AccuDiag™ HEV-IgM ELISA Kit

REF 1876-P1

IVD See External Label 2-8°C 96 Tests

HEV-IgM ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	60 minutes
Sensitivity	96.7%
Specificity	94.9%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

DAI HEV-IgM ELISA is an enzyme-linked immunosorbent assay for the qualitative detection IgM antibody to hepatitis E virus in human serum/plasma.

SIGNIFICANCE AND SUMMARY

Hepatitis E is a recurring infection in developing countries. In less frequent, isolated cases, it has also been reported in developed countries. Identified in 1990, Hepatitis E (HEV) is a non-enveloped, single-stranded RNA virus where infection can lead to acute (or subclinical) liver disease, like that associated to hepatitis A infection. Fatality rates are generally low - averaging 0.5-3 percent for most patients, while pregnant women hold elevated averages around 15-25 percent. In 1995 a theory was introduced that HEV was transmitted to humans from animals (zoonosis). Then, in 1997 and 2001, respectively, HEV was identified as transmitted from swine and from birds. After that time, HEV infection transmission has been associated with several animals, for example, wild monkeys, deer, cow, goats, rodents, dogs, and chickens. Found in both developing and developed connuntries, these HEV infections (including anti-HEV) consisted of viremia and feces excretion of HEV-affected animals, such as those listed above. A direct link to acute hepatitis E in humans from eating uncooked deer meat, was also reported. Even in supermarkets in Japan, HEV genome sequences have been identified in pork livers. Serology has been enhanced in relation to the discovery of conformational epitopes in HEV. Diagnosis, epidemiology, zoonosis-related studies, and development of a vaccine have all been strengthened by the occurrence of long-lasting and protective HEV antibodies.

ASSAY PRINCIPLE

The HEV IgM ELISA employs a solid phase, two-step incubation, antibody capture assay. Antibodies directed to human immunoglobulin M proteins (anti-u chain) are pre-coated on polystyrene microwell strips. During the first incubation stage, the patient's serum or plasma sample is added. At this point, any IgM-class antibodies will be captured in the wells. Next, all other components of the sample are washed out, especially any IgG-class antibodies. What becomes visible after adding recombinant HEV ORF2 antigens conjugated to horseradish peroxidase (HRP-Conjugate) is the specific HEV IgM captured on the solid phase. In the course of the second incubation stage, the HRP-conjugated antigens will individually react with HEV IgM antibodies. Chromogen solutions are added after the wells are washed to remove the unbound HRP-Conjugate. At this point, when (anti-u)-(anti-HEV-IgM)-(HEV Ag-HRP) immunocomplex is present, a blue-colored product appears which is the result of colorless chromogens hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antibody captured in the wells, and to the sample, respectively. Colorless wells appear when samples are negative for HEV-IgM.

SPECIMEN COLLECTION & PREPARATION

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis 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 specimen should be removed by centrifugation at 3000 RPM (round per minutes) 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 hemolytic specimens should not be used as they can 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. This ELISA kit is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of 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 frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing

local and international regulations for transportation of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations 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.
4. 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.
5. In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

REAGENTS

Materials provided with the kit

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

1. **MICROPLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains anti-IgM antibodies. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.
2. **NEGATIVE CONTROL:** (1x1.0ml per vial) preserv.0.1% ProClin 300. Blue-colored liquid filled in a vial with green screw cap.Protein-stabilized buffer tested non-reactive for HEV IgM antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.
3. **POSITIVE CONTROL:** (1x1.0ml per vial) preserv.0.1% ProClin 300. Red-colored liquid filled in a vial with red screw cap. HEV IgM antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8°C.
4. **CONJUGATE:** (1x12ml per vial) preserv.0.1% ProClin 300 Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated recombinant HEV antigens. Ready to use as supplied. Once open, stable for one month at 2-8°C.
5. **SAMPLE DILUENT:** (1x12ml per vial) preserv 0.1% ProClin 300 Green-colored in a vial with blue screw cap. Serum base, casein, and sucrose solution.
6. **WASH BUFFER:** (1x40ml per bottle) DILUTE BEFORE USE! detergent Tween-20 Colorless liquid filled in a white bottle with white screw cap.PH 7.4, 20 × PBS The concentrate must be diluted 1 to 19 with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.
7. **SUBSTRATE SOLUTION A:** (1x6ml per vial) Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.
8. **SUBSTRATE SOLUTION B:** (1x6ml per vial) Colorless liquid filled in a black vial with black screw cap. **TMB (Tetramethyl benzidine) solution**. Ready to use as supplied. Once open, stable for one month at 2-8°C.
9. **STOP SOLUTION:** (1x6ml per vial) Colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4).Ready to use as supplied. Once open, stable for one month at 2-8°C.
10. **PLASTIC SEALABLE BAG:** For enclosing the strips not in use 1unit
11. **PACKAGE INSERT** 1copy

Materials required but not provided

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

ASSAY PROCEDURE

1. **Reagents preparation:** Allow the reagents to reach room temperature (18-30°C). Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are READY TO USE AS SUPPLIED.
2. **Preparation:** Format the microplate's wells for control and patient specimen to be assayed. Replace any unused microwell strips back into the aluminum bag seal and store at 2-8°C.
3. **Adding Sample Diluent:** Add 100µl of Sample Diluent into their respective wells except the Blank.
4. **Adding Sample:** Add 10µl of the control or the specimen into the assigned well.
5. **Incubating:** Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
6. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
7. **Adding Conjugate:** Add 100µl of CONJUGATE into each well except the Blank.
8. **Incubating:** Cover the plate with the plate cover and incubate for 20 minutes at 37°C.
9. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
10. **Adding Substrate:** Add 50µl of Substrate Solution A and 50µl of Substrate Solution B into each well. Incubate for 10 minutes at 37°C avoiding light.
11. **Adding Stop Solution:** Using a multichannel pipette or manually, add 50µl of Stop Solution into each well and mix gently.
12. **Measuring the Absorbance:** 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: read the absorbance within 10 minutes after stopping the reaction).

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.) = 2.1×NC (NC = the mean absorbance value for two negative controls).

Important: If the NC is lower than 0.05, take it as 0.05.
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.

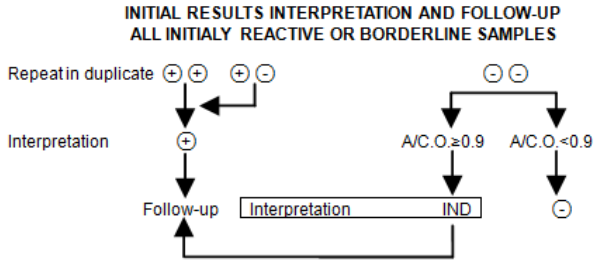
If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded values do not meet the Quality Control Range specifications, the test is invalid and must be repeated. And the mean value calculated again using the remaining value. If more than one Negative control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

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**	
Negative control A values after blanking:		0.028	0.030	
Well No.:		**D1**	**E1**	
Positive control A values after blanking:		2.421	2.369	
All control values are within the stated quality control range				
2. Calculation of Nc: = $\frac{(0.028+0.030)}{2}$ = 0.029				
3. Calculation of the Cut-off: (C.O.) = 2.1 + 0.05 = 0.105				

INTERPRETATION

Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HEV IgM has been detected with this ELISA kit.
Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HEV IgM has probably been detected using this ELISA kit. All initially reactive specimens should be retested in duplicates using this ELISA kit before the final assay results interpretation.

Borderline (A / C.O. = 0.9-1.1): Specimens with 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 specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.



IND = non interpretable

1. 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.
2. 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 HEV IgM.
3. 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

Clinical Sensitivity and Specificity: The clinical performances of this assay have been evaluated by a panel of samples obtained from 1105 healthy blood donors and undiagnosed hospitalized patients. The evaluation results are given below.
The clinical sensitivity is 96.7%; the clinical specificity is 94.9%.

LIMITATIONS OF THE ASSAY

1. Non- repeatable reactive results may be obtained with any ELISA test due to the general characteristics of this diagnostic method. A negative result with an antibody detection test does not preclude the possibility of infection. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. 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.
3. Any positive results must be interpreted in conjunction with the patient clinical information and other laboratory results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures and wrong washing buffer concentration, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during

washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. False negative results can occur from inhibition of specific IgM in the presence of high titers of specific IgG. The removal of IgG can be helpful to prevent false negative results and methods for this are given elsewhere.
7. 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.

STORAGE CONDITIONS

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this ELISA kit, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS

To be used only from qualified professionals

1. The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
2. 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.
3. 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.
4. 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.
5. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
6. 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.
7. 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.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. 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.
10. Assure that the incubation temperature is 37°C inside the incubator.
11. When adding specimens, do not touch the well's bottom with the pipette tip.
12. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
13. 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.
14. 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.
15. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

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. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClin 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

Indications of instability deterioration of the reagent

Values of the Positive or 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 case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one.

REFERENCES

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4. Sjogren MH, Tanno H, Fay O, et al. Hepatitis A virus in stool during clinical relapse. Ann Intern Med 1987; 106:221-6.
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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Woodland Hills, California 91367 USA

Date Adopted	2023-10
Brand Name	AccuDiag™
REF 1876-P1	AccuDiag™ - HEV IgM ELISA
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu
Revision Date: 2021-06-04	