

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

A. 510(k) Number:

K063318

B. Purpose for Submission:

New device clearance

C. Measurand:

Total Antibody to Hepatitis A virus

D. Type of Test:

Qualitative, ELISA

E. Applicant:

Bio-Rad.

F. Proprietary and Established Names:

MONOLISA™ Anti-HAV 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. Intended use(s):

The MONOLISA™ anti-HAV EIA is an *in vitro* enzyme immunoassay kit intended for use in the qualitative detection of total antibodies (anti-HAV IgG and IgM) to Hepatitis A virus (anti-HAV) in human (adult and pediatric) serum or plasma (EDTA, Heparin, Citrate, ACD). This kit can be used as an aid in the diagnosis of acute or past Hepatitis A Virus (HAV) infection or as an aid in the identification of HAV-susceptible individuals for vaccination. However, any diagnosis should take into consideration the patient's clinical history and symptoms, as well as serological data.

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 (competitive assay format) for the detection of total antibodies to Hepatitis A virus.

J. Substantial Equivalence Information:

1. Predicate device name(s):

DiaSorin ETI-AB-HAVK PLUS

2. Predicate K number(s):

P890019

3. Comparison with predicate:

Table 1: Similarities between kit components and materials

Similarities in Components / Materials	MONOLISA™ Anti-HAV EIA	ETI-AB-HAVK PLUS
Solid Phase	Microplate wells coated with mouse Monoclonal anti-HAV antibodies.	Microplate wells coated with mouse Monoclonal anti-HAV antibodies.
Conjugate	Peroxidase-labeled mouse monoclonal antibody to HAV.	Peroxidase-labeled mouse monoclonal antibody to HAV.
Negative Control	Human serum, negative for total anti-HAV antibodies.	Human serum/plasma, negative for total anti-HAV antibodies.
Calibrator	Human serum, positive for anti-HAV antibodies, diluted in human serum pool negative for anti-HAV antibodies.	Human serum/plasma, containing anti-HAV antibodies.

Positive Control	Human serum, positive for anti-HAV antibodies, diluted in human serum pool negative for anti-HAV antibodies.	Human serum/plasma, reactive for anti-HAV antibodies.
Chromogen	Tetramethylbenzidine (TMB)	Tetramethylbenzidine (TMB)
Substrate	Hydrogen Peroxide	Hydrogen Peroxide
Washing Solution	Concentrated buffered solution with Tween 20.	Concentrated buffered solution with detergents.

Table 2: Differences between kit components and materials

Differences in Components / Materials	MONOLISA™ Anti-HAV EIA Catalog# 72496	ETI-AB-HAVK PLUS Catalog# P001926
Conjugate	Ready-to-use.	To be diluted.
Incubation buffer	NA	Buffer, containing protein stabilizers and an inert blue dye.
Viral Antigen / Neutralizing Solution	Tris-buffer, containing inactivated HAV-virus, proteins and sample indicator dye.	Buffer, containing HAV, human serum/plasma and protein stabilizers.
Stopping Solution	1N H ₂ SO ₄ .	0.4N H ₂ SO ₄ .

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 EIA is an enzyme immunoassay (competitive assay format) for the detection of total antibodies to Hepatitis A virus. In the assay procedure, patient specimens, a calibrator and controls are incubated with HAV antigen in microwells that have been coated with mouse monoclonal anti-Hepatitis A antibodies. Antibodies to HAV present in a specimen or control will complex with the HAV antigen reagent and with antibodies coated on the microwells. Excess sample and HAV Viral antigen reagent are removed by a wash step. The conjugate (containing horseradish peroxidase-labeled mouse monoclonal antibody to HAV) is subsequently added to the microwells and incubated. The conjugate binds to the HAV antigen bound to the microwell in the absence of antibodies to HAV from the specimen. Excess conjugate is removed by a wash step, and a TMB Chromogen / Substrate solution is added to the microwells and allowed to incubate. If a sample does not contain anti-HAV antibodies, the bound enzyme (HRP) causes the colorless

tetramethylbenzidine (TMB) in the Chromogen solution to change to blue. The blue color turns yellow after the addition of a Stopping Solution. If a sample contains anti-HAV antibodies, 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 determined by the mean of the Calibrator absorbance values.

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 tables.

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

Panel Member	N	Mean CO/S	Within run ¹		Between Run ²		Between Day ³		Total ⁴	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative Control C0	40	0.282	NA	NA	0.02	6.4%	0.01	5.0%	0.02	8.1%
Positive Control C1	40	4.093	NA	NA	0.54	13.5%	0.00	0.0%	0.49	12.3%
Serum 1	80	0.395	0.02	4.1%	0.03	6.3%	0.01	1.9%	0.03	7.7%
EDTA K2 1	80	0.379	0.02	5.0%	0.03	6.8%	0.01	1.4%	0.03	8.6%
EDTA K3 1	80	0.376	0.01	3.4%	0.04	9.3%	0.00	0.0%	0.04	9.9%
Sodium Citrate 1	80	0.387	0.04	10.1%	0.01	3.3%	0.02	5.1%	0.05	11.8%
Sodium Heparin 1	80	0.363	0.01	3.4%	0.03	7.6%	0.00	0.0%	0.03	8.3%
Lithium Heparin 1	80	0.364	0.01	3.4%	0.03	7.0%	0.01	3.5%	0.03	8.5%
ACD 1	80	0.402	0.02	4.3%	0.04	10.4%	0.00	0.0%	0.05	11.3%
Serum 2	80	0.691	0.03	4.9%	0.06	9.6%	0.01	2.3%	0.06	11.0%
EDTA K2 2	80	0.657	0.02	3.2%	0.05	6.6%	0.01	1.8%	0.05	7.5%
EDTA K3 2	80	0.686	0.03	4.9%	0.06	8.2%	0.00	0.0%	0.07	9.5%
Sodium Citrate 2	80	0.636	0.03	3.7%	0.05	7.0%	0.03	4.8%	0.06	9.2%
Sodium Heparin 2	80	0.628	0.02	3.1%	0.04	6.0%	0.03	4.2%	0.06	7.9%
Lithium Heparin 2	80	0.685	0.05	6.6%	0.06	8.6%	0.00	0.0%	0.08	10.8%
ACD 2	80	0.746	0.04	5.6%	0.05	6.4%	0.03	4.7%	0.07	9.7%
Serum 3	80	1.506	0.06	4.2%	0.14	9.4%	0.00	0.4%	0.15	10.3%
EDTA K2 3	80	1.261	0.07	4.7%	0.11	7.0%	0.07	4.8%	0.15	9.7%
EDTA K3 3	80	1.257	0.04	2.4%	0.09	6.0%	0.05	3.6%	0.11	7.4%
Sodium Citrate 3	80	1.462	0.08	5.1%	0.13	8.7%	0.07	4.9%	0.17	11.2%
Sodium Heparin 3	80	1.380	0.11	7.5%	0.12	8.0%	0.05	3.4%	0.17	11.4%
Lithium Heparin 3	80	1.346	0.08	5.6%	0.11	7.1%	0.03	1.8%	0.14	9.2%
ACD 3	80	1.344	0.05	3.4%	0.08	5.6%	0.09	6.0%	0.13	8.9%

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 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 EIA Reproducibility Results by Panel Member Cutoff to Signal (CO/S)

Test site	Panel Member	N	Mean CO/S	Within Run ¹		Between Day ²		Between Lot ³		Total ⁴	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Site #1	P1	18	0.33	0.01	3.03	0.09	28.1	0 ⁵	0	0.09	28.3
	P2	18	0.68	0.03	4.9	0.07	10.0	0 ⁵	0	0.07	11.1
	P3	18	1.02	0.05	5.0	0.05	5.2	0 ⁵	4.1	0.08	8.3
	P4	18	1.98	0.09	4.7	0.32	16.0	0 ⁵	0	0.33	16.7
	P5	18	2.48	0.18	7.4	0.36	14.8	0 ⁵	0	0.41	16.5
	P6	18	3.66	0.23	6.2	0.20	5.5	0 ⁵	0	0.3	8.3
Site#2	P1	18	0.35	0.01	1.6	0.02	4.7	0.01	1.2	0.02	5.1
	P2	18	0.92	0.03	3.0	0.07	8.0	0 ⁵	0	0.08	8.5
	P3	18	1.28	0.04	3.5	0.00	0.0	0.01	0.62	0.05	3.6
	P4	18	2.32	0.08	3.6	0.20	8.5	0.02	0.95	0.21	9.3
	P5	18	3.10	0.13	4.1	0.20	6.4	0 ⁵	0	0.23	7.5
	P6	18	4.16	0.13	3.2	0.36	8.7	0 ⁵	0	0.39	9.3
Site #3	P1	27	0.36	0.01	4.0	0.02	5.4	0.02	6.6	0.03	9.4
	P2	27	0.81	0.03	3.4	0.03	3.9	0.06	7.2	0.07	8.8
	P3	27	1.27	0.08	6.6	0.04	3.6	0.14	11.2	0.17	13.5
	P4	27	2.16	0.11	5.0	0.05	2.2	0.34	15.7	0.36	16.6
	P5	27	3.09	0.11	3.7	0.15	4.9	0.49	15.7	0.52	16.9
	P6	27	4.47	0.11	2.5	0.33	7.3	1.01	22.5	1.06	23.8

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.

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:*

See assay cut-off below

e. *Analytical specificity:*

The potential for cross reactivity to other disease states, or viruses was evaluated for the MONOLISA™ Anti-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	Comparative assay Positive			Comparative assay BRD			Comparative assay Negative			Total
	MONOLISA™ Anti-HAV EIA			MONOLISA™ Anti-HAV EIA			MONOLISA™ Anti-HAV EIA			
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
Hepatitis C (HCV)	7	0	1	0	0	0	0	0	7	15
Hepatitis B (HBV) HBs Ag	9	0	0	0	0	0	0	0	6	15
Hepatitis B (HBV) anti HBc	10	0	0	0	0	0	3	1	1	15
Human Immunodeficiency Virus (HIV)	6	0	1	1	0	0	0	0	7	15
Epstein Barr Virus (EBV) IgG	1	0	0	1	0	0	0	0	13	15
Epstein Barr Virus (EBV) IgM	15	0	0	0	0	0	0	0	0	15
Cytomegalovirus (CMV) IgG	6	0	0	0	0	0	0	0	9	15
Cytomegalovirus (CMV) IgM	7	0	0	0	0	0	0	0	8	15
Rubella IgG	5	0	1	0	0	0	0	0	9	15

Toxoplasmosis IgG	10	0	0	0	0	0	0	0	5	15
Toxoplasmosis IgM	8	2	0	0	0	1	0	0	4	15
Mumps IgG	3	0	0	0	0	0	1	0	11	15
Varicella Zoster Virus(VZV) IgG	1	0	0	0	0	0	0	0	14	15
Varicella Zoster Virus(VZV) IgM	6	0	0	1	0	1	0	0	7	15
Anti Nuclear Antibody (ANA)	7	0	0	0	0	1	0	0	7	15
Human Anti Mouse Antibody (HAMA)	2	0	0	0	0	1	0	0	12	15
Rheumatoid Arthritis	12	0	0	0	0	0	0	0	3	15
Total	115	2	3	3	0	4	4	1	123	255

7 samples were discrepant: 4 reactive on MONOLISA™ Anti-HAV EIA, nonreactive on comparative assay and 3 were nonreactive on MONOLISA™ Anti-HAV EIA and reactive on comparative assay.

Warning: Assay interference for anti EBV IgM, anti HBc Ab, HBs Ag and rheumatoid factor, has not been evaluated sufficiently. The user is responsible for establishing cross-reactivity performance with these cross-reactants.

f. Assay cut-off:

The assay calibrator is equivalent to 20 mIU/mL standardized to the WHO 2nd Reference Standard for Anti-Hepatitis Immunoglobulin. However, assay results cannot be considered quantitative and no clinical claims for immunity can be determined from the cutoff.

2. Comparison studies:

a. Method comparison with predicate device:

MONOLISA™ Anti-HAV EIA were compared to the DiaSorin ETI-AB-HAVK 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 Studies

A multi-center prospective and retrospective study was conducted to evaluate the clinical performance of the MONOLISA™ Anti-HAV EIA assay among individuals with signs or symptoms and those at high risk of Hepatitis infection. Specimens were collected in 3 different geographical areas: 404 specimens were collected in the US and 928 were collected in Europe (France and Italy).

The US population consisted of 174 subjects with signs and symptoms of Hepatitis.

Of these, 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 1.1% represented by multiple ethnic groups. The remaining 36.8% were unknown. Among these 174 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), and 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. Of these, 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 252 specimens collected from patients with signs and symptoms of Hepatitis. Of these, 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).

Three hundred and forty five (345) specimens were from an asymptomatic hospitalized population. Of these, 51% were male and 49% were female, and they ranged in age from 18 to 87 years (mean age of 59). Thirty four specimens were from healthcare workers (for HAV pre-vaccination screening). One hundred and fifty one (151) patients had recovered HAV

infection. Among these 844 European samples, 35 (4.1%) were from pediatric subjects.

Vaccinated subjects:

Sixty-two (62) pre- and post-vaccination samples from 38 individuals were tested. Fourteen (14) individuals were enrolled in a vaccination program. They received the TWINRIX® vaccine, a combined Hepatitis A and Hepatitis B vaccine from GlaxoSmithKline. A pre-vaccination sample was collected the day of the first vaccination dose. A second sample was collected before the second vaccination dose was injected (one month after the first dose). A third dose of vaccine was scheduled 6 months after the first injection. The sample after the third vaccination dose was not available.

Twenty samples were collected from 10 subjects, aged 24 to 45 years, who had received the HAVRIX® vaccine. These subjects received HAVRIX® 1440, an inactivated Hepatitis A vaccine from GlaxoSmithKline, in a two-dose schedule (at 0 and 6 to 12 months). For each subject, a pre- and a post-vaccination specimen was obtained. All post-vaccination samples were obtained 4 weeks after vaccination. Fourteen purchased post-vaccination samples were tested; 8 were from individuals vaccinated with HAVRIX® and 6 were from individuals vaccinated with VAQTA® from Merck & Co.

MONOLISA™ Anti-HAV 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 EIA			MONOLISA™ Anti-HAV EIA			MONOLISA™ Anti-HAV EIA			
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
Subjects with signs and symptoms	123	0	2	1	0	0	1	3	44	174
Subjects with high risk for Hepatitis	114	0	0	2	1	1	4	1	107	230
Total	237	0	2	3^b	1^d	1^c	5	4^a	151	404

	Positive percent agreement	95% Exact Confidence interval	Negative percent agreement	95% Exact Confidence interval
Total	98,8% (237/240)	96.4 – 99.7	92.6% (151/163)	87.5 – 96.1

R: Reactive, NR: Nonreactive, BRD: Borderline

^athe Borderline results with MONOLISA™ Anti-HAV EIA were considered as false positives.

^bthe specimens that were Borderline with the comparative assay and reactive with MONOLISA™ Anti-HAV EIA were considered as false positives with MONOLISA™ Anti-HAV EIA.

^cthe specimens that were Borderline with the comparative assay and nonreactive with MONOLISA™ Anti-HAV EIA were considered as false negative with MONOLISA™ Anti-HAV EIA.

^dthe results that were borderline with both the MONOLISA™ Anti-HAV EIA and with the comparative assay were not included in the negative agreement or the positive agreement calculations.

Comparison of Results for MONOLISA™ Anti-HAV EIA versus the comparative assay in the European Population (N= 844)

Subject category	Comparative assay: Positive			Comparative assay: Borderline			Comparative assay: Negative			Total
	MONOLISA™ Anti-HAV EIA			MONOLISA™ Anti-HAV EIA			MONOLISA™ Anti-HAV EIA			
	R	BRD	NR	R	BRD	NR	R	BRD	NR	
General hospitalized population	236	0	1	0	0	0	0	0	108	345
Sign / Symptoms of Hepatitis	190	0	0	0	2	1	1	1	57	252
Subjects with high risk for Hepatitis	28	0	0	0	0	0	0	0	34	62
Healthcare workers	6	0	0	0	0	0	0	0	28	34
Infected/ recovered HAV	150	0	0	1	0	0	0	0	0	151
Total	610	0	1	1^b	2^d	1^c	1	1^a	227	844

	Positive percent agreement	95% Exact Confidence interval	Negative percent agreement	95% Exact Confidence interval
Total	99.7% (610/612)	98.8 – 99.9	98.7% (227/230)	96.2 – 99.7

R: Reactive, NR: Nonreactive, BRD: Borderline

^athe Borderline result with MONOLISA™ Anti-HAV EIA was considered as false positive

^bthe specimen that was Borderline with the comparative assay and reactive with MONOLISA™ Anti-HAV EIA was considered as false positive with MONOLISA™ Anti-HAV EIA.

^cthe specimen that was Borderline with the comparative assay and nonreactive with MONOLISA™ Anti-HAV EIA was considered as false negative with MONOLISA™ Anti-HAV EIA.

^dthe 2 borderline results with both MONOLISA™ Anti-HAV EIA and with the comparative assay were not included in the calculation of the negative agreement or the positive agreement.

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

HAVRIX	Pre-vaccination	0	0	0	0	0	0	0	0	10	10
	Post-vaccination	18	0	0	0	0	0	0	0	0	18
TWINRIX	Pre-vaccination	1	0	0	0	0	0	1*	0	12	14
	Post 1 st injection	9	1	0	0	0	2	0	0	2	14

R: reactive, NR: Nonreactive, BRD: Borderline

* Result close to the cutoff value (CO/S=1.2)

In pre-vaccination samples, MONOLISATM Anti-HAV EIA was in overall agreement with the comparative assay for 21/22 (95.5%) of samples tested. For TWINRIX® vaccine on post first dose vaccination, MONOLISATM Anti-HAV EIA demonstrated reactivity in 9/14 (64.3%) samples. The reference method demonstrated reactivity in 10/14 (71.4%) samples. For HAVRIX® post-vaccination samples, MONOLISATM Anti-HAV EIA demonstrated reactivity in 18/18 (100%) samples. The reference method demonstrated reactivity in 18/18 (100%) samples. For VAQTA® post-vaccination samples, MONOLISATM Anti-HAV EIA demonstrated reactivity in 6/6 (100%) samples. The reference method demonstrated reactivity in 6/6 (100%) samples.

4. Clinical cut-off:

See assay cut-off previously described in this document

5. Expected values/Reference range:

The expected results of the MONOLISA™ Anti-HAV EIA assay were determined in presumably healthy individuals from the Mid-west US (St Louis, Missouri), 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 ranging 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 this study, 41 % were found reactive for Anti-HAV total antibodies, and 57% were found nonreactive.

In the Western US, 73% were from California, 27% were from Washington. The population was 56% female and 44% male, and their ages ranged from 15 to 90 years.

In this population, 38% were found reactive for Anti-HAV total antibodies, and 62% were found nonreactive. In Europe, the population was 50% female and 50% male, with ages ranging from 18 to 87 years. In this group, 69% were found reactive for Anti-HAV total antibodies and 31% were found nonreactive. The expected results for the US and for presumably healthy individuals living in Europe are presented below (Tables 5, 6 and 7).

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

MONOLISA™ Anti-HAV EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 10	Female	10	28.6%	0	N/A	25	71.4%	35
	Male	7	18.4%	2	5.3%	29	76.3%	38
10 -19	Female	14	36.8%	2	5.3%	22	57.9%	38
	Male	9	39.1%	0	N/A	14	60.9%	23
20- 29	Female	3	60.0%	0	N/A	2	40.0%	5
	Male	3	100.0%	0	N/A	0	N/A	3
30 -39	Female	5	50.0%	0	N/A	5	50.0%	10
	Male	3	33.3%	0	N/A	6	66.7%	9
40 -49	Female	3	23.1%	0	N/A	10	76.9%	13
	Male	4	50.0%	0	N/A	4	50.0%	8
50 -59	Female	10	55.6%	0	N/A	8	44.4%	18
	Male	8	47.1%	0	N/A	9	52.9%	17
60 -69	Female	8	57.1%	0	N/A	6	42.9%	14
	Male	4	30.8%	0	N/A	9	69.2%	13
70-79	Female	6	66.7%	1	11.1%	2	22.2%	9
	Male	5	83.3%	0	N/A	1	16.7%	6
80-89	Female	9	69.2%	0	N/A	4	30.8%	13
	Male	3	50.0%	0	N/A	3	50.0%	6
>=90	Female	0	N/A	0	N/A	0	N/A	0
	Male	1	50.0%	0	N/A	1	50.0%	2
Total		115	41.1%	5	1.8%	160	57.1%	280

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

MONOLISA™ Anti-HAV EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
<19	Female	3	60.0%	0	N/A	2	40.0%	5
	Male	1	20.0%	0	N/A	4	80.0%	5
20- 29	Female	11	42.3%	0	N/A	15	57.7%	26
	Male	5	20.8%	0	N/A	19	79.2%	24
30 -39	Female	10	50.0%	0	N/A	10	50.0%	20
	Male	5	27.8%	0	N/A	13	72.2%	18
40 -49	Female	6	33.3%	0	N/A	12	66.7%	18
	Male	10	45.5%	0	N/A	12	54.5%	22
50 -59	Female	15	38.5%	1	2.6%	23	59.0%	39
	Male	5	23.8%	0	N/A	16	76.2%	21
60 -69	Female	6	50.0%	0	N/A	6	50.0%	12
	Male	4	33.3%	0	N/A	8	66.7%	12
70-79	Female	1	11.1%	0	N/A	8	88.9%	9
	Male	1	50.0%	0	N/A	1	50.0%	2
80-89	Female	6	100%	0	N/A	0	N/A	6
	Male	3	75.0%	0	N/A	1	25.0%	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		92	37.6%	1	0.4%	152	62.0%	245

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

MONOLISA™ Anti-HAV 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	1	33.3%	0	N/A	2	66.7%	3
	Male	0	N/A	0	N/A	2	100.0%	2
30-39	Female	1	14.3%	0	N/A	6	85.7%	7
	Male	2	28.6%	0	N/A	5	71.4%	7
40-49	Female	7	33.3%	0	N/A	14	66.7%	21
	Male	3	15.8%	0	N/A	16	84.2%	19
50-59	Female	10	45.5%	0	N/A	12	54.5%	22
	Male	14	51.9%	0	N/A	13	48.1%	27
60-69	Female	37	86.0%	0	N/A	6	14.0%	43
	Male	23	85.2%	0	N/A	4	14.8%	27
70-79	Female	31	96.9%	0	N/A	1	3.1%	32
	Male	32	86.5%	0	N/A	5	13.5%	37
80-89	Female	13	100.0%	0	N/A	0	N/A	13
	Male	23	100.0%	0	N/A	0	N/A	23
Total		197	69.1%	0	N/A	88	30.9%	285

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), and 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. Of these subjects, 81% were male and 19% were female, and they ranged in age from 18 to 70 years (mean age of 45). The data are reported in Table below. The percent of Anti-HAV reactive results with MONOLISA™ Anti-HAV EIA in this high-risk asymptomatic population was 53%.

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 9.

The percent of Anti-HAV reactive results with MONOLISA™ Anti-HAV EIA in this high-risk asymptomatic population was 45%.

Expected results for MONOLISATM Anti-HAV EIA in the US High Risk Group for Viral Hepatitis A (N=230)

MONOLISA™ Anti-HAV EIA								
Age Range	Gender	Reactive		Borderline		Nonreactive		Total
		N	%	N	%	N	%	
< 19	Female	1	100.0%	0	N/A	0	N/A	1
	Male	1	100.0%	0	N/A	0	N/A	1
20-29	Female	1	33.3%	0	N/A	2	66.7%	3
	Male	1	50.0%	0	N/A	1	50.0%	2
30-39	Female	4	57.1%	0	N/A	3	42.9%	7
	Male	12	33.3%	0	N/A	24	66.7%	36
40-49	Female	15	62.5%	0	N/A	9	37.5%	24
	Male	37	43.5%	1	1.2%	47	55.3%	85
50-59	Female	6	85.7%	0	N/A	1	14.3%	7
	Male	31	60.8%	1	2.0%	19	37.3%	51
60-69	Female	1	100.0%	0	N/A	0	N/A	1
	Male	9	90.0%	0	N/A	1	10.0%	10
70-79	Female	0	N/A	0	N/A	0	N/A	0
	Male	2	100.0%	0	N/A	0	N/A	2
Total		121	52.6%	2	0.9%	107	46.5%	230

Expected results for MONOLISATM Anti-HAV EIA in the European High Risk Group for Viral Hepatitis A (N=62)

MONOLISA™ Anti-HAV 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	1	50.0%	0	N/A	1	50.0%	2
	Male	5	35.7%	0	N/A	9	64.3%	14
40-49	Female	1	100.0%	0	N/A	0	N/A	1
	Male	9	64.3%	0	N/A	5	35.7%	14
50-59	Female	0	N/A	0	N/A	0	N/A	0
	Male	9	81.8%	0	N/A	2	18.2%	11
60-69	Female	0	N/A	0	N/A	0	N/A	0
	Male	1	50.0%	0	N/A	1	50.0%	2
70-79	Female	0	N/A	0	N/A	0	N/A	0
	Male	2	100.0%	0	N/A	0	N/A	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		28	45.2%	0	N/A	34	54.8%	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-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.¹⁸⁻²⁰

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.

Conclusion:

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