

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

A. 510(k) Number:

K063319

B. Purpose for Submission:

New device clearance

C. Measurand:

IgM Antibody to Hepatitis A Virus

D. Type of Test:

Qualitative, ELISA

E. Applicant:

Bio-Rad.

F. Proprietary and Established Names:

MONOLISA™ Anti-HAV IgM EIA

G. Regulatory Information:

1. Regulation section:

21 CFR Part 866.3310, Hepatitis A virus (HAV) Serological Reagents

2. Classification:

Class II

3. Product Code:

LOL

4. Panel:

83 Microbiology

H. Intended Use:

1. Indication(s) for use:

The MONOLISA™ Anti-HAV IgM EIA is an *in vitro* enzyme immunoassay kit intended for use in the qualitative detection of IgM antibodies to Hepatitis A virus (anti-HAV IgM) in human (adult and pediatric) serum or plasma (EDTA, Heparin, Citrate, ACD). The assay is indicated for testing specimens from individuals who have signs and symptoms consistent with acute Hepatitis. Assay results, in conjunction with other serological or clinical information, may be used for the laboratory diagnosis of individuals with acute or recent Hepatitis A.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, and core blood or neonatal specimens.

Warning: This assay is not intended for screening blood or solid or soft tissue donors.

2. Special condition for use statement(s):

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”.

3. **Special instrument Requirements:**

Bio-Rad microwell plate or strip reader or equivalent. The spectrophotometer should have the following specifications at wavelength 450 nm:

Bandwidth: 10 nm HBW (Half Band Width) or equivalent

Absorbance Range: 0 to 3.0 Repeatability: \pm (0.5% + 0.005) Linearity or

Accuracy: 1% from 0 to 3.0

The instrument should contain a reference filter for reading at 615 to 630 nm. An instrument without a reference filter can be used; however, areas in the bottoms of the wells that are opaque, scratched or irregular may cause absorbance readings that are falsely elevated.

I. Device Description:

Enzyme immunoassay (antibody capture format) for the detection of IgM antibodies to Hepatitis A virus.

J. Substantial Equivalence Information:

1. Predicate device name(s):

DiaSorin ETI-HA-IGMK PLUS

2. Predicate K number(s):

P890014

3. Comparison with predicate:

The following tables summarize similarities and differences between the MONOLISA™ Anti-HAV IgM EIA kit and the predicate device ETI-HA-IGMK PLUS.

Table 1: Similarities between kit components and materials

Similarities in Components / Materials	MONOLISA™ Anti-HAV IgM EIA Catalog# 72495	ETI-HA-IGMK PLUS Catalog# P001925
Conjugate	Peroxidase-labeled mouse monoclonal antibody to HAV.	Peroxidase-labeled mouse monoclonal antibody to HAV.
Positive Control	Human plasma, positive for IgM anti-HAV antibodies, diluted in human plasma negative for anti-HAV antibodies.	Human serum/plasma reactive for IgM anti-HAV, diluted with buffer.
Chromogen	Tetramethylbenzidine (TMB)	Tetramethylbenzidine (TMB)
Substrate	Hydrogen Peroxide	Hydrogen Peroxide
Washing Solution	Concentrated buffered solution with Tween 20.	Concentrated buffered solution with detergents.

Sample diluent	Buffered solution with proteins and sample indicator dye.	Buffered solution with proteins and an inert blue dye.
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Table 2: Differences between kit components and materials

Differences in Components / Materials	MONOLISA™ Anti-HAV IgM EIA Catalog# 72495	ETI-HA-IGMK PLUS Catalog# P001925
Solid Phase	Microplate wells coated with polyclonal anti-human IgM antibodies.	Microplate wells coated with mouse monoclonal antibodies to human IgM.
Negative Control	Human plasma, negative for IgM anti-HAV antibodies and total anti-HAV antibodies.	Human serum/plasma non-reactive for IgM anti-HAV and reactive for IgG anti-HAV, diluted in buffer.
Calibrator	Human plasma, positive for IgM anti-HAV antibodies diluted in buffer.	Human serum/plasma non-reactive for IgM anti-HAV and reactive for IgG anti-HAV diluted with buffer.
Conjugate	Ready-to-use	To be diluted.
Stopping Solution	1N H ₂ SO ₄ .	0.4N H ₂ SO ₄ .
Required sample volume	20 µl	10 µl

Table 3: Similarities between kits with regard to function and use

Similarities in Function and Use	MONOLISA™ Anti-HAV IgM EIA Catalog# 72495	ETI-HA-IGMK PLUS Catalog# P001925
Test Method	EIA (antibody capture)	EIA (antibody capture)
Specimen Storage Requirements	Samples may be stored at 2-8°C for up to 24 hours.	Samples may be stored at 2-8°C for up to 24 hours.
Format	96-well microplate	96-well microplate
Intended Use	Assay for the qualitative detection of anti-HAV IgM antibodies in human serum or plasma.	Assay for the qualitative detection of anti-HAV IgM antibodies in human serum or plasma.
Wavelength	Dual wavelength reading at 450 nm and 615/630 nm.	Dual wavelength reading at 450 nm and 630 nm.

Interpretation of results	Obtained absorbance readings for patient specimens compared to cut-off value determined by the mean of the calibrator absorbance values.	Obtained absorbance readings for patient samples compared to cut-off value determined by the mean of the calibrator absorbance values.
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Table 4: Differences between kits with regard to function and use

Differences in Function and Use	MONOLISA™ Anti-HAV IgM EIA Catalog# 72495	ETI-HA-IGMK PLUS Catalog# P001925
Spectrophotometric Verification of Sample and Reagent Pipeting	Possible (but optional)	NA
Cutoff calculation	Mean absorbance of calibrator values divided by 4	Mean absorbance of the calibrator values + 0.250

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

Class II Special Control Guidance Document, Hepatitis A Virus Serological Assays, issued February 9, 2006.

L. Test Principle:

The MONOLISA™ Anti-HAV IgM EIA is an enzyme immunoassay (IgM antibody capture format) for the detection of IgM antibodies to Hepatitis A Virus. In the assay procedure, patient specimens, a calibrator, and controls are incubated with anti-human IgM antibodies coated on the microwells. If IgM antibodies to HAV are present in a specimen or control, they bind to the antibody. Excess sample is removed by a wash step. The HAV Viral Antigen and the Conjugate (containing horseradish peroxidase – labeled mouse monoclonal antibody to HAV) are successively added to the microwells and allowed to incubate. The presence of IgM anti-HAV in the sample enables the HAV Viral Antigen and the Conjugate to bind to the solid phase. Excess Conjugate and HAV Viral Antigen are removed by a wash step, and a TMB Chromogen / Substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HAV IgM, the bound enzyme (HRP) causes the colorless TMB in the Chromogen solution to change to blue. The blue color turns yellow after the addition of a Stopping Solution. If a sample does not contain anti-HAV IgM, the Chromogen / Substrate solution in the well remains colorless during the substrate incubation, and after the addition of the Stopping Solution. The color intensity is measured spectrophotometrically. Absorbance value readings for patient specimens are compared to the cutoff value.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. **Precision/Reproducibility:**

Within-Laboratory Precision Study:

A 21-member panel was tested: serum samples with the 6 corresponding plasma samples (EDTA K2, EDTA K3, Sodium Citrate, Sodium Heparin, Lithium heparin, ACD) at 3 different levels (1 negative, 1 negative near the cutoff, 1 low positive near the cutoff) were tested on 1 lot, in duplicate, in 2 different runs per day (am and pm), by the same operator for a period of 20 days. The data were analyzed following the CLSI guidance EP5A2. The mean ratio, the Standard Deviation (SD) and percent coefficient of variation (%CV) were calculated for each panel member.

The data summary is shown in the following table:

MONOLISA™ Anti-HAV IgM EIA Precision Results by Panel Member Signal to Cutoff (S/CO)

Panel Member	N	Mean S/CO	Within run ¹		Between Run ²		Between Day ³		Total ⁴	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative Control C0	40	0.08	NA	NA	0.01	11.0%	0.00	0.8%	0.01	11.0%
Positive Control C1	40	2.03	NA	NA	0.08	4.2%	0.05	2.5%	0.10	4.8%
serum 1	80	0.05	0.00	5.5%	0.01	14.1%	0.00	7.7%	0.01	17.0%
EDTA K2 1	80	0.05	0.00	6.3%	0.01	13.4%	0.00	6.4%	0.01	16.1%
EDTA K3 1	80	0.05	0.00	6.3%	0.01	15.2%	0.00	4.6%	0.01	17.1%
Sodium Citrate 1	80	0.05	0.00	6.7%	0.01	14.0%	0.00	0.0%	0.01	15.5%
Sodium Heparin 1	80	0.05	0.00	6.7%	0.01	12.6%	0.00	5.6%	0.01	15.4%
Lithium Heparin 1	80	0.05	0.00	6.7%	0.01	14.5%	0.00	7.6%	0.01	17.7%
ACD A	80	0.05	0.00	6.3%	0.01	14.0%	0.00	0.4%	0.01	15.3%
Serum 2	80	0.55	0.02	3.7%	0.02	4.0%	0.02	3.6%	0.04	6.5%
EDTA K2 2	80	0.66	0.02	3.5%	0.03	4.9%	0.03	5.3%	0.05	8.0%
EDTA K3 2	80	0.65	0.02	3.3%	0.04	6.1%	0.03	4.7%	0.05	8.3%
Sodium Citrate 2	80	0.65	0.03	5.0%	0.03	5.0%	0.02	3.8%	0.05	8.0%
Sodium Heparin 2	80	0.57	0.01	2.5%	0.02	3.7%	0.03	4.8%	0.04	6.6%
Lithium Heparin 2	80	0.57	0.02	2.7%	0.04	6.1%	0.02	4.0%	0.05	7.8%
ACD 2	80	0.68	0.03	5.2%	0.04	6.3%	0.03	4.4%	0.06	9.2%
Serum 3	80	1.33	0.03	2.1%	0.06	4.8%	0.06	5.2%	0.09	7.4%
EDTA K2 3	80	1.44	0.03	2.5%	0.06	4.8%	0.06	4.7%	0.09	7.2%
EDTA K3 3	80	1.35	0.07	6.2%	0.07	5.4%	0.05	4.5%	0.11	9.4%
Sodium Citrate 3	80	1.44	0.04	3.1%	0.05	4.2%	0.06	5.2%	0.09	7.4%
Sodium Heparin 3	80	1.36	0.02	2.1%	0.06	5.1%	0.06	4.7%	0.09	7.2%
Lithium Heparin 3	80	1.35	0.05	4.1%	0.07	5.8%	0.05	4.3%	0.10	8.3%
ACD 3	80	1.47	0.04	3.5%	0.10	8.4%	0.06	5.1%	0.13	10.5%

NA: Not Applicable

¹ Within Run: variability of the assay performance from replicate to replicate

² Between Run: variability of the assay performance from Run to Run

³ Between Day: variability of the assay performance from Day to Day

⁴ Total: total variability of the assay performance includes within run, between run and between day.

Reproducibility Study:

A 6 member panel consisting of diluted plasma specimens (negative and different levels of positive) was tested in triplicate, once a day for 3 days on 3 lots* of MONOLISA™ Anti-HAV IgM EIA at 3 separate clinical trial sites. Each panel was coded with a different number on each day tested in order to blind the operator to the expected value of the sample. *3 different lots were used at the Bio-Rad site and 2 lots were used on each of the external sites. The data from all reagent lots and sites were combined to obtain standard deviation (SD) and percent coefficient of variation (CV) for within run, between day, between lot, between site and total variance. The data were analyzed according to the principles described in the Clinical Laboratory Standards Institute guidance EP5-A2, revised November 2004 and ISO/TR 22971:2005. The PROC GLM procedure in SAS® was used to estimate the variance components of the model. The model was $y = \text{site} + \text{lot}(\text{site}) + \text{day}(\text{lot site}) + \text{error}$.

The summaries are shown in the following tables:

MONOLISA™ Anti-HAV IgM EIA Reproducibility Results by Panel Member Signal to Cutoff (S/CO)

Test site	Panel Member	N	Mean	Within Run ¹		Between Day ²		Between Lot ³		Total ⁴	
			S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Site #1	P1	18	0.05	0.03	74.6	0 ⁵	0	0 ⁵	0	0.03	74.6
	P2	18	0.69	0.03	4.7	0.06	8.1	0 ⁵	0	0.06	9.4
	P3	18	1.10	0.04	3.6	0.07	6.7	0 ⁵	0	0.08	7.6
	P4	18	1.69	0.05	2.7	0.13	7.6	0 ⁵	0	0.14	8.1
	P5	18	3.26	0.07	2.2	0.03	1.1	0 ⁵	0	0.08	2.4
	P6	18	4.19	0.19	4.6	0.00	0.0	0.05	1.1	0.20	4.7
Site#2	P1	18	0.06	0.00	6.4	0.01	12.2	0.00	6.7	0.01	15.4
	P2	18	0.82	0.02	2.8	0.06	7.0	0 ⁵	0	0.06	7.5
	P3	18	1.27	0.05	3.8	0.08	6.6	0.11	8.6	0.15	11.5
	P4	18	2.01	0.12	5.7	0.14	7.0	0 ⁵	0	0.18	9.1
	P5	18	3.8	0.15	4.0	0.20	5.2	0 ⁵	0	0.25	6.6
	P6	18	4.8	0.14	2.8	0.38	7.9	0 ⁵	0	0.40	8.3
	P1	27	0.04	0.00	6.7	0.01	13.0	0.00	11.7	0.01	18.7

Site #3	P2	27	0.71	0.02	3.3	0.03	4.0	0.04	6.1	0.06	8.0
	P3	27	1.12	0.05	4.3	0.02	2.1	0.09	7.7	0.10	9.1
	P4	27	1.77	0.06	3.4	0.08	4.3	0.10	5.8	0.14	8.0
	P5	27	3.26	0.09	2.8	0.10	3.1	0.13	4.0	0.19	5.8
	P6	27	3.93	0.10	2.6	0.10	2.5	0.20	5.1	0.25	6.3

¹ Within Run: variability of the assay performance from replicate to replicate

² Between Day: variability of the assay performance from Day to Day

³ Between Lot: variability of the assay performance from Lot to Lot

⁴ Total: total variability of the assay performance includes within run, between day and between lot.

⁵ Negative variances were rounded to zero, per statistical convention.

MONOLISA™ Anti-HAV IgM EIA Reproducibility summary by Panel Member Signal to Cutoff (S/CO).

Panel Member	N	Mean S/CO	Within Run ¹		Between Day ²		Between Lot ³		Between Site ⁵		Total ⁴	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
P1	63	0.05	0.02	37.8	0.00	0.0	0.00	8.1	0.01	19.6	0.02	43.3
P2	63	0.74	0.03	3.6	0.05	6.4	0.02	2.6	0.06	8.0	0.08	11.1
P3	63	1.16	0.05	3.9	0.06	5.3	0.08	6.8	0.07	5.8	0.13	11.1
P4	63	1.82	0.08	4.2	0.11	6.3	0.05	3.0	0.15	8.1	0.21	11.5
P5	63	3.41	0.11	3.0	0.13	3.7	0.09	2.5	0.29	8.5	0.35	10.1
P6	63	4.25	0.14	3.3	0.21	5.0	0.14	3.2	0.43	10.1	0.52	12.2

¹ Within Run: variability of the assay performance from replicate to replicate

² Between Day: variability of the assay performance from Day to Day

³ Between Lot: variability of the assay performance from Lot to Lot

⁵ Between site: variability of the assay performance from Site to Site

⁴ Total: total variability of the assay performance includes within run, between day between lot and between Site.

Reproducibility study on Negative and Positive Controls:

The negative and positive controls were tested in triplicate, once a day by 3 different operators for 3 days. The data were analyzed according to the principles described in the Clinical Laboratory Standards Institute guidance EP5-A2, revised November 2004 and ISO/TR 22971:2005.

b. Linearity/assay reportable range:

NA

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

Controls and calibrators are provided specifically designed for use in conjunction with the performance of the assay.

d. Detection limit:

NA

e. Analytical specificity:

The potential for cross reactivity to other disease states, or viruses was evaluated for the MONOLISA™ Anti-IgM HAV EIA Assay and the comparative assay. In addition, samples containing rheumatoid factors, auto-antibodies, anti-mouse antibodies were tested. In total, 255 specimens (including both serum and plasma) from 16 groups of potential cross-reactivity were tested. FDA approved methods were used to confirm the disease state of each specimen. The results are summarized in the following table.

Potential cross reactivity study

Clinical condition	Number tested	MONOLISA™ Anti-HAV IgM EIA nonreactive
Hepatitis C (HCV)	15	15
Hepatitis B (HBV) HBs Ag	15	15
Hepatitis B (HBV) anti HBc	15	15
Human Immunodeficiency Virus (HIV)	15	15
Epstein Barr Virus (EBV) IgG	15	15
Epstein Barr Virus (EBV) IgM	15	15
Cytomegalovirus (CMV) IgG	15	15
Cytomegalovirus (CMV) IgM	15	15
Rubella IgG	15	15
Toxoplasmosis IgG	15	15
Toxoplasmosis IgM	15	15
Mumps IgG	15	15
Varicella Zoster Virus(VZV) IgG	15	15
Varicella Zoster Virus(VZV) IgM	15	15
Anti Nuclear Antibody (ANA)	15	15
Human Anti Mouse Antibody (HAMA)	15	15
Rheumatoid Arthritis	15	15
Total Samples tested	255	255

All the 255 specimens were found nonreactive with HAV IgM with MONOLISA™ Anti-HAV IgM and with the predicate assay.

f. Assay cut-off: N/A

2. Comparison studies:
 - a. **Method comparison with predicate device:**
MONOLISA™ Anti-IgM HAV EIA was compared to the DiaSorin ETI-HA-IGMK PLUS assays.
 - b. **Matrix comparison:** N/A

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 Performance

A multi-center prospective and retrospective study was conducted to evaluate the clinical performance of the MONOLISA™ Anti-HAV IgM EIA assay among individuals with signs or symptoms and those at high risk for Hepatitis infection. Specimens were collected in 3 different geographical areas: 404 specimens were collected in the US and 929 were collected in Europe (France and Italy).

The US population consisted of 174 subjects with signs and symptoms of Hepatitis. In this group, 60% were male and 40% were female, and they ranged in age from 17 to 72 years (mean age of 38). The group was Caucasian (13.2%), Black or African American (4.6%), Hispanic or Latino (2.9%), and Asian (41.9%), with the remaining 1.1% represented by multiple ethnic groups. The ethnicity of 36.8% was unknown. Among these subjects, 23 (13.2%) were pediatric samples.

The 230 subjects from the high-risk group for Hepatitis A include intravenous drug users (N= 55), homosexual males (N=15), sex workers (N=39), prison history (N= 92), high-risk sex partners (N=25), high-risk occupation/health care workers (N=4). Many had more than 1 high-risk behavior or risk factor. The group was Caucasian (7.4%), Black or African American (74.3%), Hispanic or Latino (15.2%), Asian (0.4%), Native Hawaiian or other Pacific Islander (0.4%), and American Indian or Alaska native (0.9%), with the remaining 1.3% represented by multiple ethnic groups. In this group, 81% were male and 19% were female, and they ranged in age from 18 to 70 years (mean age of 45). Among these 230 subjects, 2 (0.9%) were pediatric samples.

The European population consisted of 253 specimens collected from patients with signs and symptoms of Hepatitis. In this group, 51% were male and 49% were female and they ranged in age from 1 to 105 years (mean age of 53).

Sixty-two (62) specimens were collected from a population at high risk for hepatitis composed of intravenous drug users (30), subjects who had clotting factor disorders (7) and MSM patients (25). The group was 87% male and 13% female and ranged in age from 21 to 75 years (mean age of 40). There were 345 specimens from an asymptomatic hospitalized population and 34 were from healthcare workers (for HAV pre-vaccination screening). One hundred and fifty one (151) patients had recovered HAV infection. Among these 845 European samples, 36 (4.3%) were from pediatric subjects.

Percent Agreement:

The results obtained with MONOLISA™ Anti-HAV IgM EIA were compared with the results obtained using the comparative assay. The positive and negative percent agreements and the 95% exact confidence between MONOLISA™ Anti-HAV IgM EIA and the comparative assay were calculated. To determine the percent agreement on borderline results the following criteria were used:

- Specimens that were borderline with the comparative assay and reactive with MONOLISA™ Anti-HAV IgM EIA were considered as false positives for MONOLISA™ Anti-HAV IgM EIA assay
- Specimens that were borderline with the comparative assay and non reactive with MONOLISA™ Anti-HAV IgM EIA were considered as false negatives for MONOLISA™ Anti-HAV IgM EIA

The results obtained with the US specimens and with the European specimens are presented in the following tables:

MONOLISA™ Anti-HAV IgM EIA versus the comparative assay results in the US population (N=404)

Subject category	Comparative assay: Positive			Comparative assay : Borderline			Comparative assay: Negative			Total
	MONOLISA™ Anti-HAV IgM EIA			MONOLISA™ Anti-HAV IgM EIA			MONOLISA™ Anti-HAV IgM EIA			
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
Subjects with signs and symptoms	1	0	0	0	0	0	2	0	171	174
Subjects with high risk for Hepatitis	0	0	0	0	0	0	0	1	229	230
Total	1	0	0	0	0	0	2^b	1^{a b}	400	404

R: Reactive, NR: Nonreactive, BRD: Borderline

^athe borderline sample with MONOLISA was considered as “false positive”

^bthese samples were found HAV IgG reactive.

	Positive percent agreement	95% Exact Confidence interval	Negative percent agreement	95% Exact Confidence interval
Total	100% (1/1)	NA	99.3% (400/403)	97.8– 99.9%

MONOLISATM Anti-HAV IgM EIA versus the comparative assay results in the European population (N= 845)

Subject category	Comparative assay: Positive			Comparative assay: Borderline			Comparative assay: Negative			Total
	MONOLISA^T_M Anti-HAV IgM EIA			MONOLISA^T_M Anti-HAV IgM EIA			MONOLISA^T_M Anti-HAV IgM EIA			
	R	BR D	NR	R	BR D	NR	R	BR D	NR	
General hospitalized population	1	0	0	0	0	0	1	1 ^b	342	345
Sign / Symptoms of Hepatitis	0	0	0	0	0	0	3	0	250	253
Subjects with high risk for Hepatitis	0	0	0	0	0	0	0	0	62	62
Healthcare workers	0	0	0	0	0	0	0	0	34	34
Infected/ recovered HAV	0	0	0	2 ^a	0	0	1	0	148	151
Total	1	0	0	2^c	0	0	5^c	1^c	836	845

	Positive percent agreement	95% Exact Confidence interval	Negative percent agreement	95% Exact Confidence interval
Total	100% (1/1)	N/A	99.0% (836/844)	98.1-99.6

R: Reactive, NR: Nonreactive, BRD: Borderline

^athe 2 borderline samples with the comparative assay were considered “false positive” with MONOLISA

^bthe borderline sample with MONOLISA was considered as “false positive”

^cthese samples were found HAV IgG Reactive

Acute HAV Infection:

Among the retrospective samples, 84 were from subjects with a medical history and laboratory results indicative of acute Hepatitis A. The subjects included 56% male, 37% female; the gender was not available for 7%. The mean age was 21, and subjects ranged from 1 to 55 years. Among them 39 were pediatric subjects.

The results are presented in the following table:

Comparison of Results for MONOLISA™ Anti-HAV IgM EIA versus the comparative assay on Acute HAV infection in the adult and pediatric European Population (N= 84):

	Comparative assay: Positive			Comparative assay: Borderline			Comparative assay: Negative			total
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
Adults	45	0	0	0	0	0	0	0	0	45
Pediatrics	39	0	0	0	0	0	0	0	0	39
Total	84	0	0	0	0	0	0	0	0	84

R: Reactive, NR: Nonreactive, BRD: Borderline

The positive agreement was 100% (84/84) with a 95% exact confidence interval of 96.5% to 100%.

Performance of MONOLISA™ Anti-HAV EIA in Pediatric subjects:

Sixty-one (61) pediatric samples were tested during the US and European clinical studies in addition to the 39 samples from acute HAV infection. Among the US population, 23 had signs and symptoms of hepatitis and 2 were from the high risk group. In the European population, 3 belonged to the general hospitalized population, 23 had signs and symptoms of hepatitis, 2 were from the high risk group, 3 were healthcare workers, 5 had recovered from Hepatitis A infection. The results from these pediatric samples are summarized in the following table.

Comparison of Results for MONOLISA™ Anti-HAV IgM EIA versus the comparative assay in the Pediatric European and US Population (N= 61)

Subject category	Comparative assay: Positive			Comparative assay: Borderline			Comparative assay : Negative			Total
	MONOLISA™ Anti-HAV IgM EIA			MONOLISA™ Anti-HAV IgM EIA			MONOLISA™ Anti-HAV IgM EIA			
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
European pediatric s	0	0	0	0	0	0	1	0	35	36
US pediatric s	0	0	0	0	0	0	1	0	24	25
Total	0	0	0	0	0	0	0^a	0	59	61

R: Reactive, NR: Nonreactive, BRD: Borderline

^athese samples were found HAV IgG Reactive

	Positive percent agreement	95% Exact Confidence interval	Negative percent agreement	95% Exact Confidence interval
Total	0	N/A	96.7% (59/61)	88.6 – 99.6

Including the combined US and European Sites, the positive percent agreement of the MONOLISA™ Anti-HAV IgM EIA with the comparative assay was **100% (86/86)**, with a 95% exact confidence interval of **96.6% to 100%**. The negative percent agreement of the MONOLISA™ Anti-HAV IgM EIA with the comparative assay was **99.1% (1233/1244)** with a 95% exact confidence interval of **98.4% to 99.6%**.

Seroconversion Panels:

Eight commercially available HAV seroconversion panels were tested using MONOLISA™ Anti-HAV IgM EIA and the comparative assay to determine the sensitivity of the assay. The results are summarized in the following table:

MONOLISA™ Anti-HAV IgM EIA Seroconversion panels Results

Panel ID	MONOLISA™ Anti-HAV IgM EIA	Anti-HAV IgM Comparative Assay	
	Post bleed day of first reactive result	Post bleed day of first reactive result	Difference in Days to Reactive result
07467A	0	0	0
60160K	0	0	0
60162K	0	0	0
HAV01	0	0	0
RP-004	6	6	0
RP-013	8	8	0
PHT901	12	12	0
PHT902	16	16	0

Panel ID	MONOLISA™ Anti-HAV IgM EIA	Anti-HAV IgM Comparative Assay	
	Post bleed day of last reactive result*	Post bleed day of last reactive result*	Difference in Days from last Reactive result
HAV01	91 ^a	77	+14

a: last bleed of the panel

For all seroconversion panels, both MONOLISA™ Anti-HAV IgM EIA and the comparative assay detected HAV IgM antibodies at the same first bleed. MONOLISA™ Anti-HAV IgM EIA appears to detect IgM for a longer period than the comparator assay for qualitative determination of IgM antibody to Hepatitis A.

Among seroconversion panels beginning with samples negative for anti-HAV antibodies and having subsequent samples to 5-6 months, one (PHT-902) becomes borderline after 5 months and one (PHT-901) gives a negative result after more than 20 months. Another seroconversion panel (RP-013) with samples collected through 6 months has a declining ratio but still remains positive. The other panels contain members collected through 2 to 3 months.

4. Clinical cut-off:

See assay cut-off previously described in this document

5. Expected values/Reference range:

Healthy individuals:

The expected results of the MONOLISA™ Anti-HAV IgM EIA assay were determined in presumably healthy individuals from the Mid-west US (St Louis), the Western US (California and Washington) and from Europe (Parma, Italy).

In the Mid-west, the population was 55% female and 45% male, with ages that ranged from 1 to 96 years. 48% (134) were pediatric specimens. The majority of the subjects were White/Caucasian (64%) and 32% were black or African American; for 4% data were not available. In the Western US, 73% were from California and 27% were from Washington. The population was 56% female and 44% male, with ages that ranged from 15 to 90 years. In Europe, the population was 50% female and 50% male, with ages that ranged from 18 to 87 years.

The expected results for the US and for Europe are presented below. The percent of Anti-HAV IgM reactive results with MONOLISA™ Anti-HAV IgM EIA were 0.4% for the US and 0.7% for Europe.

Expected Results for MONOLISA™ Anti-HAV IgM EIA in subjects from the Mid-west US (N= 280)

MONOLISA™ Anti-HAV IgM EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 10	Female	0	N/A	0	N/A	35	100.0%	35
	Male	0	N/A	0	N/A	38	100.0%	38
10 -19	Female	0	N/A	0	N/A	38	100.0%	38
	Male	0	N/A	0	N/A	23	100.0%	23
20- 29	Female	0	N/A	0	N/A	5	100.0%	5
	Male	0	N/A	0	N/A	3	100.0%	3
30 -39	Female	0	N/A	0	N/A	10	100.0%	10
	Male	0	N/A	0	N/A	9	100.0%	9
40 -49	Female	0	N/A	0	N/A	13	100.0%	13
	Male	0	N/A	0	N/A	8	100.0%	8
50 -59	Female	1	5.6%	1	5.6%	16	88.9%	18
	Male	0	N/A	0	N/A	17	100.0%	17
60 -69	Female	0	N/A	0	N/A	14	100.0%	14
	Male	0	N/A	0	N/A	13	100.0%	13
70-79	Female	0	N/A	0	N/A	9	100.0%	9
	Male	0	N/A	0	N/A	6	100.0%	6
80-89	Female	0	N/A	0	N/A	13	100.0%	13
	Male	0	N/A	0	N/A	6	100.0%	6
>=90	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	2	100.0%	2
Total		1*	0.4%	1**	0.4%	278	99.3%	280

*1 subject was reactive with a result of 2.25 (S/CO)

** 1 subject gave a borderline result of 1.04 (S/CO).

Expected Results for MONOLISA™ Anti-HAV IgM EIA in subjects from the Western US (N= 245)

MONOLISA™ Anti-HAV IgM EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
<19	Female	0	N/A	0	N/A	5	100.0%	5
	Male	0	N/A	0	N/A	5	100.0%	5
20-29	Female	0	N/A	0	N/A	26	100.0%	26
	Male	0	N/A	0	N/A	24	100.0%	24
30-39	Female	0	N/A	0	N/A	20	100.0%	20
	Male	0	N/A	0	N/A	18	100.0%	18
40-49	Female	0	N/A	0	N/A	18	100.0%	18
	Male	0	N/A	0	N/A	22	100.0%	22
50-59	Female	1	2.6%	0	N/A	38	97.4%	39
	Male	0	N/A	1	4.8%	20	95.2%	21
60-69	Female	0	N/A	0	N/A	12	100.0%	12
	Male	0	N/A	0	N/A	12	100.0%	12
70-79	Female	0	N/A	0	N/A	9	100.0%	9
	Male	0	N/A	0	N/A	2	100.0%	2
80-89	Female	0	N/A	0	N/A	6	100.0%	6
	Male	0	N/A	0	N/A	4	100.0%	4
>=90	Female	0	N/A	0	N/A	1	100.0%	1
	Male	0	N/A	0	N/A	0	N/A	0
Unknown	Female	0	N/A	0	N/A	1	100.0%	1
Total		1*	0.4%	1**	0.4%	243	99.2%	245

*1 subject was reactive with a result of 1.34 (S/CO)

** 1 subject gave a borderline result of 0.96 (S/CO).

Expected Results for MONOLISA™ Anti-HAV IgM EIA in subjects from Italy, Europe (N= 285)

MONOLISA™ Anti-HAV IgM EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 19	Female	0	N/A	0	N/A	1	100.0%	1
	Male	0	N/A	0	N/A	1	100.0%	1
20-29	Female	0	N/A	0	N/A	3	100.0%	3
	Male	0	N/A	0	N/A	2	100.0%	2
30-39	Female	0	N/A	0	N/A	7	100.0%	7
	Male	0	N/A	0	N/A	7	100.0%	7
40-49	Female	0	N/A	0	N/A	21	100.0%	21

	Male	0	N/A	0	N/A	19	100.0%	19
50-59	Female	0	N/A	0	N/A	22	100.0%	22
	Male	0	N/A	0	N/A	27	100.0%	27
60-69	Female	1	2.5%	1	2.5%	41	95.0%	43
	Male	0	N/A	0	N/A	27	100.0%	27
70-79	Female	1	3.6%	0	N/A	31	96.4%	32
	Male	0	N/A	0	N/A	37	100%	37
80-89	Female	0	N/A	0	N/A	13	100%	13
	Male	0	N/A	0	N/A	23	100%	23
Total		2*	0.7%	1**	0.4%	282	98.9%	285

*2 subjects gave reactive results of 3.4 and 1.2 (S/CO)

** 1 subject gave a borderline result of 1.07 (S/CO)

Adult subjects at high risk for viral hepatitis:

Expected results of asymptomatic prospective high-risk subjects determined from a multi-center study in the US and in Europe are reported in the following tables.

A total of 230 US Subjects were at high risk for viral hepatitis including intravenous drug users (N= 55), homosexual males (N=15), sex workers (N=39), prison history (N= 92), high-risk sex partners (N=25), high-risk occupation/health care workers (N=4). Many had more than 1 high-risk behavior or risk factor. Subjects were from Los Angeles, CA, (86.5%), Santa Ana, CA (4.3%), or Miami, FL (9.1%). The group was Caucasian (7.4%), Black or African American (74.3%), Hispanic or Latino (15.2%), Asian (0.4%), Native Hawaiian or other Pacific Islander (0.4%), and American Indian or Alaska native (0.9%), with the remaining 1.3% represented by multiple ethnic groups.

The subjects were 81% male and 19% female, and ranged in age from 18 to 70 years (mean age of 45). The data are reported in Table 4.

The percent of Anti-HAV IgM reactive results with MONOLISA™ Anti-HAV IgM EIA in this high-risk asymptomatic population was 0%.

The European group (N= 62) was 87% male and 13% female and ranged in age from 21 to 75 years (mean age of 40). It consisted of intravenous drug users (30), subjects who had clotting factor disorders (7) and MSM patients (25). The data are reported in Table 5.

The percent of Anti-HAV IgM reactive results with MONOLISA™ Anti-HAV IgM EIA in this high-risk asymptomatic population was 0%.

Expected results for MONOLISA™ Anti-HAV IgM EIA in the US High risk Group for Viral Hepatitis A (N=230)

MONOLISA™ Anti-HAV IgM EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 19	Female	0	N/A	0	N/A	1	100.0%	1
	Male	0	N/A	0	N/A	1	100.0%	1
20- 29	Female	0	N/A	0	N/A	3	100.0%	3

	Male	0	N/A	0	N/A	2	100.0%	2
30 -39	Female	0	N/A	0	N/A	7	100.0%	7
	Male	0	N/A	0	N/A	36	100.0%	36
40 -49	Female	0	N/A	0	N/A	24	100.0%	24
	Male	0	N/A	1	1.2 %	84	98.8%	85
50 -59	Female	0	N/A	0	N/A	7	100.0%	7
	Male	0	N/A	0	N/A	51	100.0%	51
60 -69	Female	0	N/A	0	N/A	1	100.0%	1
	Male	0	N/A	0	N/A	10	100.0%	10
70-79	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	2	100.0%	2
Total		0	N/A	1	0.4%	229	99.6%	230

Expected results for MONOLISA™ Anti-HAV IgM EIA in the European High risk group for Viral Hepatitis A (N=62)

MONOLISA™ Anti-HAV IgM EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 19	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	0	N/A	0
20- 29	Female	0	N/A	0	N/A	5	100.0%	5
	Male	0	N/A	0	N/A	11	100.0%	11
30 -39	Female	0	N/A	0	N/A	2	100.0%	2
	Male	0	N/A	0	N/A	14	100.0%	14
40 -49	Female	0	N/A	0	N/A	1	100.0%	1
	Male	0	N/A	0	N/A	14	100.0%	14
50 -59	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	11	100.0%	11
60 -69	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	2	100.0%	2
70-79	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	2	100.0%	2
>80	Female	0	N/A	0	N/A	0	N/A	0
	Male	0	N/A	0	N/A	0	N/A	0
Total		0	N/A	0	N/A	62	100.0%	62

N. Proposed labeling:

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

WARNINGS and PRECAUTIONS:

For in vitro diagnostic use only

1. The MONOLISA™ Anti-IgM HAV EIA contains human source material used in

- the preparation of Negative Control (C0), Positive Control (C1) and Calibrator (C2) that has been tested with either FDA or CE approved methods and found non-reactive for Hepatitis B surface antigen (HBsAg), antibodies to Hepatitis C Virus (HCV) and antibodies to Human Immunodeficiency Viruses (HIV-1 and HIV-2).
2. The HAV Viral Antigen reagent (R6) has been treated with formalin to inactivate the virus.
 3. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
 4. The following is a list of potential chemical hazards contained in some kit components (See section 4: REAGENTS):
 - 4.1 ProClin™ 300 (0.1% and 0.25%) are biocidal preservatives that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
 - 4.2 The 1N Sulfuric Acid (H₂SO₄) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases, reducing agents and metals; do not pour water into this component. Waste from this material is considered hazardous acidic waste. However, if permitted by local, regional, and national regulations, it can be neutralized to pH 6-9 for non-hazardous disposal if operators are trained and equipped to do so.
 - 4.3 Sodium azide (< 0.1%), a biocidal preservative, may be detrimental if enough is ingested. Sodium azide may react with certain metals, including lead or copper often found in plumbing, to form highly explosive metal azides. Flush with copious amounts of water when pouring dilute solutions down the drain to prevent explosive build-up.
 5. Biological spills: Human source material spills should be treated as potentially infectious. Spills not containing acid should be immediately decontaminated, including the spill area, materials, and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus], or a phenolic, etc.) and wiped dry.
 6. Spills containing acid should be appropriately absorbed or neutralized, and wiped dry. The area should be decontaminated with an appropriate agent. Materials used to absorb the spill should be disposed of as biohazardous waste.

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

7. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

O. Conclusion:

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