

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

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

K092353

**B. Purpose for Submission:**

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

**C. Measurand:**

Antibody to Hepatitis A (IgM)

**D. Type of Test:**

Enzyme immunoassay (competitive assay format)

**E. Applicant:**

Bio-Rad Laboratories

**F. Proprietary and Established Names:**

MONOLISA Anti-HAV IgM EIA/Hepatitis A test (IgM 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 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) in human (adult and pediatric) serum or plasma (EDTA, Heparin, Citrate, ACD). This 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. The MONOLISA Anti-HAV IgM EIA is intended for manual use and with the Evolis Automated Microplate System in the detection of IgM 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 IgM EIA 192 test kit contains the following components:

- 2 Microwell strip plates. Wells are coated with polyclonal anti-human IgM
- Wash Solution Concentrate – Tris NaCl buffer, ProClin, Tween 20
- Negative Control – Human serum negative for anti-HAV IgM and total antibodies
- Positive Control – Human serum positive for anti-HAV IgM antibodies
- Calibrator – Human serum positive for anti-HAV IgM antibodies
- Sample Diluent – Tris buffer containing protein and sample indicator dye
- 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<sub>2</sub>O<sub>2</sub>, buffer, DMSO
- Chromogen - TMB
- Stopping solution – 1N H<sub>2</sub>SO<sub>4</sub>

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 name: MONOLISA Anti-HAV IgM EIA
2. Predicate 510(k) number: K063319
3. Comparison with predicate:

| <b>Similarities</b>                               |   |   |
|---|---|---|
| Item  | Device  | Predicate   |
| Intended Use/Indications for Use                  | An in vitro enzyme immunoassay kit intended for use in the qualitative detection of IgM antibodies 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 IgM antibodies 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   |

| <b>Differences</b>            |  |   |
|-------------------------------|--|---|
| 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 wand |

| Differences  |  |  |
|--|--|--|
| Item   | Device   | Predicate  |
| 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 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 anti-HAV IgM 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 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 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:

##### 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)]. Two replicates each of the twenty-four (24) member panel were assayed twice a day for 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.

| Panel Member        | N  | Mean<br>S/CO | Within run <sup>1</sup> |        | Between Run <sup>2</sup> |        | Between Day <sup>3</sup> |        | Total <sup>4</sup> |        |
|---------------------|----|--------------|-------------------------|--------|--------------------------|--------|--------------------------|--------|--------------------|--------|
|                     |    |              | SD                      | CV (%) | SD                       | CV (%) | SD                       | CV (%) | SD                 | CV (%) |
| Positive Control    | 80 | 1.97         | 0.035                   | 1.8    | 0.091                    | 4.6    | 0.163                    | 8.3    | 0.190              | 9.7    |
| High Negative       | 80 | 0.10         | 0.006                   | 6.1    | 0.015                    | 14.9   | 0.015                    | 14.7   | 0.022              | 21.8   |
| Cutoff Control      | 80 | 3.78         | 0.146                   | 3.9    | 0.132                    | 3.5    | 0.166                    | 4.4    | 0.256              | 6.8    |
| Serum (1)           | 80 | 1.55         | 0.036                   | 2.3    | 0.076                    | 4.9    | 0.138                    | 8.9    | 0.161              | 10.4   |
| EDTA K2 (1)         | 80 | 1.44         | 0.020                   | 1.4    | 0.075                    | 5.2    | 0.131                    | 9.1    | 0.152              | 10.6   |
| EDTA K3 (1)         | 80 | 1.49         | 0.030                   | 2.0    | 0.083                    | 5.6    | 0.126                    | 8.5    | 0.154              | 10.3   |
| Sodium Citrate (1)  | 80 | 1.48         | 0.033                   | 2.2    | 0.086                    | 5.8    | 0.140                    | 9.5    | 0.168              | 11.3   |
| Sodium Heparin (1)  | 80 | 1.41         | 0.024                   | 1.7    | 0.080                    | 5.7    | 0.132                    | 9.4    | 0.156              | 11.1   |
| Lithium Heparin (1) | 80 | 1.39         | 0.026                   | 1.9    | 0.077                    | 5.5    | 0.120                    | 8.7    | 0.145              | 10.5   |
| ACD (1)             | 80 | 1.64         | 0.021                   | 1.3    | 0.107                    | 6.6    | 0.144                    | 8.8    | 0.181              | 11.0   |
| Serum (2)           | 80 | 0.62         | 0.016                   | 2.7    | 0.031                    | 5.0    | 0.059                    | 9.5    | 0.068              | 11.1   |
| EDTA K2 (2)         | 80 | 0.69         | 0.016                   | 2.3    | 0.034                    | 5.0    | 0.077                    | 11.3   | 0.086              | 12.5   |
| EDTA K3 (2)         | 80 | 0.69         | 0.014                   | 2.0    | 0.046                    | 6.6    | 0.073                    | 10.5   | 0.087              | 12.5   |
| Sodium Citrate (2)  | 80 | 0.74         | 0.014                   | 1.9    | 0.044                    | 5.9    | 0.075                    | 10.1   | 0.088              | 11.9   |
| Sodium Heparin (2)  | 80 | 0.66         | 0.011                   | 1.6    | 0.041                    | 6.2    | 0.061                    | 9.2    | 0.074              | 11.2   |
| Lithium Heparin (2) | 80 | 0.66         | 0.020                   | 3.0    | 0.040                    | 6.1    | 0.058                    | 8.9    | 0.073              | 11.1   |
| ACD (2)             | 80 | 0.78         | 0.012                   | 1.5    | 0.052                    | 6.7    | 0.072                    | 9.2    | 0.090              | 11.5   |
| Serum (3)           | 80 | 0.10         | 0.004                   | 3.6    | 0.010                    | 9.7    | 0.010                    | 10.1   | 0.015              | 14.5   |
| EDTA K2 (3)         | 80 | 0.11         | 0.005                   | 4.7    | 0.011                    | 10.3   | 0.009                    | 8.2    | 0.015              | 14.0   |
| EDTA K3 (3)         | 80 | 0.10         | 0.004                   | 4.2    | 0.010                    | 9.5    | 0.011                    | 10.6   | 0.015              | 14.8   |
| Sodium Citrate (3)  | 80 | 0.10         | 0.003                   | 2.9    | 0.009                    | 9.2    | 0.010                    | 9.6    | 0.014              | 13.8   |
| Sodium Heparin (3)  | 80 | 0.10         | 0.004                   | 3.8    | 0.009                    | 8.7    | 0.010                    | 9.9    | 0.014              | 13.7   |
| Lithium Heparin (3) | 80 | 0.10         | 0.015                   | 4.5    | 0.010                    | 9.5    | 0.010                    | 9.2    | 0.014              | 14.0   |
| ACD (3)             | 78 | 0.10         | 0.005                   | 4.3    | 0.010                    | 10.0   | 0.009                    | 8.7    | 0.015              | 13.9   |

<sup>1</sup> Within Run: variability of the assay performance from replicate to replicate

<sup>2</sup> Between Run: variability of the assay performance from Run to Run

<sup>3</sup> Between Day: variability of the assay performance from Day to Day

<sup>4</sup> Total: Total variability of the assay performance includes within run, between run and between days

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 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. One (1) lot was used at each of 3 sites.

| Panel Member | N  | Mean | Within Run <sup>1</sup> |      | Between Day <sup>2</sup> |      | Between Site <sup>3</sup> |     | Total <sup>4</sup> |      |
|--------------|----|------|-------------------------|------|--------------------------|------|---------------------------|-----|--------------------|------|
|              |    |      | CO/S                    | SD   | %CV                      | SD   | %CV                       | SD  | %CV                | SD   |
| P1           | 90 | 0.16 | 0.02                    | 13.5 | 0.01                     | 8.9  | 0.00 <sup>5</sup>         | 0.0 | 0.026              | 16.1 |
| P2           | 89 | 0.72 | 0.02                    | 3.3  | 0.03                     | 3.8  | 0.021                     | 2.9 | 0.042              | 5.8  |
| P3           | 90 | 1.18 | 0.04                    | 3.4  | 0.03                     | 2.9  | 0.031                     | 2.6 | 0.061              | 5.2  |
| P4           | 90 | 1.17 | 0.45                    | 3.9  | 0.04                     | 3.1  | 0.032                     | 2.8 | 0.066              | 5.7  |
| P5           | 90 | 3.05 | 0.08                    | 2.6  | 0.10                     | 3.2  | 0.084                     | 2.8 | 0.151              | 5.0  |
| P6           | 88 | 3.63 | 0.18                    | 4.8  | 0.14                     | 4.0  | 0.000 <sup>5</sup>        | 0.0 | 0.227              | 6.3  |
| P7           | 90 | 1.90 | 0.08                    | 4.0  | 0.07                     | 3.7  | 0.044                     | 2.3 | 0.113              | 5.9  |
| P8           | 88 | 0.11 | 0.01                    | 9.7  | 0.02                     | 13.0 | 0.000 <sup>5</sup>        | 0.0 | 0.018              | 16.2 |
| P9           | 88 | 3.46 | 0.23                    | 6.6  | 0.23                     | 6.6  | 0.106                     | 3.1 | 0.339              | 9.8  |

<sup>1</sup> Within run: Variability of the assay performance from replicate to replicate

<sup>2</sup> Between day: Variability of the assay performance from day to day

<sup>3</sup> Between site: Variability of the assay performance from site to site

<sup>4</sup> Total: Total variability of the assay performance includes within run, between days and between sites

<sup>5</sup> Negative variances were rounded to zero, per statistical convention

*b. Linearity/assay reportable range:*

K063319

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

See K063319

*d. Detection limit:*

See K063319

*e. Analytical specificity:*

See K063319

*f. Assay cut-off:*

See K063319

2. Comparison studies:

*a. Method comparison with predicate device:*

Six-hundred ninety-one retrospective samples were tested on the MONOLISA Anti-HAV IgM 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 IgM 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.

| Manual Anti-HAV Results | EVOLIS Anti-HAV IgM Results |            |             |       |
|-------------------------|-----------------------------|------------|-------------|-------|
|                         | Reactive                    | Borderline | Nonreactive | Total |
| Reactive                | 94                          | 0          | 0           | 94    |
| Borderline              | 1                           | 0          | 0           | 1     |
| Nonreactive             | 1                           | 0          | 595         | 596   |
| <b>Total</b>            | 96                          | 0          | 595         | 691   |

The positive percent agreement with the reference method, manual testing, is 100% (94/94) with a 95% confidence interval of 96.1 – 100%. The negative percent agreement with the reference method is 99.7% (595/597) with a 95% confidence interval of 98.8 – 99.9%.

The EVOLIS was also evaluated by performing a combination of 2 assays on the same plate. In this study 313 samples were tested with the MONOLISA Anti-HAV IgM assay on a combination plate on the EVOLIS (both the Anti-HAV IgM EIA and Anti-HAV 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 IgM 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).

| Manual Anti-HAV IgM Results - Individual Plate | EVOLIS™ Anti-HAV IgM Results - Combination Plate |            |             |       |
|--|--|------------|-------------|-------|
|  | Reactive   | Borderline | Nonreactive | Total |
| Reactive                                       | 49   | 0          | 0           | 49    |
| Borderline                                     | 1  | 0          | 0           | 1     |
| Nonreactive                                    | 0  | 1          | 262         | 263   |
| <b>Total</b>                                   | 50   | 1          | 262         | 313   |

The positive percent agreement with the reference method, manual testing, is 100% (49/49) with a 95% confidence interval of 92.7 – 100%. The negative percent agreement with the reference method is 99.2% (262/264) with a 95% confidence interval of 97.3 – 99.8%.

*b. Matrix comparison:*

See K063319

3. Clinical studies:

*a. Clinical Sensitivity:*

See K063319

*b. Clinical specificity:*

See K063319

*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 K063319

**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.