Epitope Diagnostics, Inc.

EDI™ Hepatitis E Virus IgM ELISA Kit

Enzyme-linked immunosorbent assay for the detection of IgM antibody against HEV in serum or plasma

REF KT 1019 EU: IVD CE Σ 96 +2 +8 °C

INTENDED USE

This kit is an enzyme-linked immunosorbent assay (ELISA, IgM Capture Method) intended for the qualitative determination of human IgM antibody against Hepatitis E Virus in human plasma and serum. This test is for in-vitro diagnostic use and suitable for the aid of clinical diagnostic procedures.

SUMMARY OF PHYSIOLOGY

Hepatitis E (HEV) viral particles are 27 to 34 nanometers in diameter, non-enveloped and contain single stranded positive-sense RNA that is approximately 7300 bases in length. The RNA molecule contains three discontinuous and partially overlapping open reading frames (ORFs) along with 5' and 3' cis-acting elements, which have important roles in HEV replication and transcription. ORF1 encodes a methyltransferase, protease, helicase and replicase. ORF2 encodes the capsid protein and ORF3 encodes a protein of undefined function.

HEV was not recognized as a distinct human disease until 1980. Although humans are considered the natural host for HEV, antibodies to HEV or closely related viruses have been detected in primates and several other animal species. Animals have been reported as a reservoir for the hepatitis E virus, with some surveys showing infection rates exceeding 95% among domestic pigs. HEV is transmitted via the fecal-oral route. Hepatitis E is a waterborne disease; contaminated water or food supplies have been implicated in major outbreaks. Consumption of contaminated drinking water has given rise to epidemics, and the ingestion of raw or uncooked shellfish has been the source of sporadic cases in endemic areas. There is a possibility of zoonotic spread of the virus, since several non-human primates, pigs, cows, sheep, goats and rodents are susceptible to infection. The risk factors for HEV infection are related to HEV shedding in feces and poor sanitation. Person-to-person transmission is uncommon. There is no evidence for sexual transmission or transmission by transfusion.

The incubation period following exposure to HEV ranges from 3 to 8 weeks, with a mean of 40 days. The period of communicability is unknown. There are no chronic infections reported. Hepatitis E virus causes acute sporadic and epidemic viral hepatitis. Symptomatic HEV infection is most common in youth and adults aged 15-40 years. Although HEV infection is frequent in children, it is mostly asymptomatic or causes a very mild illness without jaundice (anicteric) that goes undiagnosed. Typical signs and symptoms of hepatitis include jaundice (yellow discoloration of the skin and sclera of the eyes, dark urine and pale stools), anorexia (loss of appetite), an enlarged, tender liver (hepatomegaly), abdominal pain and tenderness, nausea and vomiting, and fever, although the disease may range in severity from subclinical to fulminant.

Both IgM and IgG subtypes of antibodies can be detected in patients with hepatitis E after 3 – 4 weeks of infection. The IgM subtype of anti-HEV antibody disappears after 15 weeks, whereas, the IgG subtype titer continues to be high.

ASSAY PRINCIPLE

The assay is based on the principle of "IgM capture". Wells of a microtiter plate are coated with an anti-human IgM specific antibody. During the first incubation of the test serum or plasma sample, human IgM antibody is captured by the solid-phase coated anti-human IgM. After a washing step, all the other components of the sample including human IgG are removed. Addition of peroxidase (HRP)-conjugated HEV antigen allows the binding of the previously captured anti-HEV IgM. After the second incubation, a complex of "well coated anti-human IgM antibody – human IgM – HRP conjugated HEV antigen" is formed. Unbound HRP conjugates are removed with a wash step. The enzyme complex is detected by adding a chromogen/substrate into each well. A blue color is developed in proportion to the amount of anti-HEV IgM antibody in the specimen. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of controls and specimens is determined by using an ELISA plate reader with wavelength at 450 nm.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

Materials provided in this kit:

1. **Anti-human IgM Microtiter Plate (Cat. No. 30900):** One microplate with 12 by 8 strips (96 wells total) coated with Anti-human IgM Specific antibody. The plate is framed and sealed in a foil zipper bag with desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. **HEV IgM Negative Control (Cat. No. 30582):** One vial contains 1 ml of a normal human serum-based control. This reagent is ready to use. It should be stored at 2-8°C and is stable until the expiration date on the kit box.

3. **HEV IgM Positive Control (Cat. No. 30583):** One vial contains 0.5 ml of an anti-HEV IgM positive serum-based control. This reagent is ready to use. It should be stored at 2-8°C and is stable until the expiration date on the kit box.

4. **HRP-Conjugated HEV Antigen (Cat. No. 30584):** One bottle contains 11 ml of ready-to-use HRP-conjugated HEV antigen. It should be stored at 2-8°C and is stable until the expiration date on the kit box.

5. **Assay Buffer (Cat. No. 30585):**
One bottle contains 15ml of assay buffer. This reagent is ready to use. It should be stored at 2-8°C and is stable until the expiration date on the kit box.

6. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains **30 mL** of 30-fold concentrate. Before use, the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working (1x) wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

7. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle contains **12 mL** of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent is ready to use and should be stored at 2 – 8°C. It is stable until the expiration date on the kit box.

8. **ELISA Stop Solution (Cat. No. 10030)**
One bottle contains 12 mL of stop solution. It is ready to use. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

STORAGE

Unopened test kits should be stored at 2-8°C upon receipt. The microtiter plate, once opened, should be kept in its sealed bag with desiccant to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown at 4°C, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

SAFETY PRECAUTIONS

Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory practices. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards. Dispose of all specimens and materials used to perform the test as if they contained infectious agents. Do not mix reagents from kits with different lot numbers. Cross contamination between reagents will invalidate the test results. All reagents and components, except the conjugate, must be equilibrated at room temperature prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision pipettes: 0.01, 0.10 and 1.0 ml, etc.
2. Disposable pipette tips.
3. Distilled water.
4. Incubator of 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

SPECIMEN COLLECTION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic, or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulates should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2 - 8ºC prior to assaying. Specimens held for a longer time can be frozen at -20ºC and mixed prior to testing. Avoid repeated freeze thaw cycles. At least two wells of negative and positive controls each should be run in every assay.

ASSAY PROCEDURE

1. Reagent Preparation

(a) Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(b) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

2. Patient Sample Preparation

Samples need to be diluted 1:10 with assay buffer. These samples can be diluted directly on the plate or in a separate tube.

(a) Directly on the plate: 10µl sample + 100µl assay buffer

(b) In a tube: 15µl sample + 150µl Assay buffer.
Then Add 100µl into each well

3. Assay Procedure

(1) Place a sufficient number of microplate strips in a holder to run assay controls and desired unknown samples in duplicate. See test configuration for example.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Blank	SAMPLE 3	SAMPLE 7
B	Negative Ctrl	SAMPLE 3	SAMPLE 7
C	Negative Ctrl	SAMPLE 4	SAMPLE 8
D	Positive Ctrl	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

(3) Set one blank well as a background control. Add **100µl** of Assay Buffer (30585) into this well.

(4) First, dispense **100 µL** of Negative Control (30582) in duplicate into designated wells.

(5) Dispense **100 µL** of Positive Control (30583) in singlet into designated wells.

*(6) Add **10 µl** of serum or plasma samples directly into respective sample wells then dispense **100 µl of** Assay Buffer (Catalog No. 30585) into these wells.*

*NOTE: If samples were diluted (1:10) using assay buffer in a separate tube before starting the assay, add **100µl** of diluted serum or plasma samples into respective sample wells.*

(7) Seal the plate with a plate sealer and incubate at **37ºC for 45 min**.

(8) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(9) Add **100 µl** of HRP Conjugated HEV Antigen (Catalog No. 30584) to each well, except the blank well. Mix gently by

swirling the microtiter plate on flat bench for 15 seconds. *Do not add HRP Conjugated HEV Antigen to the blank well.*

(10) Seal the plate with a plate sealer and incubate at **37ºC for 30 min**.

(11) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(12) Add **100 µl** of ELISA HRP substrate (Catalog No. 10020) to each well and incubate at room temperature for **20 min**.

(13) Add **100 µl** of Stop Solution (Catalog No. 10030) to each well to stop the color reaction. Read absorbance values of all samples immediately at **450 nm**.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

1. Internal Quality Control Standard of the Assay:

- The Blank should be less than 0.1 at OD 450 nm
- The negative control should be less than 0.12 at OD 450nm
- The positive should be over 0.5 at OD 450 nm
- If the assay does not meet the above three requirements, the assay is invalid.

2. Calculation of Cut Off Value (COV):

Positive COV = 1.1 x (NCx + 0.10)

Negative COV = 0.9 x (NCx + 0.10)

NCx: mean OD of the negative controls

3. Report Test Sample Result:

	Sample OD
Positive	≥ Positive COV
Negative	≤ Negative COV
Equivocal	> Negative COV and < Positive COV

Note: For equivocal results, a follow up test with a fresh specimen is recommended, if clinically indicated.

4. Test Example

ROW	STRIP 1 (OD 450 nm)		Corrected
A	Blank	0.073	0
B	Neg. Ctr	0.107	0.034
C	Neg. Ctr	0.100	0.027
D	Pos. Ctr.	1.332	1.259
E	Sample 1	0.592	0.519
F	Sample 2	0.635	0.562
G	Sample 3	0.090	0.017
H	Sample 4	0.198	0.125

4.1 The OD of blank, negative controls and positive control meet the Internal Quality Control Standard. The Assay is valid.

4.2 Calculate the Mean OD for negative control:

$Mean_{neg.}$ = (0.034 + 0.027)/2 = 0.031

4.3 Calculate the Positive and Negative Cut-Off Value:

Positive COV = 1.1 x (0.031 + 0.10) = 0.1441

Negative COV = 0.9 x (0.031 + 0.10) = 0.118

4.4 Interpret the Sample Result:

*Sample 1 = **0.519** ≥ Positive COV (0.1441) → Positive*
*Sample 2 = **0.562** ≥ Positive COV (0.1441) → Positive*
*Sample 3 = **0.017** ≤ Negative COV (0.118) → Negative*
*Sample 4 = 0.118 ≤ **0.125** ≤1441 → Equivocal*

LIMITATION OF THE PROCEDURE

1. The HEV IgM ELISA is limited to the detection of IgM subtype of antibody against HEV in human serum or plasma. It is recommended to run duplicates for each test sample.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. Aspirate the well thoroughly to empty the entire contents of each well before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test. A complete evaluation by physician is needed for a final diagnosis.
4. Do not use reagents from other tests; that will cause incorrect results.
5. Following the procedure instruction closely, especially with regards to incubation time and temperature.

QUALITY CONTROL

1. General Statement

To assure the validity of the results, each assay should include adequate controls with known positive levels of HEV-IgM. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

2. Precision

One positive and one negative samples are determined in 8 replicates in an assay. The mean at OD450 nm is calculated. The precision is presented as standard deviation and CV%.

Sample	Negative	Low Positive
Mean OD 450 nm	0.133	0.796
Standard Deviation	0.009	0.045
CV%	6.7%	5.7%

3. Interference

One low positive sample and one negative sample are added with one tenth volume of interference materials to reach a final concentration at hemoglobin – Low (Hb - L) 0.2 mg/ml, hemoglobin – High (Hb - H) 2 mg/ml, bilirubin 2 µg/ml and lipid 100 mg/ml. All samples are tested in an assay in duplicate. The results indicate that there is no significant interference was observed.

		Mean OD 450 nm	
	Additive	**Positive**	**Negative**
1	None	0.578	0.101
2	Hb - L	0.614	0.080
3	Hb - H	0.586	0.122
4	Bilirubin	0.509	0.099
5	Lipid	0.600	0.087

4. Clinical Sensitivity and Specificity

Seventy four normal donor serum and 9 true positive serum samples were tested in 3 assays. The normal donor sera are considered as true negative

	Negative	Positive	Equivalent
True Negative	65	4	5
True Positive	0	9	0

The calculated clinical sensitivity is 100% and the calculated clinical specificity is 94.2%.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or to place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

CE EC REP

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Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
IVD In Vitro Diagnostic Device	Use by
Read instructions before use	LOT Lot No.
EC REP Authorized Representative In Europe	

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