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User's Manual

Antigen to Hepatitis E Virus, HEV-Ag ELISA Kit

REF **DEIA077**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

HEV-Ag ELISA is an enzyme linked immunosorbent assay for the qualitative detection of Hepatitis E virus antigen in human serum and plasma samples.

General Description

Hepatitis E virus (HEV) is a non-enveloped, single- stranded RNA virus identified in 1999. 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.

Principles of Testing

This kit is a two-steps incubation, solid-phase antibody "sandwich" ELISA assay in which polystyrene microwell strips are pre-coated with anti-HEV antibodies directed against the viral antigen. Patient's serum or plasma sample is added into the microwells. In case of presence of HEV Ag in the sample, the pre-coated antibodies will bind to the viral antigen and during the first incubation step, the specific immunocomplex formed is captured on the solid phase. After washing to remove unbound sample proteins, second anti-HEV antibody conjugated to the enzyme Horseradish Peroxidase (HRP) is added into the wells. During the second incubation step, this antibody will bind to the anti-HEV-HEV Ag complexes immobilized onto the wells during the first incubation step. The unbound HRP conjugate is removed during washing and Chromogen solutions containing Tetra-methylbenzidine (TMB) and urea peroxide are then added into the wells. In presence of the antibody-antigen-antibody (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HEV Ag remain colorless.

Reagents And Materials Provided

1. Microwell Plate 96 Tests: 1 plate(12x8/8x12 well strips per plate)
2. Negative Control: 1 ml, 1 vial;
3. Positive Control: 1 ml, 1 vial;
4. HRP-Conjugate Reagent: 12ml, 1 vial;
5. Specimen Diluent: 5 ml, 1 vial;
6. Stock Wash Buffer (20×): 50 ml, 1 vial; Dilute 1 to 20 with distilled water before use. Once diluted, stable for

two weeks at 2-8°C

7. Chromogen Solution A: 7 ml, 1 vial; Ready to use and once open, stable for one month at 2-8°C
8. Chromogen Solution B: 7 ml, 1 vial; Ready to use and once open, stable for one month at 2-8°C
9. Stop Solution: 7 ml, 1 vial;
10. Plastic Sealable Bag: 1 unit;
11. Plate Cover: 1 sheet;
12. Package Inserts

Materials Required But Not Supplied

1. Freshly distilled or deionized water
2. Disposable gloves and timer
3. Appropriate waste containers for potentially contaminated materials
4. Disposable V-shaped troughs
5. Dispensing system and/or pipette, disposable pipette tips
6. Absorbent tissue or clean towel
7. Dry incubator or water bath, 37±0.5°C
8. Microshaker for dissolving and mixing conjugate with somples
9. Plate reader, single wavelength 450nm or dual wavelength 450/630nm
10. Microwell aspiration/wash system
11. Normal saline solution for dilution of the samples.

Storage

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 kit, during storage protect the reagents from contamination with microorganism or chemicals.

Specimen Collection And Preparation

Sample Collection:

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM (round per minutes for 20 minutes at room temperature or by filtration on 0.22U filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or haemolysed samples should not be used as they can give false results in the assay. Do not heat inacfivated samples. This can cause sample deterioration.

Transportation and Storage:

Store samples at 2- 8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Avoid multiple freeze-thaw cycles.

This kit is intended ONLY for testing of individual serum and plasma samples. OD not use the assay for festing of cadaver samples, saliva, urine or other body fluids or pooled mixed blood.

Assay Procedure

Special Instructions for washing

1. A good washing procedure is essential 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 350-400ul/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. We recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. 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 cycles, dispensing 350-400ul/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 liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be 1 to 20 before use. For one plate, mix diluted 7.50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. Ifless than a whole plate is used, prepare the proportional volume of solution.

Procedure

Precision Pipette must be used for ALL steps of the assay.

Step 1 Preparation: Mark three 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 Adding Diluent: Add 20ul of Specimen Diluent into each well except the Blank well.

Step 3 Adding Sample: Add 50ul of Positive control, Negative control and samples into their respective wells except the blank well. Note: Use a separate disposal pipette tip for each sample Negative control and positive control to avoid cross-contamination. Mix by tapping the plate gently.

Step 4 Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

Step 5 Adding HRP Conjugate: At the end of the incubation. Remove and discard the plate cover. Add 100ul of HRP Conjugate into each well except the blank.

Step 6 Incubating: Cover the plate with plate cover and incubate for 30 minutes at 37°C.

Step 7 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 soack for 30-60 seconds. After the final washing cycle, turn down the plate onto a blotting paper or clean towel and top it to remove any residual wash solution.

Step 8 Coloring: Add 50ul of Chromogen A and 50ul of Chromogen B solutions into each well including the Blank. Incubating the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP Conjugate produces a blue colour in Positive controls and HEV antigen positive sample wells.

Alternatively mix equal volumes of Chromogen A and Chromogen B in a clean plastic tube and immediately add to wells. For example, pipette (using a precision pipette). 1ml of Chromogen A, then using a fresh tip, pipette 1ml of Chromogen B solution, mix and use immediately.

Step 9 Stopping Reaction: Using a multichannel pipette or manually, add 50ul of stop solution into each well and mix gently, intensive yellow color develops in Positive control and HEV antigen positive sample wells.

Step 10 Measuring 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.

Interpretation Of Results

Each microplate should be considered separately when calculating and interpreting 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 from the print report values of specimens and controls.

1. **Calculation of the Cut-off value (C.O.) = Nc + 0.16**

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

   Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

   Example:

   1.Calculation of Nc:

   Well No.: B1, C1, D1

   Negative controls OD value: 0.012, 0.010, 0.011

   Nc=0.011 (AVERAGE OF 3 NCs)

   2. Calculation of Cut-off (C.O.)= 0.011 +0.16= 0.171

   Cut-off (C.o.)=0.171

   If one of the Negative control values does not meet the Quality control range speciicafions, if should be discarded and the mean value is calculated again using the remaining two values. If more than one control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. **Quality Control Range**

   1.The absorbance of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm. The absorbance value OD of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.

   2.The absorbance value OD of the Negative control must be less than 0.100 at 450/630nm or at 450nm

after blanking.

3. **Interpretations of the results:**

   (S = the individual absorbance (OD) of each specimen)

   Negative Results (S/C.O. <1): Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HEV antigens are present in the sample and there are no serological indicators for an infection with HEV.

   Positive Results (S/C.≥1): Samples giving an absorbance greater than or equal to Cut-off value are considered initially reactive, which indicates HEV antigens have been detected by this assay. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples can be considered positive for antigens to HEV. Therefore, there are indications for possible current infection with hepatitis E Virus.

   Borderline (S/CO =0.9-1.1): Samples with Borderline absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Retesting of these samples in duplicates is recommended.

Sensitivity

Human Anti-Hepatitis E Virus Antigen ELISA Kit

Interferences

NO interference was observed from rheumatoid factors up to 2000 IU/ml.

The assay characteristics performance is unaffected from elevated concentrations of bilirubin (1.7 mmol/l), haemoglobin up to 400 mg/l, and triglycerides up to 170 mmol/l.

Frozen positive/negative samples have been tested to check for interferences due to collection and storage. The performance characteristics of this kit were not affected.

Panels of specimens with elevated levels of anti-E, coli antibodies, samples from pregnant women and individuals with auto-immune diseases were tested. The performance characteristics of this kit were not affected.

Precautions

The ELISA assay is time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps 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. Allow the reagents and samples to reach room temperature(18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
5. 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. Never pipette solutions by mouth.
9. The use of automatic pipettes and disposable tips is recommended
10. Assure that the incubation temperature is 37°C inside the incubator.
11. When adding samples, do not touch the well's bottom with the pipette tip.
12. When measuring with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
13. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
14. The pipette tips, vials, strips and sample collected and should be containers 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.
15. The Stop Solution contains 2M H_2SO_4. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
16. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

Limitations

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of the ELISA assays. The test is design to achieve performance characteristics of high sensitivity and specificity. However, in very rare cases some HDV mutants or subtypes can remain undetectable. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive result should be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, incorrect assay contaminated reagents, procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay's predictive values.