HEV IgM ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAHEM1	HEV IgM ELISA	96 Tests

Intended Use:

The HEV-IgM ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgM antibody to hepatitis E virus (HEV) in human serum and plasma samples. It is intended for use in clinical laboratories for diagnosis and management of patients with infection with hepatitis E virus. This reagent is for In vitro diagnostic use by trained professionals 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 faeces 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 solid phase, two-step incubation sandwich ELISA. The microwells are pre-coated with antibody raised against the anti-µ chain of human immunoglobulin M. Sample is added to wells together with a diluent and during incubation all IgM antibodies will bind to the capture antibody and be immobilised. After washing, conjugate is added which is contains horseradish peroxidase conjugated to HEV antigens. The conjugate binds specifically to HEV-IgM amongst the IgM captured on the well surface. After washing to remove unbound HRP-Conjugate, Substrate solutions are added to the wells and the HRP on the immunocomplexes catalyses the hydrolysis of the substrate to a blue-coloured product. The reaction is stopped by the addition of acid Stop Solution which also turns the blue colour to yellow. The colour intensity can be measured spectrophotometrically and is proportional to the concentration of antibody in the sample. Wells containing samples negative for HEV-IgM remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Microwells containing anti-IgM antibody. The microwells can be broken and used separately. Place unused wells or strips in the plastic sealable bag provided 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	1.0ml	Blue-coloured liquid. Protein stabilized buffer tested non-reactive for HEV IgM antibody. Ready to use. Once open stable for 1 month at 2 - 8°C.
Positive Control	1.0ml	Red coloured liquid. IgM antibodies to HEV diluted in protein stabilized buffer. Ready to use. Once open stable for 1 month at 2 - 8°C.
HRP-Conjugate	12ml	Red coloured liquid. Recombinant HEV antigens conjugated to Horseradish Peroxidise. Once open, stable for one month at 2 - 8°C.
Specimen Diluent	12ml	Green coloured liquid. Serum base, casein, and sucrose solution. Ready to use as supplied. Once open, stable for 1 month at 2 - 8°C.
Wash Buffer (20X)	40ml	Colourless liquid, pH 7.4, 20 x PBS. The concentrate must be diluted 1 in 20 with distilled/deionised water before use. Once diluted, stable for one week at room temperature or for two weeks stored at 2 - 8°C.
Substrate A	6ml	Colourless liquid. Urea peroxide solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Substrate B	6ml	Colourless liquid. TMB (Tetramethyl benzidine) solution. Ready to use. Once open, stable for one month at 2 - 8°C
Stop Solution	6ml	Colourless liquid. Diluted sulphuric acid solution (0.5M H_2SO_4). Ready to use. Once open, stable for one month at 2 - 8°C.

Sealable plastic bag, IFU and plate covers.

Materials provided but not required:

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, pipettes, disposable pipette tips, absorbent tissue, dry incubator or water bath (37±0.5°C), plate reader (single wavelength 450nm or dual wavelength 450/630nm), microplate washer system.

Specimen Collection:

Collect serum and EDTA, sodium citrate or heparin plasma by separation after standard venipuncture technique. For serum, the blood must be allowed to clot fully but be separated as soon as possible to avoid haemolysis of the RBC. Any visible particulate matter in the serum or plasma should be removed by centrifugation. Use only serum or plasma sample type.
Samples will be stable for analysis up to 7 days at 2 - 8°C or stored at -20°C.
Do not use samples with high content of haemoglobin, bilirubin or triglyceride as these substances can interfere in the ELISA.

Storage and Stability:

The components of the kit will remain stable until the expiry date indicated on the kit when stored at 2 -8°C. Do not freeze. To assure maximum performance of this kit protect the reagents from contamination with microorganism and chemicals.

Precautions and Safety:

1. Correct ELISA results are dependent on incubation time and temperature. To avoid false results, follow the test procedure exactly as specified.
2. Do not mix reagents from different lots or substitute reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance.
3. Make sure that all reagents are from the same lot and all within expiry. Do not use reagents beyond expiry.
4. IMPORTANT: Allow the reagents and samples to reach room temperature (15 - 25°C) before use. Shake reagents gently before use. Return all components, tightly sealed, to 2 - 8°C immediately after use.
5. Use different pipette tip for each sample and reagent in order to avoid cross-contamination. Only touch the pipette tip against the side of the well, never the bottom of the well.
6. Make sure timing of reagent additions are the same across all wells and for each procedure step.
7. Do not touch the underside of the wells; fingerprints or scratches may interfere with the Absorbance. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
8. Never allow the microplate wells to dry out between wash steps. Avoid the formation of air bubbles when adding the reagents.
9. The enzyme activity of the HRP-conjugate is affected by dust and strong chemicals including sodium hypochlorite, acids and alkalis. Do not let these substances contaminate reagents.
10. If using fully automated equipment do not cover the plate with a plate cover during incubation. The tapping out of well contents after washing, can also be omitted.
11. All samples from human origin should be considered as potentially infectious. Adhere to Good Laboratory Practice regulations to ensure personal safety.
12. WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These raw materials have been tested with approved kits and proved negative for and HBsAg and antibodies to HIV 1/2, HCV, HBV and TP. However, since no analytical method that can completely assure the absence of infectious agents handle reagents and samples as if potentially infectious. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are sourced from BSE/TSE free-geographical areas.
13. Pipette tips, vials, well strips and sample 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. Reagents must be disposed of only in accordance with local or national regulations
14. Some reagent components may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Avoid contact of all reagents with skin and the mucosa but especially the Stop Solution, Chromogen reagents and the Wash Buffer.
15. The Stop Solution contains sulphuric acid. Use with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.
16. Proclin 300 is used as preservative and can cause a reaction on the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.
17. Indicators of deterioration of the reagents: The values of Positive and Negative controls fall out of the quality control range may indicate reagent instability or operator or equipment error. Where quality controls are out of range the results should be considered invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration of the reagents, immediately discard the reagents in use and use a new kit. Contact the local AMS representative.

Procedure:

Reagent preparation: Bring all reagents and samples up to room temperature (15-25°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) by adding the contents of the Wash Buffer bottle to 760 ml of distilled or deionized water. Alternatively add concentrate to distilled water in the ratio 1/20. All other reagents are supplied ready to use.

STEP 1

Preparation: Reserve 2 wells for Negative Control, 2 wells for Positive Control and one well for the Blank (e.g. A1 – ensure that neither HRP conjugate nor any samples are added to the blank well). Assign other wells according to the number of samples to be tested.

STEP 2

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

STEP 3

Addition of the Sample: Add 10 µl of Positive Control, Negative Control and samples to their assigned wells except the Blank. Mix by tapping the plate gently.

STEP 4

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

STEP 5

Wash Step: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

Addition of HRP Conjugate: Add 100ul of HRP-Conjugate into each well except the Blank. Mix by tapping the side of the plate gently.

STEP 7

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

STEP 8

Wash Step: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 9

Addition of Substrate: Add 50 µl of Substrate solution A and 50 µl of Substrate solution B into each well including the Blank. Incubate the plate at 37°C for 10 minutes. Ensure this incubation is carried out in the dark.

STEP 10

Stopping the Reaction: Add 50 µl of Stop Solution into each well and mix gently until the blue colour changes completely to yellow.

STEP 11

Measurement: Calibrate the plate reader with the Blank well and read the absorbance of all wells at 450nm within 10 minutes after stopping the reaction. If a dual filter instrument is

used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results.

Instructions for Washing:

To remove the potential for poor washing to cause false positive reactions and a high background, an automatic 5 wash cycle is recommended with 350 – 400 µl of diluted Wash Buffer used per well per wash. To avoid cross-contamination of the plate with sample or HRP conjugate, do not discard the content of the wells but allow the plate washer to aspirate contents automatically.
Ensure 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.
For manual washing, carry out 5 wash cycles, dispensing 350 – 400 µl of diluted Wash Buffer per well and aspirating the liquid by inverting the plate and flicking out the buffer. If poor results are observed with high background, increase the soak time each wash.
Treat the liquid aspirated after the reaction from the wells with 2.5% sodium hypochlorite for 24 hours before disposing off in the appropriate way.

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 run. The results are calculated by relating each sample absorbance (Abs) with the Cut off value (CO) for the run. If the reading is based on a single filter plate reader, the results are to be calculated by subtracting the Blank well Abs from the absorbances of the samples and the controls. If the results are taken from a dual filter plate reader, do not subtract the Blank well Abs value from the sample and control absorbances, just use the values obtained.

Calculation of the Cut-off value (CO) = Nc x 2.1
Where: Nc = the mean absorbance value of the Negative Control results
Note: If the mean of the control Abs (Nc) is less than 0.05, use 0.05 as the Nc value.

Assay Validation:

The assay results are only valid if the following Quality Control criteria are met.
Blank well: the absorbance must be <0.080 at 450nm.
Positive Control: the absorbance must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
Negative Control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.
If one of the Negative Control absorbance values does not match the above criteria, this value should be discarded and the mean value should be taken as the other value. If both Negative Control absorbances do not meet the criteria, the assay run is invalid and must be repeated.

Example:

1. Assay Validation
Blank well Abs: Well A1 = 0.028 at 450nm

Negative Control Abs after blanking:	0.025	0.027
Positive Control Abs after blanking:	2.125	2.235

All control values are within the stated quality control range
2. Calculation of Nc: = $\frac{(0.025 + 0.027)}{2}$ = 0.026 (Note: less than 0.05, so use 0.05 as Nc)
3. Calculation of the Cut-off: (CO) = 0.05 x 2.1 = 0.105

Interpretation of results:

Negative Results (Abs/CO < 1): Samples with an absorbance result less than the Cut-off value are negative for this assay, which indicates that no IgM antibodies have been detected with this HEV-IgM ELISA kit, therefore there are no serological indications for infection with HEV.
Positive Results (Abs/CO ≥ 1): Samples with an absorbance result equal to or greater than the Cut-off value are considered initially reactive, which indicates that IgM antibodies to HEV have probably been detected with this HEV-IgM ELISA kit. All initially reactive samples should be repeated in duplicate before final interpretation. Repeatedly reactive samples can be considered positive for IgM antibodies to HEV and therefore the patient is probably infected with HEV.
Borderline (Abs/CO = 0.9 - 1.1): Samples with an absorbance 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.
Follow up confirmation and supplementary testing of any positive sample with another analytical system such as PCR is required. Clinical diagnosis should not be established using this test as the sole source of data but integrate confirmatory test results and other clinical information.

Interpretation of repeat testing:
After re-testing of the initially reactive samples, both wells show negative results (A/CO <0.9). These samples should be considered negative and the original result must be classified as false positive. False positive results may occur due to several reasons, often associated with, but not limited to an inadequate washing procedure.
After retesting in duplicate, one or both wells give positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive samples can be considered positive for anti-HEV IgM and therefore the patient is probably infected with HEV.
After retesting in duplicate, samples with absorbance values close to the Cut-off value should be interpreted with caution and considered as "borderline" samples, or uninterpretable as at time of sample collection.

Performance Characteristics:

Clinical Sensitivity and Specificity
The clinical sensitivity and specificity of this assay was determined using a set of samples obtained from 1105 blood donors and undiagnosed hospital patients with results confirmed by a commercially available HEV IgM EIA kit. The clinical Sensitivity was determined as 96.7%. The clinical Specificity was determined as 94.9%.

Limitations:

1. Follow up confirmation and supplementary testing of any positive sample with another analytical system such as PCR is required. Clinical diagnosis should not be established using this test as the sole source of data but integrate confirmatory test results and other clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with this HEV-IgM ELISA only indicate that the sample does not contain detectable levels of IgM antibodies to HEV and does not rule out the possibility that the individual is infected with HEV.
3. Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
4. This kit is intended only for testing of serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
5. This kit is a qualitative assay and the results cannot be used to measure antibody concentration.

References:

Centres for Disease Control and Prevention. Epidemiology and Prevention of Viral Hepatitis A to E: An Overview 2000.

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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