HEV IgG Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAHEG1	HEV IgG Elisa	96 Tests

Intended Use:

HEV-IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG Antibody to hepatitis E virus in human serum or plasma samples. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis E virus. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Hepatitis E virus (HEV) is a non-enveloped, single- stranded RNA virus identified in 1990. Infection with HEV induces acute or sub-clinical liver diseases similar to hepatitis A. HEV infections, endemic and frequently epidemic in developing countries, is seen also in developed countries in a sporadic form with or without a history of traveling to endemic area. The overall case-fatality is 0.5~3%, and much higher (15~25%) among pregnant women. A hypothesis that HEV infection is a zoonosis was presented in 1995. Then a swine HEV and later an avian HEV were identified and sequenced separately in 1997 and 2001. Since then, HEV infection include anti-HEV, viremia and feces excretion of HEV was seen in a wide variety of animals, i.e., swine, rodents, wild monkeys, deer, cow, goats, dogs and chicken in both the developing and developed countries. A direct testimony was reported that the consumption of uncooked deer meat infected with HEV led to acute hepatitis E in human.

This kit is employs solid phase, indirect ELISA method for detection of IgG-class antibodies to HEV (anti-HEV) in two-step incubation procedure. Polystyrene microwell strips are pre-coated with HEV recombinant antigen. During the first incubation step, anti-HEV specific antibodies, if present, will be bound to the solid phase pre-coated HEV antigens. The wells are washed to remove unbound serum proteins and then, rabbit anti-human IgG antibodies (anti-IgG) conjugated to horseradish peroxidase (HRP-Conjugate) is added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colourless Chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HEV-IgG remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with anti-HEV antigens. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Negative Control	1x1.0ml	Blue liquid filled in a vial with green screw cap. Protein stabilized buffer tested non-reactive for HEV IgG antibodies. Ready to use. Once open stable for 1 month at 2-8°C.
Positive Control	1x1.0ml	Red coloured liquid filled in a vial with red screw cap. IgG antibodies to HEV diluted in protein stabilized buffer. Ready to use. Once open stable for 1 month at 2-8°C.
HRP-Conjugate	1x12ml	Red coloured vial in a white vial with screw cap. Horseradish peroxidise conjugated rabbit anti-human IgG antibodies. Once open, stable for one month at 2-8°C.
Specimen Diluent	1x12ml	Green coloured liquid in a white vial with blue screw cap. Serum base, casein, and sucrose solution. Ready to use as supplied. Once open, stable for 1 month at 2-8°C.
Wash Buffer (20X)	1x40ml	Colourless liquid filled in a white bottle with white screw cap. PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted 1 to 20 with distilled water before use. Once diluted, stable for one week at room temperature, or two weeks when stored at 2-8°C.
Chromogen A	1x6ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	1x6ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x6ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials provided but not required:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing systems, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration systems.

Specimen Collection:

1. No special patient preparation is required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venepuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimens should be removed by centrifugation at 3000 RPM for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric or haemolytic specimens should not be used as they give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. The AMS HEV IgG Elisa assay is used only for testing individual serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled (mixed) blood.
4. Transportation and Storage: store specimens at 2-8°C. Specimens not required for assaying within 7 days should be stored at -20oC or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. **Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.**
2. **Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.**
3. **CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.**
4. **Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.**
5. **Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
6. **Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.**
7. **Avoid assay steps long time interruptions. Assure same working conditions for all wells.**
8. **Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.**
9. **Assure that the incubation temperature is 37°C inside the incubator.**
10. **When adding specimens, do not touch the well's bottom with the pipette tip.**
11. **When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.**
12. **The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.**
13. **If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.**
14. **All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.**
15. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
16. **Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.**
17. **Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.**
18. **The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121oC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request.**
19. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: stop solution, chromogen reagents and the wash buffer.**
20. **The stop solution contains sulfuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
21. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS: The values of positive and negative controls which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and or operator or equipment errors. In such cases, the results should be considered as invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration or instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local AMS representative.

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (18-30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the wash buffer (20X) 1 volume with 19 volumes of distilled or deionized water and clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

STEP 1

Preparation: Mark 3 wells as Negative control (B1, C1, D1), two wells as Positive control (E1,F1) and one Blank (A1 – neither samples nor HRP conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

STEP 2

Addition of the Diluent: Add 100ul of Specimen Diluent into each well except the Blank.

STEP 3

Addition of the Sample: Add 10ul of Positive control, Negative Control and specimen into their respective wells except the blank. Note: Use a separate disposable tip for each specimen and standard to avoid cross-contamination.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 20 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of HRP Conjugate: Add 100ul of HRP-Conjugate into each well except the Blank.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 20 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the chromogens: Add 50ul of Chromogen A and 50ul of Chromogen B into each well including the blank. Incubate the plate at 37°C for 10 minutes avoiding light.

STEP 10

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently.

STEP 11

Measurement: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and evaluate the results. (Note: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

- To remove any effect washing on false positive reactions, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. This helps in avoiding false positive reactions and a high background.
- To avoid cross-contamination of the plate with specimen or HRP conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
- Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of wash buffer is dispensed each time into the wells.
- In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400ul/well and aspirating the liquid 5 times. If poor results are observed with high background, increase washing cycles to soak time per well.
- Treat the liquid aspirated after the reaction from the wells with Sodium hypochlorite (at a concentration of 2.5%) for 24 hours before they are disposed off in the appropriate way.
- The concentrated wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

Calculation of results:

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = Nc + 0.15

(**Nc** = the mean absorbance value for three negative controls).

Important: If the mean A value of the negative controls is lower than 0.03, take it as 0.03.

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be ≤ 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded, and the mean value should be calculated by using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control

Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range

2. Calculation of Nc: $= \frac{(0.020+0.012+0.016)}{3} = 0.016$

3. Calculation of the Cut-off: (C.O.) = 0.03+0.15=0.18

Interpretation of results:

Negative Results (A / C.O. < 1): Specimens giving A value less than the Cut-off value are negative for this assay, which indicates that no anti-HEV IgG antibodies have been detected with this HEV AG ELISA. This result should not be used alone to establish the infection state.

Positive Results (A / C.O. ≥ 1): Specimens giving A value greater than or equal to the Cut-off value are considered initially reactive, which indicates that anti-HEV IgG antibodies have probably been detected with this HEV IgG ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for anti-HEV IgG antibodies and therefore the patient is probably infected with HEV.

Borderline (A / C.O. = 0.9-1.1): Specimens with A value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

- If after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for anti-HEV IgG antibodies.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance Characteristics:

1. Detection of HEV antibodies in samples from patients with 10 years of HEV post infection history:

Reagents	Samples	Pos. rate %	Cut-off	Positive samples OD			Avr.pos S/CO
				Lowest	Average	Highest	
AMS HEV IgG	50	86	0.148	0.532	1.368	2.327	9.24
EIA 1**	50	36	0.512	0.514	1.018	2.415	1.98
EIA 2**	50	30	0.228	0.229	0.457	1.094	2.08

EIA 1, 2** - Commercially available HEV IgG Elisa tests

2. Detection of serial serums samples from acute HEV phrase

Since no golden standard for hepatitis E is available, the reference HEV IgG assay served as control reagent. Parallel comparison testing was performed with acute hepatitis E samples (Testing Center 1). If the true positive status was defined as positive for any of the two tests used in this study, the sensitivity of AMS HEV IgG and the reference HEV IgG was 97.96% and 91.84% respectively.

In the evaluation of 120 serial sera sample obtained from 30 hepatitis E (Testing Center 2).The sensitivity of AMS HEV-IgG were 100%, while the sensitivity of the reference HEV-IgG were 93.33%.

The sensitivity of AMS HEV IgM was 99.08% in the parallel testing of a total of 218 serum samples of acute hepatitis E, which was significantly higher than the reference HEV IgG tests (92.66%).

Testing Centre	Case number	AMS HEV IgG		EIA 1**	
		Positive Number	Positive rate	Positive Number	Positive rate
1	98	96	97.96%	90	91.84%
2	120	120	100.0%	112	93.33%
TOTAL	218	216	99.08%	202	92.66%

3. Total 10587 blood samples from blood donors were tested, the results are showed in the frequency distribution map, it exists two peaks of AMS IgG: the first peak was higher, of which the center was near the OD value 0.0126, representing the people who did not infect HEV. The OD logarithm of the first peak was similar to the log-normal distribution. Considering the first peak as the center, calculate the standard deviation of the data on the left. The corresponding OD values in accordance with 99%-fractile and 99.9%-fractile respectively were 0.069 and 0.120 and the probability value at the cutoff value 0.185 was 99.99%. The second peak concentrated near the OD value 3.2, representing the HEV infection population, and its logarithm behaved as negative skewed distribution. As a result, the specificity of AMS IgG was much higher, with false positive rate at 0.01%.

Limitations:

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with AMS HEV-IgG ELISA are only indication that the sample does not contain detectable level of anti-HEV IgG antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HEV.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This kit is a qualitative assay and the results cannot be used to measure antibody concentration.

References:

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2. Clayson E, Innis B, Myint K, et al. Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. Am J Trop Med Hyg,1995,53:228–232

3. Meng XJ, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA, 1997, 94: 9860–9865

4. Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362(9381):371

5. Zheng YJ, Zhang J, Xia NS. A debate about that hepatitis E is a zoonosis. Chinese J Zoonosis (in press)

6. Wang YC, Zhang HY, Xia NS, et al. Prevalence, Isolation, and Partial Sequence Analysis of Hepatitis E Virus From Domestic Animals in China. J Med Virol 2002,67:516–52

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

CE

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