualitative determination of IgM anti-HAV is an antibody capture Chemiluminescence Immunoassay (CLIA). The LIAISON<sup>®</sup> HAV IgM is an *in vitro* diagnostic device consisting of reagents provided in individual compartments within a plastic container called the Reagent Integral. The assay configuration allows performance of 100 tests. The assay is performed on the LIAISON<sup>®</sup> Analyzer, a fully automated system that combines chemiluminescence detection technology with magnetic microparticles as the solid phase.

The LIAISON<sup>®</sup> HAV IgM Reagent Integral is comprised of the following ready to use reagents:

- Magnetic particles (2.3 mL)                      Magnetic particles coated with IgG to human IgM (mouse monoclonal), BSA, buffer, < 0.1% sodium azide.
- Calibrator 1 (3.3 mL)                      Human serum/defibrinated plasma containing low levels of IgM anti-HAV diluted with non-specific human IgG (polyclonal), non-specific IgG (mouse monoclonal), BSA, PBS buffer, EDTA, preservative: ProClin<sup>®</sup> 300. The calibrator value (Index) is referenced to an in-house antibody preparation and it is encoded in the Reagent Integral bar code.
- Calibrator 2 (3.3 mL)                      Human serum/defibrinated plasma containing high levels of IgM anti-HAV diluted with non-specific human IgG (polyclonal), non-specific IgG (mouse monoclonal), BSA, PBS buffer, EDTA, preservative: ProClin<sup>®</sup> 300, an inert blue dye. The calibrator value (Index) is referenced to an in-house antibody preparation and it is encoded in the Reagent Integral bar code.
- Specimen diluent (28 mL)                      Non-specific human IgG (polyclonal), non-specific IgG (mouse monoclonal), BSA, PBS buffer, EDTA, preservative: ProClin<sup>®</sup> 300, an inert blue dye.
- Conjugate (8.0 mL)                      Mouse monoclonal antibodies to HAV conjugated to an isoluminol derivative, human serum/plasma, BSA, PBS buffer, preservative: ProClin<sup>®</sup> 300.
- HAV Antigen (18 mL)                      Human serum/defibrinated plasma, HAV, BSA, HEPES buffer, EDTA, preservative: ProClin<sup>®</sup> 300, an inert red dye.

The LIAISON<sup>®</sup> Control HAV IgM Kit is comprised of the following ready to use reagents:

- Negative control (2 x 0.6 mL)                      Human serum/defibrinated plasma negative for HAV IgM antibodies, preservative: ProClin<sup>®</sup> 300 and Gentamicin Sulfate.
- Positive control (2 x 0.6 mL)                      Human serum/defibrinated plasma containing HAV IgM antibodies, diluted in human serum matrix IgM free, preservative: ProClin<sup>®</sup> 300 and Gentamicin Sulfate.

## **J. Substantial Equivalence Information:**

### 1. Predicate device name(s):

PMA: Diasorin Inc. Enzyme Immunoassay for detection of IgM Antibody to Hepatitis A Virus (IgM anti-HAV) in human serum or plasma (ETI-HA-IGMK Plus)

### 2. Predicate 510(k) number(s):

P890014/S002

3. Comparison with predicate:

<b>Characteristic</b>	<b>New Device LIAISON<sup>®</sup> HAV IgM</b>	<b>Predicate Device ETI-HA-IGMK PLUS PMA No. P890014/S002</b>
Intended Use	Qualitative detection of IgM antibodies to hepatitis A virus (IgM anti-HAV) in human serum or plasma (sodium citrate, potassium EDTA, lithium and sodium heparin and citrate dextrose (ACD)).	Qualitative determination of IgM antibody to hepatitis A virus (IgM anti-HAV) in human serum or plasma
Controls	2 (Negative and Positive)	2 (Negative and Positive)
Sample Matrix	Serum or plasma (sodium citrate, potassium EDTA, lithium and sodium heparin and citrate dextrose (ACD))	Serum or plasma
Reagent Storage	2-8°C, On-board or in Refrigerator	2-8°C Refrigerator only

<b>Characteristic</b>	<b>New Device LIAISON<sup>®</sup> HAV IgM</b>	<b>ETI-HA-IGMK PLUS PMA No P890014/S002</b>
Type of Assay	Chemiluminescence Immunoassay	Enzyme Immunoassay
Sample Handling/processing	Automated	Manual
Calibrators	Two	One (Cut-Off)
Detector	Mouse Monoclonal to HAV conjugated to an isoluminol derivative	Horseradish peroxidase-labeled mouse monoclonal antibody to HAV
Capture Reagent	Magnetic particles coated with IgG to human IgM (mouse monoclonal)	Microwells coated with mouse monoclonal antibody to human IgM
Equivocal zone	Index Value of $\geq 0.90$ and $< 1.1$	Absorbance within 80 – 100% of assay cutoff
Sample Volume	20 $\mu$ L	10 $\mu$ L
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA Microtiter plate reader)
Total incubation	40 minutes	3.5 hours

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

1. CLSI Guideline EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods.
2. CLSI Guideline EP07-A2, Interference Testing in Clinical Chemistry.
3. CLSI EP15-A3; User Verification of Precision and Estimation of Bias.
4. Class II Special Controls Guidance Document: Hepatitis A Virus Serological Assays, Feb. 9, 2006.

**L. Test Principle:**

The LIAISON<sup>®</sup> HAV IgM assay is an antibody capture chemiluminescence immunoassay (CLIA). The principal components of the test are magnetic particles (solid phase) coated with IgG to human IgM (mouse monoclonal) and a conjugate of a mouse monoclonal antibody to HAV linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, IgM antibodies present in calibrators, samples or controls bind to the solid phase. After a wash step, HAV antigen and conjugate are dispensed into the reaction module and incubated. During this second incubation, the antibody conjugate reacts with HAV antigen and the immune complex thus formed reacts with IgM already bound to the solid phase. Unbound material is then 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 as relative light units (RLU) and is indicative of anti-HAV IgM present in calibrators, samples or controls.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

REPRODUCIBILITY STUDY:

A 5 day reproducibility study was conducted at 3 external laboratories. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol. A coded panel comprised of 6 frozen serum samples was prepared by DiaSorin. The coded panel was prepared by either spiking or diluting samples as necessary to contain negative, low positive and mid positive samples.

The coded panel was tested at all 3 sites, using 3 replicates per run in 2 runs per day for 5 operating days. The mean, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the sites and across sites.

The LIAISON<sup>®</sup> Control HAV IgM (negative and positive) were also included in the 5 day study. The LIAISON<sup>®</sup> HAV IgM Negative Control as well as negative panel sample (HAVM-P00) read below the detectable limit of the curve (0.10); therefore, standard deviation (SD) and %CV were calculated using the Relative Light Units (RLUs) for these two samples.

The results from the reproducibility study are summarized in Table 4 below (combined sites). The mean Index value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for intra- run, run to run, between site, and total across sites.

**Table 4: Summary of Reproducibility Results Across All Three Sites**

Sample ID	Mean Index	Intra-Run		Run to Run		Between Site		Total	
		S.D.	%CV	S.D.	%CV	S.D.	%CV	S.D.	%CV
HAVM Negative*	334	18.70	5.6	7.45	2.2	8.28	2.5	21.80	6.5
HAVM Positive	2.22	0.26	11.7	0.05	2.2	0.05	2.3	0.26	11.8
HAVM-P00*	535	20.50	3.8	16.10	3.0	0.00	0.0	26.00	4.9
HAVM-P01	1.48	0.12	8.4	0.01	0.7	0.03	2.1	0.13	8.6
HAVM-P04	0.60	0.07	11.0	0.01	1.7	0.01	1.2	0.07	11.2
HAVM-P14	1.18	0.08	6.6	0.02	1.9	0.02	1.3	0.08	6.7
HAVM-P15	2.42	0.21	8.6	0.03	1.3	0.03	1.2	0.21	8.8
HAVM-P16	5.62	0.48	8.5	0.00	0.0	0.31	5.5	0.57	10.1

\* Samples below the reading range of the assay, precision calculations are based on signal (RLU).

**PRECISION STUDY:**

A 20 day precision study was performed at DiaSorin, Inc. A coded panel comprised of 6 frozen serum samples was prepared by DiaSorin. The coded panel was prepared by either spiking or diluting samples as necessary to contain negative, low positive and mid positive samples. The controls of the LIAISON® Control HAV IgM (negative and positive) were also tested in the 20-day study. The CLSI document EP5-A3 was consulted in the preparation of the testing protocol.

The panel samples and kit controls were tested on 2 LIAISON® HAV IgM kit lots in 2 replicates per run, 2 runs per day for 20 operating days on one LIAISON® Analyzer. The mean, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens.

The 20-day combined 2 lot results are summarized in Table 5 as sample overall mean result, SD, and %CV computed for Between-Lot/Within-Site and Total (Across Lots Within-Site).

<b>Table 5: Combined Two Lot Precision Results</b>					
Sample ID	Mean Index	Between-Lot Within-Site		Total (Across Lots Within-Site)	
		S.D.	%CV	S.D.	%CV
HAVM Negative*	346	31.78	9.2	23.63	6.8
HAVM Positive	2.20	0.06	2.9	0.18	8.0
HAVM-P00*	446	46.68	10.5	41.51	9.3
HAVM-P01	1.59	0.04	2.4	0.12	7.7
HAVM-P04	0.61	0.02	2.9	0.03	5.5
HAVM-P14	1.20	0.02	1.8	0.07	6.1
HAVM-P15	2.65	0.04	1.6	0.17	6.6
HAVM-P16	6.20	0.10	1.6	0.44	7.1

\* Samples below the reading range of the assay, precision calculations are based on signal (RLU).

b. *Linearity/assay reportable range:*

Not applicable.

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

*Stability:* The stability parameters are summarized in Table 6 below.

<b>Table 6: Stability Parameters</b>	
LIAISON <sup>®</sup> HAV IgM	
Study	Stability
Calibration Curve	4 weeks
Open Use storage On-board Analyzer	8 weeks
Open Use storage at 2-8°C	8 weeks
LIAISON <sup>®</sup> HAV IgM	
Study	Stability
Calibration Curve	4 weeks
Open Use storage On-board Analyzer	8 weeks
Open Use storage at 2-8°C	8 weeks
LIAISON <sup>®</sup> HAV IgM	
Study	Stability
Calibration Curve	4 weeks
Open Use storage On-board Analyzer	8 weeks
Open Use storage at 2-8°C	8 weeks

d. *Detection limit:*

This device is for the qualitative detection of HAV IgM antibody and contains an equivocal zone. The detection limit is therefore not applicable.

e. *Analytical specificity:*

Cross Reactivity:

The cross-reactivity panel was comprised of 251 specimens to evaluate potential interference from antibodies to other viruses that may cause symptoms similar to HAV infection (i.e., EBV, CMV, Rubella, HBV, HCV), other organisms that may cause infectious disease (i.e., VZV, HSV, Measles, Mumps, *Toxoplasma gondii*) and from other conditions that may result from atypical immune system activity (i.e., rheumatoid factor (RF), antinuclear autoantibodies (ANA), hyper  $\gamma$ -globulins). The cross-reactivity experiments were conducted at DiaSorin. The IgM anti-HAV serological status of the sample panels was determined using the DiaSorin ETI-HA IgMK PLUS (P890014) FDA approved kit as the reference. The serological reactivity for the potential cross-reactant was confirmed using various commercially available FDA cleared/approved kits (when applicable). The results obtained are summarized in Table 7 below. No cross-reactivity was noted in the specimens tested.

**Table 7: Summary of Cross Reactivity Studies**

Organism/Condition	N	Comparator HAV IgM Assay	LIAISON <sup>®</sup> HAV IgM		
			Negative	Equivocal	Reactive
HAV antibodies (IgG)	14	Negative	14	0	0
Measles IgM	10	Negative	10	0	0
Mumps IgM	16	Negative	16	0	0
EBV IgM	14	Negative	14	0	0
CMV IgM	11	Negative	11	0	0
Rubella IgM	11	Negative	11	0	0
Toxo IgM	10	Negative	10	0	0
HSV 1/2 IgM	11	Negative	11	0	0
Treponema total antibodies	10	Negative	10	0	0
VZV IgM	14	Negative	14	0	0
HTLV I/II antibodies	10	Negative	10	0	0
HCV antibodies	13	Negative	13	0	0
HBc IgM	17	Negative	17	0	0
HIV antibodies	10	Negative	10	0	0
RF (Anti Fc immunoglobulin)	11	Negative	11	0	0
ANA IgG	16	Negative	16	0	0
Anti ds DNA IgG	11	Negative	11	0	0
HAMA	10	Negative	10	0	0
Heterophilic antibodies reactive	10	Negative	10	0	0
Hypergammaglobulinemia	12	Negative	12	0	0
PARVO VIRUS B19 IgM	10	Negative	10	0	0
Total	251	Negative	251	0	0

Interference:

The potential interference of each of the following substances at the indicated concentration (indicated in the table below) was tested on three samples close to the decision point. For each of the interfering substance, the three samples were divided into equal aliquots, and spiked with the indicated concentration of the interfering substance. The matched spiked and unspiked samples were tested in the same run, on one instrument with one LIAISON<sup>®</sup> HAV IgM kit lot in twenty-six replicates each, alternating the replicates of the two samples. The results are indicated in Table 8 below. No interference was observed from the potentially interfering substances at the tested concentration.

<b>Table 8: Summary of Testing of Potentially Interfering Substances</b>	
<b>Substance Tested</b>	<b>No Interference at Listed Concentration</b>
Triglycerides	3000 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	20 mg/dL
Conjugated bilirubin	20 mg/dL
Albumin	6000 mg/dL
Cholesterol	510 mg/dL
Gamma-globulin	4000 mg/dL

*f. Assay cut-off:*

The cut-off values for the modified LIAISON<sup>®</sup> HAV IgM assay were set at Index values of < 0.9 (Negative)  $\geq$  1.1 (Reactive), i.e., the same cut-off Index values as used for the LIAISON<sup>®</sup> HAV IgM cleared under K082050. There is an equivocal zone for index values  $\geq$  0.90 and < 1.10. The cut-off values were validated in the clinical study.

2. Comparison studies:

*a. Method comparison with predicate device:*

See Clinical Study Section below (Section 3).

*b. Matrix comparison:*

Sample sets of matched serum and multiple types of plasma were used in the matrix comparison study to demonstrate the equivalence of different sample matrices. Forty (40) matched samples were assayed for each comparison. The samples were collected as serum, serum in serum separator tubes (SST), and plasma in the following anticoagulants: Sodium Citrate, Potassium EDTA, Lithium and Sodium heparin and Citrate Dextrose (ACD). The serum specimen in the set

was taken as the reference for the purposes of comparison. Samples belonging to each set were tested in triplicate with one LIAISON<sup>®</sup> HAV IgM reagent lot on one instrument in a single run. The results from the SST tube and all plasma samples were compared to serum by Passing-Bablok regression and Bland-Altman analysis. All slopes were between 0.90-1.10, and the bias was within  $\pm 10\%$ . Human serum, SST serum, Sodium Citrate Plasma, Potassium EDTA Plasma, Lithium Heparin Plasma, Sodium Heparin Plasma, or ACD Plasma are acceptable sample types for use in the LIAISON<sup>®</sup> HAV IgM assay.

### 3. Clinical studies:

#### *a. Clinical Sensitivity*

Not applicable

#### *b. Clinical Specificity*

Not applicable

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

#### Verification of Cut-off Values:

The cut-off values for the LIAISON<sup>®</sup> HAV IgM assay (K082050) are set at Index values of 0.9 and 1.1. There is an Equivocal Zone for samples giving Index values  $\geq 0.9$  and  $< 1.1$ . A cut-off study was carried out to verify that the cut-off values for the K082050 assay apply also to the modified LIAISON<sup>®</sup> HAV IgM assay (K160650). A total of 380 serum specimens were tested with the LIAISON<sup>®</sup> HAV IgM assay in the cut-off verification study and the results were compared to those obtained with the DiaSorin ETI-HA-IGMK Plus assay (P890014/S002). Testing included the following three sets of specimens:

- i. 121 adult patient samples sent to the laboratory for HAV testing (prospectively collected specimens).
- ii. 121 samples from a pediatric population (prospectively collected specimens).
- iii. 138 samples from patients with an acute HAV infection (retrospective, archived specimens).

The results for each set of specimens are presented separately below.

#### i. Adult Prospectively Collected Specimens.

The specimens were prospectively collected from adults in the United States. The 121 individuals from the HAV testing population were 66.1% female (n=80), ranging in age from 21 to 90, and 33.9% male (n=41) ranging in age from 21 to 80. Table 9 shows the comparison of the LIAISON<sup>®</sup> HAV IgM to the ETI-HA-IGMK PLUS for these specimens.

LIAISON <sup>®</sup> HAV IgM	ETI-HA-IGMK PLUS			Total
	Positive	Borderline	Negative	
Positive	0	0	0	0
Equivocal	0	0	0	0
Negative	0	0	121	121
Total	0	0	121	121

Negative Percent Agreement = 100% (121/121)  
Exact 95% Confidence Interval (CI) = 97.6 – 100.0%

ii. Pediatric Prospectively Collected Specimens.

The pediatric samples were prospectively collected from children in the United States. Of these 121 samples, 43.8% were female subjects (n=53) and 56.2% were male subjects (n=68), ranging in age from 8 months to 20 years of age. Table 10 shows the comparison of the LIAISON<sup>®</sup> HAV IgM to the ETI-HA-IGMK PLUS for the pediatric specimens.

LIAISON <sup>®</sup> HAV IgM	ETI-HA-IGMK PLUS			Total
	Positive	Borderline	Negative	
Positive	0	0	0	0
Equivocal	0	0	0	0
Negative	0	0	121	121
Total	0	0	121	121

Negative Percent Agreement = 100% (121/121)  
Exact 95% CI = 97.6 – 100.0%

iii. Specimens from Patients with Acute HAV Infection.

This set of archived specimens consisted of 138 serum samples from individuals with acute HAV infection that had been diagnosed serologically. Table 11 shows the comparison of the LIAISON<sup>®</sup> HAV IgM to the ETI-HA-IGMK PLUS for these specimens.

**Table 11: Cut-off Verification Using Specimens From Patients With Acute HAV Infection**

LIAISON <sup>®</sup> HAV IgM	ETI-HA-IGMK PLUS			Total
	Positive	Borderline	Negative	
Positive	138	0	0	138
Equivocal	0	0	0	0
Negative	0	0	0	0
Total	138	0	0	138

Positive Percent Agreement = 100% (138/138)

Exact 95% CI = 97.9 – 100.0%

#### Seroconversion Panels:

The performance of the LIAISON<sup>®</sup> HAV IgM was compared to that of the ETI-HA-IGMK PLUS with five seroconversion panels. The results are summarized in Table 12 and demonstrate equivalent performance of the two assays.

**Table 12: Summary of Results With Seroconversion Panels**

Panel ID	LIAISON <sup>®</sup> HAV IgM		ETI-HA-IGMK PLUS		Difference in days from last reactive result
	Post-bleed day of earliest reactive result	Post-bleed day of last reactive result	Post-bleed day of earliest reactive result	Post-bleed day of last reactive result	
0615-0026	14	27	14	27	0
PHT902 seroconversion	16	21	16	21	0
PHT903 seroconversion	38	108	38	108	0
RP004 seroconversion	6	62	6	62	0
RP013 seroconversion	8	189	8	189	0

#### Clinical Study Results:

Please refer to the Decision Summary for K082050 for a summary of clinical study results.

#### 4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Please refer to the Decision Summary for K082050.

**N. Proposed Labeling:**

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

**O. Conclusion:**

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