HEV IgM Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAHEM1	HEV IgM Elisa	96 Tests

Intended Use:

HEV-IgM ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgM-class antibodies 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.

HEV-IgM ELISA is a two-step incubation, solid-phase antibody capture ELISA assay in which polystyrene microwell strips are pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain). The patient's serum/plasma sample is added, and during the first incubation step, any IgM-class antibodies will be captured in the wells. After washing out all the other substances of the sample and in particular IgG-class antibodies, the specific HEV IgM captured on the solid phase is detected by the addition of recombinant HEV ORF2 antigen conjugated to the enzyme horseradish peroxidase (HRP-conjugate). During the second incubation, the HRP-conjugated antigens will specifically react only with anti-HEV IgM antibodies. After washing to remove the unbound HRP-conjugate, chromogen solutions are added into the wells. In presence of (anti-μ) - (anti-HEV-IgM) - (HEV Ag-HRP) immunocomplex, the colourless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-coloured product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for HEV IgM remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with anti-IgM antibodies. 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 coloured liquid filled in a vial with green screw cap. Protein stabilized buffer tested non-reactive for HEV IgM 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. Purified HEV IgM class antibodies 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 recombinant HEV antigens. Once open, stable for one month at 2-8°C.
Specimen Diluent	1x12ml	Blue 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 IgM 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 121°C 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 and Positive control and Negative control wells. (ADD SPECIMEN DILUENT ONLY TO THE SAMPLE WELLS)

STEP 3

Addition of the Sample: Add 100ul of Positive control, 100ul Negative Control and **10ul of 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 30-60 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. Intensive yellow colour develops in the positive control and HEV IgM positive sample wells.

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).

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:	0.012	0.010	0.011
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.363	2.436	

All control values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.012+0.010+0.011)}{3}$ = 0.011

3. Calculation of the Cut-off: (C.O.) = 0.011+0.15=0.161

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 IgM 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 IgM antibodies have probably been detected with this HEV IgM ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for anti-HEV IgM 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:

Specificity: The specificity of AMS HEV-IgM ELISA was determined in 11 non-hepatitis E groups (included hepatitis A cases, hepatitis B cases, hepatitis C cases, inoculated with HBV vaccine groups, population with routine virus testing, blood donors and healthy individuals). Each sample group was no less than 150 members, with 7113 participants for the largest testing groups. The specificity ranged of 95.3%~100.0%.

No interference was observed from rheumatoid factors up to 2000U/ml.
- The assay performance characteristics are unaffected from elevated concentrations of bilirubin (up to 1.71mmol/L), haemoglobin (up to 400mg/L), and triglyceride (up to 170mmol/L).
- Frozen positive/negative specimens have been tested to check for interferences due to collection and storage. The performance characteristics of HEV-IgM ELISA were not affected.
- Panels of specimens with elevated levels of anti-E.coli antibodies, specimens from pregnant women and individuals with auto-immune diseases were tested. The performance characteristics of HEV-IgM ELISA were not affected.

Testing Center	Testing Groups	Samples	Pos. No.	Specificity (%)
1	Acute hepatitis A	168	1	99.4
	Acute hepatitis B	164	1	99.4
	Hepatitis C	168	4	97.6
	HBV vaccinated	871	25	97.1
	Routine virus testing group	445	3	99.3
2	Healthy individuals	273	0	100.0
	Acute hepatitis A	298	14	95.3
3	Blood donors	7113	124	98.3
4	Healthy individuals	883	1	99.9
5	Nature population	388	1	99.7
	Blood donors	355	3	99.2
Total	Healthy individuals	9012	129	98.6
	Other groups	2114	48	97.7
	Total	11126	177	98.4

Sensitivity: In the evaluation of 126 serial sera samples obtained from 31 hepatitis E patients (Testing Center 3), the sensitivity of AMS HEV-IgM ELISA for the first serum sample and serial samples were 100% and 97.62% respectively, while the sensitivity of the reference HEV-IgM test was 90.32% and 86.51% respectively. Parallel comparison testing was performed with 202 non-A-non-B hepatitis samples (Testing Center 1). Since no golden standard for hepatitis E is available, reference HEV IgM assay served as control reagent. The concordance rate between AMS HEV-IgM ELISA and the reference IgM assay was 83.66%. If the true positive status was defined as positive for any of the two tests used in this study, the sensitivity of AMS HEV-IgM ELISA and the reference HEV IgM was 96.43% and 80% respectively.

Sensitivity Summary: The sensitivity of AMS HEV-IgM ELISA was 97.1% (95% confidence interval: 94.6%-98.5%) in the parallel testing of a total of 314 serum samples of acute hepatitis E, which was significantly higher than the reference HEV IgM tests (81.5%, 95% confidence interval: 76.9%-85.4%) and the reference HEV IgG test (93.8%, 95% confidence interval: 83.1%-97.7%).

Testing Centre	**Samples**	**AMS HEV IgM**		**Reference HEV IgM Elisa**		**Reference HEV IgM Elisa**	
		Positive	**Positive rate**	**Positive**	**Positive rate**	**Positive Number**	**Positive rate**
1	140	135	96.4%	112	80.0%	45	93.8%
2	48	47	97.9%	35	72.9%		
3	126	123	97.6%	109	86.5%		
TOTAL	314	305	97.1%	256	81.5%	45/48	93.8%

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-IgM ELISA are only indication that the sample does not contain detectable level of anti-HEV IgM 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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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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