510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K092355

B. Purpose for Submission:

This is a new 510k application for a new indication for the MONOLISA™ Anti-HAV EIA with the EVOLIS™ Automated Microplate System. The MONOLISA™ Anti-HAV EIA was previously cleared with a manual assay procedure.

C. Measurand:

Antibody to Hepatitis A (Total)

D. Type of Test:

Enzyme immunoassay (competitive assay format)

E. Applicant:

Bio-Rad Laboratories

F. Proprietary and Established Names:

MONOLISA Anti-HAV EIA/Hepatitis A test (Total Antibody)
EVOLIS Automated Microplate System/Automated Laboratory Analyzer

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LOL	Class II	21 CFR 866.3310	Microbiology (83)
JJE	Class I	21 CFR 862.2160	Chemistry (75)

H. Intended Use:

1. Intended use`:
   The MONOLISA Anti-HAV EIA is an in vitro enzyme immunoassay kit intended for use in the qualitative detection of total antibodies (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. The MONOLISA Anti-HAV EIA is intended for manual use and with the Evolis™ Automated Microplate System in the detection of total antibodies to hepatitis A virus.

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

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

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   The assay may be run using a manual method or with the EVOLIS Automated Microplate System.

I. Device Description:

The MONOLISA Anti-HAV EIA 192 test kit contains the following components:

- 2 Microwell strip plates. Wells are coated with monoclonal anti-HAV
- Wash Solution Concentrate – Tris NaCl buffer, ProClin, Tween 20
- Negative Control – Human serum negative for total anti-HAV antibodies
- Positive Control – Human serum positive for anti-HAV antibodies
- Calibrator – Human serum positive for anti-HAV antibodies
- HAV Viral antigen – inactivated HAV virus in Tris buffer and ProClin
- Conjugate – Peroxidase labeled mouse monoclonal antibody to HAV in Tris buffer
- Substrate buffer – H_2O_2, buffer, DMSO
- Chromogen - TMB
- Stopping solution – 1N H_2SO_4

The EVOLIS Automated Microplate System is an automated microplate analyzer that performs all functions necessary for processing microplate assays. Functions include: barcode scanning, sample pre-dilutions, sample and reagent dispensing, plate incubations, plate wash cycles, photometric measurement of completed assay plates and results evaluation. The analyzer instrument is controlled via the EVOLIS software, a Windows 2000 application running on a separate dedicated PC. An operator loads the appropriate microplates, assay reagents, and patient and control samples, then selects assay parameters, loads sample information, initiates instrument processing, and generates results reports.

J. Substantial Equivalence Information:

1. Predicate device names:

   MONOLISA Anti-HAV EIA

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

   K063318

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use/Indications for Use	An in vitro enzyme immunoassay kit intended for use in the qualitative detection of total antibodies (IgG and IgM) to hepatitis A virus (anti-HAV) in human (adult and pediatric) serum or plasma (EDTA, Heparin, Citrate, ACD)	An in vitro enzyme immunoassay kit intended for use in the qualitative detection of total antibodies (IgG and IgM) to hepatitis A virus (anti-HAV) in human (adult and pediatric) serum or plasma (EDTA, Heparin, Citrate, ACD)
Assay procedure	Per the instructions in the package insert	Per the instructions in the package insert
Plate incubation	60 ± 5 minutes at 37°C + 2°C	60 ± 5 minutes at 37°C + 2°C
Plate washing	Wash with ≥ 370 µL of Working Wash Solution per well, and 30 - 60 second soak between each wash cycle for a total of 5 cycles.	Wash with ≥ 370 µL of Working Wash Solution per well, and 30 - 60 second soak between each wash cycle for a total of 5 cycles.
Result interpretation	Result interpretations, based on sample O.D.s, are determined according to package insert criteria.	Result interpretations, based on sample O.D.s, are determined according to package insert criteria.
Photometric measurement of completed assay plates	Read absorbance using 450 nm filter with 620 nm as the reference	Read absorbance using 450 nm filter with 615 to 630 nm as the reference

Differences		
Item	Device	Predicate
Sample and reagent dispensing	Samples and reagents are dispensed by the automated system	Samples and reagents are dispensed manually
Barcode reading	Sample and reagent ID are verified automatically	NA or can be performed manually with barcode

Differences		
Item	Device	Predicate
		wand
Plate incubation	Plates are automatically moved to the incubation chamber	Plates are moved manually to an incubation chamber
Plate wash cycles	Plates are automatically washed	Plates are moved manually to an automated plate washer
Data management	Archives and retrieves data and sample information	NA
Spectrophotometric verification of sample and reagent pipeting	Performed automatically	Optional verification visually or with microplate reader

K. Standard/Guidance Documents Referenced:

- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006)
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Guidance for Industry and FDA Reviewers (March 2007)
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (May 2005)
- Evaluation of Precision Performance of Qualitative Measurement Methods, CLSI EP5-A2
- User Protocol for Evaluation of Qualitative Test Performance, CLSI EP15-A2

L. Test Principle:

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:

1. Analytical performance:

a. Precision/Reproducibility:

A 21-member panel consisting of the following was tested: three (3) serum samples with six (6) corresponding plasma samples (EDTA K2, EDTA K3, Sodium Citrate, Sodium Heparin, Lithium Heparin, ACD) at three (3) different levels [1 low positive near the cutoff (Panel Set 1), 1 negative near the cutoff (Panel Set 2) and 1 negative (Panel Set 3)]. The kit controls and calibrator were also tested for a total of 24 samples. Two replicates each of the twenty-four (24) samples were assayed twice a day for 20 days. The data were analyzed per CLSI guidance EP5A2. The mean ratio, the Standard Deviation (SD) and percent coefficient of variation (%CV) were calculated for each panel member.

Panel Member	N	Mean CO/S	Within run[1] SD	Within run[1] CV (%)	Between Run[2] SD	Between Run[2] CV (%)	Between Day[3] SD	Between Day[3] CV (%)	Total[4] SD	Total[4] CV (%)
Positive Control	80	4.63	0.21	4.5	0.31	6.7	0.75	16.2	0.84	18.1
High Negative	76[5]	0.36	0.01	3.4	0.02	6.4	0.01	4.1	0.03	8.3
Cutoff Control	80	0.91	0.03	3.5	0.07	7.4	0.10	10.8	0.12	13.6
Serum (1)	80	1.13	0.05	4.6	0.06	5.3	0.11	9.5	0.13	11.8
EDTA K2 (1)	80	1.16	0.04	3.7	0.06	5.5	0.08	6.9	0.11	9.6
EDTA K3 (1)	80	1.16	0.04	3.8	0.09	7.9	0.08	6.7	0.13	11
Sodium Citrate (1)	80	1.12	0.05	4.2	0.09	8.0	0.08	7.5	0.13	11.8
Sodium Heparin (1)	80	1.24	0.06	4.9	0.08	6.8	0.11	9.0	0.15	12.3
Lithium Heparin (1)	80	1.18	0.04	3.1	0.08	6.6	0.12	10.2	0.15	12.5
ACD (1)	80	0.4	0.01	2.7	0.03	6.4	0.04	9.4	0.05	11.7
Serum (2)	80	0.64	0.02	3.6	0.03	5.1	0.02	3.4	0.05	7.1
EDTA K2 (2)	80	0.63	0.02	3.4	0.04	6.0	0.03	4.8	0.05	8.4
EDTA K3 (2)	80	0.62	0.02	3.1	0.04	7.0	0.03	4.9	0.06	9.1
Sodium Citrate (2)	80	0.63	0.02	3.1	0.04	7.0	0.04	5.9	0.06	9.7
Sodium Heparin (2)	80	0.68	0.02	3.3	0.04	6.2	0.07	10.4	0.09	12.6
Lithium Heparin (2)	80	0.61	0.02	3.2	0.04	6.1	0.04	7.1	0.06	9.9
ACD (2)	80	0.59	0.01	2.4	0.04	7.0	0.06	9.8	0.07	12.3
Serum (3)	80	0.41	0.01	1.9	0.03	6.3	0.02	4.7	0.03	8.1
EDTA K2 (3)	80	0.41	0.02	4.6	0.03	7.7	0.02	4.1	0.04	9.9
EDTA K3 (3)	80	0.41	0.01	3	0.03	7.1	0.02	4.6	0.04	8.9
Sodium Citrate (3)	80	0.43	0.01	2.7	0.03	6.5	0.02	4.9	0.04	8.6
Sodium Heparin (3)	80	0.43	0.01	2	0.03	5.9	0.04	9.1	0.05	11
Lithium Heparin (3)	80	0.4	0.01	3.5	0.03	6.8	0.04	9.2	0.05	12
ACD (3)	80	1.07	0.04	3.4	0.08	7.6	0.14	13.2	0.17	15.6

[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 days
[5] 4 replicates did not meet volume verification requirements

A 6-member panel consisting of diluted plasma specimens (negative and different levels of positive) was tested in triplicate, once a day for 5 days with the 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. One (1) lot was used at each of 3 sites.

Panel Member	N	Mean	Within Run[1]		Between Day[2]		Between Site[3]		Total[4]	
		CO/S	SD	%CV	SD	%CV	SD	%CV	SD	%CV
P1	90	0.41	0.02	5.7	0.01	1.2	0.00	1.1	0.02	5.9
P2	90	0.8	0.05	5.6	0.03	4.1	0.01	1.7	0.06	7.2
P3	90	1.3	0.06	4.9	0.05	4.2	0.005	0	0.08	6.4
P4	90	2.13	0.14	6.6	0.09	4.3	0.005	0	0.17	7.9
P5	90	2.82	0.13	4.5	0.16	5.6	0.005	0	0.20	7.1
P6	90	4.04	0.16	3.9	0.27	6.8	0.005	0	0.32	7.8
P7	90	3.69	0.17	4.6	0.28	7.5	0.005	0	0.32	8.8
P8	87	0.38	0.02	5.1	0.01	3.1	0.005	0	0.02	6.0
P9	87	0.95	0.06	5.9	0.04	4.0	0.005	0	0.07	7.1

[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 site: Variability of the assay performance from site to site
[4] Total: Total variability of the assay performance includes within run, between days and between sites
[5] Negative variances were rounded to zero, per statistical convention

b. Linearity/assay reportable range:

See K063318

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

See K063318

d. Detection limit:

See K063318

e. Analytical specificity:

See K063318

f. Assay cut-off:

See K063318

2. Comparison studies:

a. Method comparison with predicate device:

Six-hundred eighty-eight retrospective samples were tested on the MONOLISA Anti-HAV assay, using a total of four (4) EVOLIS instruments at three sites. The same samples were tested manually (reference method) on the MONOLISA Anti-HAV assay. Specimens that were borderline with the reference assay and negative with EVOLIS were considered as false negative for the EVOLIS; specimens that were borderline with the reference assay and reactive with EVOLIS were considered as false positive for the EVOLIS.

	EVOLIS Anti-HAV Results			
Manual Anti-HAV Results	**Reactive**	**Borderline**	**Nonreactive**	**Total**
Reactive	336	2	1	339
Borderline	2	0	1	3
Nonreactive	2	5	339	346
Total	340	7	341	688

The positive percent agreement with the reference method, manual testing, is 98.8% (336/340) with a 95% confidence interval of 97.0 – 99.5%. The negative percent agreement with the reference method is 97.4% (339/348) with a 95% confidence interval of 95.2 – 98.6%.

The EVOLIS was also evaluated by performing a combination of 2 assays on the same plate frame. In this study 315 samples were tested with the MONOLISA Anti-HAV assay on a combination plate on the EVOLIS (both the MONOLISA Anti-HAV EIA and MONOLISA Anti-HAV IgM EIA assays were run in a single microplate frame). Results were compared to the same samples tested manually (the reference method, individual plate format) on the MONOLISA Anti-HAV assay. Specimens that were borderline with the reference assay (manual individual plate) and negative with EVOLIS (combination plate) were considered as false negative for the EVOLIS (combination plate).

	EVOLIS™ Anti-HAV Results - Combination Plate			
Manual Anti-HAV Results - Individual Plate	**Reactive**	**Borderline**	**Nonreactive**	**Total**
Reactive	161	0	0	161
Borderline	0	0	0	0
Nonreactive	0	0	154	154
Total	161	0	154	315

The positive percent agreement with the reference method, manual testing, is 100% (161/161) with a 95% confidence interval of 97.7 – 100%. The negative percent agreement with the reference method is 100% (154/154) with a 95% confidence interval of 97.6 – 100%.

b. Matrix comparison:

See K063318

3. Clinical studies:

   a. Clinical Sensitivity:

   See K063318

   b. Clinical specificity:

   See K063318

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

   Not applicable.

4. Clinical cut-off:

   Not applicable.

5. Expected values/Reference range:

   See K063318

N. Instrument Name:

EVOLIS Automated Microplate System

O. System Descriptions:

1. Modes of Operation:
   The EVOLIS Automated Microplate System is an open tube, batch mode analyzer with a continuous load option. The reagent bottles used from the test kit are placed on the instrument with the caps removed. The sample tubes can be the primary tubes with stoppers removed or the serum/plasma can be poured off into identified test tubes.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X___ or No _____

3. Specimen Identification:

   Specimen information may be entered either by EVOLIS system barcode reading directly off the specimen tube or entered manually by the user.

4. Specimen Sampling and Handling:
   The system can store and distribute samples from different types of vessels into dilution vessels and microplates. The samples can be accessed in any order. Sample addition is via a 300 µL disposable tip. The system can load and unload samples and assay reagents while it is operating.

   The pipetting system utilizes a liquid syringe pump and system fluid. The system uses disposable tips (300 µL and 1100 µL), and can aspirate and dispense fluids from a variety of different vessels. Key functions of the system are liquid level detection, using capacitive sensing, verification of fluid distribution, and the detection of clots and blocked tips. If the pipettor does not detect a sufficient volume an error is displayed. The pipettor automatically flushes with system fluid between each aspirate/dispense cycle of samples and reagent during a pipetting sequence. Mixing occurs during the transfer of sample, addition of diluents, and other reagents.

   Intermediate vessels are used to dilute samples when the level of dilution exceeds the volume available in the final reaction vessel. Mixing is utilized to obtain a homogeneous mixture after preparing the dilution. The instrument has space for at least one microplate to be used as a dilution position.

5. Calibration:
   The system performs a self-test each time EVOLIS software is launched. During the self-test the instrument hardware is initialized and the status of all instrument modules is verified. The self-test evaluates the following systems: Pipettor, washer, photometer, plate transport, incubators, system communications, and other user-defined maintenance.

   Users are instructed in the Operator's Manual to perform the following Performance Evaluation Procedures monthly: Plate Transport Check, Photometer Verification Check, Fluidics Panel Check.

6. Quality Control:
   Assay includes positive and negative controls that are run with each batch.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:
N/A

Q. Proposed Labeling:

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

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