Epitope Diagnostics, Inc.

EDI™ Hepatitis E Virus IgG ELISA Kit

Enzyme-linked immunosorbent assay for the detection of IgG antibody against Hepatitis E Virus in serum or plasma

REF KT 1018 EU: IVD CE Σ 96 +2 +8 °C

I.INTENDED USE

This ELISA is a qualitative enzyme immunoassay for the detection of human antibody (specifically for IgG subtype) against HEV human specimen.

II. 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.

III. ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the qualitative measurement of HEV IgG level in test sample. The assay utilizes the microplate based enzyme immunoassay technique
by coating highly purified HEV antigen onto the wall of microtiter well.

Assay controls and human serum or plasma samples containing HEV IgG antibodies are added to microtiter wells of a microplate that was coated with a highly purified HEV antigen on its wall. After the first incubation period, the unbound protein matrix is removed in the subsequent washing step. A horseradish peroxidase conjugated goat anti-human IgG subclass specific antibody (tracer antibody) is added to each well. After an incubation period an immunocomplex of “HEV Ag– anti-HEV IgG Antibodies– HEV IgG tracer antibody” is formed if there is HEV IgG antibody present in the test sample. The unbound tracer antibody is removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the human IgG on the wall of the microtiter well is directly proportional to the amount of HEV IgG antibody level in the sample

IV. 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.

1. **HEV Antigen Coated Plate (Cat. No. 30326):**
   One microplate with 12 by 8 strips (96 wells total) coated with HEV recombinant Antigen. 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 IgG Negative Control (Cat. No. 30592):**
   One vial contains 1.0 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 IgG Positive Control (Cat. No. 30593):**
   One vial contains 1.0 ml of an anti-HEV IgG 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. **HEV IgG Tracer Antibody (Cat. No. 30594):**
   One bottle contains 11 ml of ready-to-use HEV IgG Tracer Antibody and must be diluted prior to use. It should be stored at 2-8°C and is stable until the expiration date on the kit box.

5. **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.

6. **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.

7. **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.

8. **Concentrated Sample Diluent (31129)**
   One bottle contains 30 mL of 5-fold concentrate. Before use the content must be diluted with 120 mL of deminerialized water and mixed well. Upon dilution, this yields a ready-to-use patient

V. STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in the sealed pouch to minimize exposure to air.

VI. SAFETY PRECAUTIONS

The reagents must be used in a laboratory setting. Reagents of bovine serum were derived in the contiguous 48 United States, and have been obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases.

Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

VII. MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µl, 65 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

VIII. SPECIMEN COLLECTION

Only 10 µL of human serum or plasma (EDTA or Heparin) is required for this kit. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples may be stored at –20ºC or below until measurement. Plasma samples should be stored at 2-8°C or -20°C for long term storage. Avoid more than three freeze-thaw cycles of specimen.

IX. ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
(2) Concentrated Sample Diluent and ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

Patient serum or plasma sample needs to be diluted 1:100 with working Sample Diluent (1x) before being measured.

(1) Label test tube (12x75 mm).
(2) Add 1 mL of assay buffer to each tube.
(3) Pipet 10 µL of patient serum or plasma sample to tube and mix well.

3. Assay Procedure

(1) Place a sufficient number of HEV Antigen coated microwell strips in a holder to run assay controls and unknown samples in duplicate.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Control Negative	SAMPLE 3	SAMPLE 7
B	Control Negative	SAMPLE 3	SAMPLE 7
C	Control Positive	SAMPLE 4	SAMPLE 8
D	Control Positive	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) Add **100 µL** of Controls and diluted (1:100) patient samples
(4) Cover the plate with an aluminum foil to avoid exposure to light. Incubate the plate at room temperature static for **30 minutes.**
(5) 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.
(6) Add **100 µL** HEV IgG Tracer Antibody
(7) Cover the plate with an aluminum foil to avoid exposure to light. Incubate the plate at room temperature, static for **30 minutes**.
(8) 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 ELISA HRP Substrate into each of the wells.

(10) Cover the plate with an aluminum foil to avoid exposure to light. Incubate plate at room temperature static for **20 minutes**
(11) Remove the aluminum foil and plate sealer. Add **100 µl** of ELISA Stop Solution into each of the wells. Mix gently.
(12) Read the absorbance at 450 nm within 10 minutes using a microplate reader.

X. PROCEDURAL NOTES

1. It is recommended that all control and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles. Store any unused antibody coated strips in the foil zip-seal bag with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may affect the results.
5. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate readings.
6. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

XI. INTERPRETATION OF RESULTS

Visual:

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result.

ELISA Reader:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off
The positive cut-off and the negative cut-off are established by using following formula.

Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.10)
Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

3. Interpret test result
   i. Positive: patient sample extinction is greater than the Positive Cut-Off
   ii. Negative: patient sample extinction is less than the Negative Cut-Off
   iii. Equivocal: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

4. Assay quality control
   1. Positive control must show an average OD reading greater than 0.5.
   2. Negative control should show an average OD reading less than 0.12.

XII. EXAMPLE DATA AND CALCULATED CUT-OFF

A typical absorbance data and the resulting negative control and positive controls are represented. This absorbance must not be used in lieu of control values run with each assay.

ROW	STRIP 1 (OD 450 nm)	
A	Neg. Ctr	0.044
B	Neg. Ctr	0.041
C	Pos. Ctr.	2.120
D	Pos. Ctr.	2.061
E	Sample 1	0.249
F	Sample 2	2.215
G	Sample 3	0.082
H	Sample 4	0.135

1. The OD of negative controls and positive control meet the Internal Quality Control Standard. The Assay is valid.
2. Calculate the Mean OD for negative control:

   $Mean_{neg.} = (0.044 + 0.041)/2 = 0.043$

3. Calculate the Positive and Negative Cut-Off Value:

Positive Cut-Off = 1.1 x (0.043 + 0.10) = 0.157
Negative Cut-Off = 0.9 x (0.043 + 0.10) = 0.129
Equivocal = 0.130 ~ 0.156

4. Interpret the Sample Result:

Sample 1 = 0.249 ≥ Positive COV (0.157) → *Positive*
Sample 2 = 2.215 ≥ Positive COV (0.157) → *Positive*
Sample 3 = 0.082 ≤ Negative COV (0.129) → *Negative*
Sample 4 = 0.135 ≤Pos. COV; ≥ Neg COV → *Equivocal*

XIII. LIMITATION OF THE PROCEDURE

1. This HEV IgG ELISA is limited to the detection of human IgG subtype of antibody against HEV in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. Aspirate the well or get rid of the entire content of wells completely before adding the washing solution.
3. A negative result does not exclude the possibility of exposure or infection with HEV.
4. A positive result in neonates must be interpreted with caution, since IgG is transferred passively from mother to the fetus before birth.
5. As with all laboratory tests, a definitive clinical decision should not be made based only on the results of a single test. A complete evaluation by physician is needed.
6. Samples with positive or equivocal result must be re-analyzed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for HEV IgG antibody. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.
7. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
8. Do not use reagents from different tests that will cause incorrect results.
9. Follow the procedure instructions closely, especially the incubation time and temperature.

XIV. QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.12 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

XV. PERFORMANCE CHARACTERISTICS

Reproducibility and Precision

The reproducibility of this assay is validated by measuring two samples both in a single assay of 16-replicate determinations (intra-assay) and in 10 different assays run on different dates (inter-assay).

Inter-Assay		
	Sample 1	**Sample 2**
Mean	0.270	2.135
Std Dev	0.021	0.086
%CV	7.8%	4.0%

Intra-Assay		
	Sample 1	**Sample 2**
Mean	0.273	2.089
Std Dev	0.019	0.108
%CV	6.9%	5.2%

Interference

One positive sample is added with 5% volume of interference materials to reach a final concentration shown in the table below. All samples are tested in an assay in duplicate.

Sample	OD @450nm	OD @450nm	Interferant added (mg/mL)
Test Control	**0.443**	**3.587**	-
Billirubin	0.220	1.313	10
	0.309	2.727	2
	0.444	2.729	0.4
Test Control	**0.292**	**0.615**	-
Hemoglobin	0.417	0.469	10
	0.311	0.521	2
	0.334	0.620	0.4
Lipid	0.258	0.655	200
	0.275	0.465	40
	0.293	0.603	8

XVI. 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

XVII. REFERENCES

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
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CE EC REP

MDSS GmbH
Schiffgraben 41
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Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
EC REP Authorized Representative In Europe	

Short Assay Procedure:

1. Add 100 µL of positive and negative controls and diluted 1:100 samples into the designated microwell.
2. Cover and incubate at room temperature for 30 minutes.
3. Wash each well 5 times.
4. Add 100 µL of Tracer Antibody into each well.
5. Cover and incubate the plate at room temperature for 30 minutes.
6. Wash each well 5 times.
7. Add 100 µL of TMB Substrate
8. Cover and incubate plate at room temperature for 20 minutes
9. Add 100 µL of ELISA Stop Solution into each of the wells.
10. Read the absorbance at 45