CTK Biotech, Inc.
The New Wave of Diagnosis

HAV IgM ELISA Test

REF

E0100

IVD

- 96-well ELISA kit for the qualitative detection of IgM anti-HAV in human serum or plasma
- For export only, not for re-sale In the USA
- Store at 2℃ - 8℃ upon receipt

INTENDED USE

The HAV IgM ELISA Test is a solid phase enzyme linked immunoabsorbent assay for the qualitative detection of IgM anti-hepatitis A virus in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HAV. Any reactive specimen with the HAV IgM ELISA Test must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

HAV is a positive RNA virus, a unique member of picornavirdae[1]. Its transmission depends primarily on serial transmission from person to person by the fecal-oral route. Although hepatitis A is not ordinarily a sexually transmitted disease, the infection rate is high among male homosexuals, as result of oral-anal contact [2,3].

The presence of specific anti-HAV IgM in blood specimens suggests acute or recent HAV infection 4-6. The IgM antibody rapidly increases in titer over a period of 4-6 weeks post infection, and then declines to non-detectable levels within 3 to 6 months in most patients 7.

TEST PRINCIPLE

HAV IgM ELISA Test is a solid phase enzyme linked immunoabsorbent assay based on the principle of the IgM capture technique for the detection of IgM anti-HAV in human serum or plasma.

The HAV IgM ELISA Test is composed of two key components:

1) Solid microwells pre-coated with monoclonal anti human IgM antibody;

2) Liquid conjugates composed of HAV antigens conjugated with horse reddish peroxidase (HRP-HAV conjugates).

During the assay, the test specimen is first incubated with the coated microwells. IgM anti-HAV, if present in the specimen, binds to the antibody coated on the microwell surface.

In the second incubation with the HRP-HAV conjugates, the IgM anti-HAV antibody absorbed on the surface of microwell reacts to the HRP-HAV conjugates, forming a complexed conjugates.

Unbounded conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with Stop Solution and absorbances are read using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with anti-human IgM antibody	12 wells x 8 strips	E0100W
2	HAV IgM negative control	1 mL	E0100N
3	HAV IgM positive control	1 mL	E0100P
4	HRP-HAV conjugates	6 mL	E0100H
5	Wash buffer (30 x concentrate)	20 mL	WE3000
8	TMB substrate A	6 mL	TME2000A
7	TMB substrate B	6 mL	TME2000B
8	Stop solution	6 mL	SE1000
9	ELISA Working Sheet	2 sets	E0001ES
10	Product insert	1 set	PI-E0100

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 50 µL and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3OD or greater at 450 nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells.
4. Parafilm or other adhesive film sealant for sealing plate.
5. Timer.
6. Distilled water or de-ionized water.

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8℃.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18℃-28℃) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
13. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16. Avoid strong light during color development.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2℃-8℃. If storage period greater than three days are anticipated, the specimen should be frozen (-20℃). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (18℃-28˚C).
2. Dilute concentrated Wash Buffer 30 fold with water as following:

Plate	DI water	30 X wash buffer	Final volume
Full plate	580 mL	20 mL	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

   Warm up the concentrated Washing Buffer at 37˚C to dissolve the precipitant if it appears.

3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet

   2.1 Blank well: Leave the blank well alone. Don't add any reagents.

   2.2 Control wells: Add 50 µL of HAV IgM Positive, Negative Control into the designated control wells, respectively.

   2.3 Test wells: Add 50 µL of test specimens into each test well, respectively.

   To ensure better precision, use pipette to handle solution.

3. Incubate the wells at 37℃ for 30 minutes.

4. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.

5. Add 50 µL of HRP-HAV antigen conjugate into each well except the blank well, cover the plate, and incubate at 37℃ for 30 minutes.

7. Wash the plate 5 times as step 5 described.

8. Add 50 µL (or 1 drop) of TMB substrate A and 50 µL (or 1 drop) of TMB substrate B into each well including the blank well.

9. Incubate at 37℃ in dark for 10 minutes.

10. Stop the reaction by adding 50 µL (1 drop) of stop buffer to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**

11. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620 –690 nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = N x 2.1

N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if the mean OD is less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the HAV IgM positive controls should be ≥ 0.80.
The mean OD value of the HAV IgM negative controls should be ≤ 0.10.

Check the procedure and repeat assay if above conditions are not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	**<** 1.00
Positive	**≥** 1.00

1. The negative result indicates that there is no detectable IgM anti-HAV in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the HAV IgM Test. They should be retested in duplicate before final interpretation.
   If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the HAV IgM Test.
   Non repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens by serum or plasma with a high antibody titer,
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the stopping solution

   If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the HAV IgM Test, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 400 specimens from susceptible subjects were tested by the HAV IgM ELISA and by a reference HAV IgM ELISA test. Comparison for all subjects is showed in the following table:

	HAV IgM Test		
Ref. HAV IgM	**Positive**	**Negative**	**Total**
Positive	39	0	39
Negative	0	361	361
Total	39	361	400

Relative Sensitivity:100%, Relative Specificity:100%, Overall Agreement: 100%

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of IgM anti-HAV in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The HAV IgM Test is limited to the qualitative detection of IgM anti-HAV in human serum or plasma. The intensity of color does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable IgM anti-HAV. However, a negative test result does not preclude the possibility of exposure to or infection with HAV.
4. A negative result can occur if the quantity of IgM anti-HAV present in the specimen is below the detection limit of the assay, or the antibodies that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Minor P. Picornaviridae. In: Francki RIB, Fauquet CM, Knudson DL, et al., eds. Classification and nomenclature of viruses (Arch Virol Supp 2). Wien: Springer-Verlag, 1991: 320-326.
2. Keeffe EB. Clinical approach to viral hepatitis in homosexual men. Med Clin North Am. 1986;70(3):567-86.
3. Ballesteros J, Dal-Re R, Gonzalez A, del Romero J. Are homosexual males a risk group for hepatitis A infection in intermediate endemicity areas? Epidemiol Infect. 1996;117(1):145-8.
4. Bradley DW, Maynard JE, Hindman SH, et al: Serodiagnosis of viral hepatitis A: Detection of acutephase immunoglobulin M anti-hepatitis A virus by radioimmunoassay. J Clin Microbiol 1977; 5: 521-530.

EC REP **European Authorized Representative:**
CEpartner4U , Esdoornlaan 13, 3951DB Maarn.
The Netherlands. Tel.: +31 (0)6.516.536.26

Manufacturer:
CTK Biotech, Inc.
6748 Nancy Ridge Drive, San Diego, CA 92121, USA
Tel: 858-457-8698, Fax: 858-535-1739,
E-mail: info@ctkbiotech.com

PI-E0100 Rev. C Effective date: 10-10-2006

Index of CE Symbol

	Attention, see instructions for use
IVD	For *in vitro* diagnostic use only
REF	Catalog #
LOT	Lot Number
	Use by
Σ	Tests per kit
2℃ 8℃	Store between 2-8°C
2	Do not reuse
	Manufacturer
	Date of manufacture