Human Anti-Hepatitis A Virus IgM Antibody, Anti-HAV IgM ELISA Kit

Cat. No.: DEIA008
Pkg. Size: 96T

Intended use

HAV-IgM ELISA is an enzyme-linked immunosorbent assay for qualitative determination of hepatitis A virus IgM-class antibodies in human serum or plasma samples. The assay is intended to be used in clinical laboratories for diagnosis and management of patients related to infection with hepatitis A virus.

General Description

Hepatitis A is a self-limited disease and chronic stage or other complications are rare. Infections occur early in life in areas where sanitation is poor and living conditions are crowded. With improved sanitation and hygiene, infections are delayed and consequently the number of persons susceptible to the disease increases. Because the disease is transmitted through the fecal-oral route in dense populated regions, an outbreak can arise from single contaminated source. The cause of hepatitis A is hepatitis A virus (HAV)-non enveloped positive strand RNA virus with a linear single strand genome, encoding for only one known serotype. HAV has four major, structural polypeptides and it localizes exclusively in the cytoplasm of human hepatocytes. The infection with HAV induces strong immunological response and elevated levels first of IgM and then IgG are detectable within a few days after the onset of the symptoms. The presence of anti–HAV IgM is an important serological marker for early detection and observation of the clinical manifestation of the disease. Increasing levels of anti-HAV IgM are detectable about three weeks after exposure with highest titter after four to six weeks later. Within six months after infection IgM concentration declines to non-detectable levels.

Principle Of The Test

This kit is a solid phase, two-step incubation, 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, any IgM antibodies will be captured in the wells. After washing out all the other components of the sample and in particular IgG antibodies, the specific HAV IgM captured on the solid phase is detected by the addition of HAV antigens conjugated to horseradish peroxidase (HRP-Conjugate). During the second incubation, the HRP-conjugated antigens will specifically react only with the HAV IgM antibodies and after washing to remove unbound HRP-conjugate, Chromogen solutions are added to the wells. In presence of the (anti-μ)-(HAV-IgM)-(antigen-HRP) 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 is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for HAV-IgM remain colorless.

Reagents And Materials Provided

Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with anti-IgM antibodies (anti-μ chain);
Negative Control: 0.5ml, 1 vial;
Positive Control: 0.5ml, 1 vial;
HRP-Conjugate: 12ml, 1 vial;
TMB Solution A: 6ml, 1 vial;
TMB Solution B: 6ml, 1 vial;
TMB Stop Solution: 6ml, 1 vial;
Wash Buffer (20×): 50ml, 1 vial;
Microtiterplate sealers: 2 sheets;
Plastic Sealable Bag: 1 unit.

**Materials Required But Not Supplied**

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multichannel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

**Storage**

Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.
Opened/Reconstituted Reagents: TMB Solution A; TMB Solution B; TMB Stop Solution; Wash Buffer; HRP-Conjugate
The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zipseal. May be stored for up to 1 month at 2 - 8°C.

**Specimen Collection And Handling**

Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20° C or -80°C. Avoid repeated freeze-thaw cycles.

**Assay Steps**

1. Allow all the reagents and samples to reach room temperature (18-30°C) for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer 1 to 20 with distilled or deionized water. Only use clean vessels to dilute the buffer.
2. Set the strips needed in strip-holder. And number sufficient number of wells including three for the Negative control, two for the Positive control and one Blank (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.
3. Add 50 μl of samples, and 50 μl Positive and Negative controls into their respective wells. Note: Use a separate disposable pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.
4. Cover the plate with the plate cover and incubate for 20 minutes at 37°C.
5. After the end of the incubation, 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 blotting paper or clean towel and tap it to remove any remainders.
6. Add 100 μl of HRP-Conjugate into each well except for the Blank. Cover the plate with the plate cover and incubate for 40 min. at 37°C, and Washing as Step 5.
7. Dispense 50 μl of TMB Solution A and 50 μl TMB Solution B into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the TMB solutions and the HRP-Conjugate produces blue color in Positive control and HAV IgM positive sample wells.
8. Using a multichannel pipette or manually, add 50 μl Stop solution into each well and mix gently. Intensive yellow color
develops in Negative control and negative sample wells.

9. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cutoff value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction.)

**Quality Control**

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 less than 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 less than 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again 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: A = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)
   - Negative control A values: B1 = 0.012, C1 = 0.010, D1 = 0.011
   - Positive control A values: E1 = 2.363, F1 = 2.436
   - All control values are within the stated quality control range

2. Calculation of Nc:
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   Nc = \frac{(0.012 + 0.010 + 0.011)}{3} = 0.011 \text{ (take it as 0.05)}
   \]

3. Calculation of the Cut-off (C.O.) = 0.05 x 2.1 = 0.105

**Calculation**

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 x 2.1 (Nc = the mean absorbance value for three negative controls).

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

**Interpretation of Results**

Negative Results (A / C.O. <1): Samples giving A value less than the Cut-off value are negative for this assay, which indicates that no HAV IgM antibodies have been detected with HAV IgM ELISA kit, therefore there are no serological indications for current infection with HAV.

Positive Results (A / C.O. ≥ 1): Samples giving A value which is equal to, or greater than the Cut-off value are considered initially reactive, which indicates that hepatitis A virus antigens have probably been detected using this ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for HAV IgM antibodies and therefore there are serological indications of current or recent infection with hepatitis A virus.

Borderline: (A / C.O. = 0.9-1.1): Samples 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.

**Precautions**

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

**Limitations**

1. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the "capture model" minimizes the unspecific reactions due to interference with unknown matters in specimen (false positive results).
2. Antibodies may be undetectable during the early stage of the disease. Therefore, negative results obtained with HAV IgM ELISA are only indication that the sample does not contain detectable level of HAV antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HAV.
3. The most common sources for mistakes are: 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 haemolytic specimens or specimens containing fibrin, incompletely clotted serum specimens.
4. The prevalence of the marker will affect the assay’s predictive values.
5. HAV IgM ELISA is a qualitative assay and the results cannot be use to measure antigen concentrations.

**REFERENCES**