Hepatitis A virus IgM ELISA Kit

Catalog Number KA0285
96 assays
Version: 04

Intended for research use only

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Introduction

Intended Use

Hepatitis A virus IgM ELISA kit (TMB) is a fast test for the qualitative detection of IgM antibody to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes Anti-human IgM on microtiter wells as solid phase and HAV Ag and peroxidase-conjugated Anti-HAV in liquid phase in an “IgM capture” principle to detect Anti-HAV IgM levels in serum or plasma. Specimens with absorbance values greater than the Cutoff Value are considered REACTIVE for Anti-HAV IgM.

Specimens with absorbance values less or equal than the Cutoff Value are considered NONREACTIVE for Anti-HAV IgM.

The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.

If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples.

Background

The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae (1-2). Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice (2). Hepatitis A antigen can be detected in the feces only briefly before or at the onset of infection becoming generally undetectable during the late acute stage (3). The antibody specific to HAV during the acute phase of hepatitis A is the IgM type (Anti-HAV IgM), which decreases then being replaced by IgG type (Anti-HAV IgG) during early and late convalescence (4). Anti-HAV IgM usually disappears 3 to 4 months after the acute phase. An acute hepatitis A virus infection can be assumed if anti-HAV IgM antibody is detected (5). Anti-HAV IgM antibody develops only very rarely after vaccination (6). Assays to detect anti-HAV IgM antibodies are useful in distinguishing hepatitis A infection from other types of infections.
**Principle of the Assay**

Hepatitis A virus IgM ELISA kit (TMB) is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) — based on the principle of “IgM capture”. The solid phase of the microtiter plate is made of polystyrene wells coated with anti-human IgM, while peroxidase-conjugated Anti-HAV acts as liquid phase.

When a serum or plasma specimen containing Anti-HAV IgM is added to the Anti-human IgM-coated wells and incubated, IgM antibodies present in the specimen bind to the Anti-human IgM on the wells. After addition of an HAV Ag-containing solution and a solution containing Peroxidase-conjugated anti-HAV a further incubation takes place, during which (Anti-h IgM) • (Anti-HAV IgM) • (HAV Ag) • (Anti-HAV• Peroxidase) complex is formed on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. If Anti-HAV IgM is present in the specimen, after washing, the activity of peroxidase on the wells reflects the content of anti-HAV IgM in a specimen. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 650 nm.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IgM Coated Plate</td>
<td>Microtiter plate coated with purified antibody to human IgM.</td>
<td>1 plate</td>
</tr>
<tr>
<td>Anti-HAV · Peroxidase Solution</td>
<td>Anti-HAV · Peroxidase (horseradish) conjugate in buffer with protein stabilizers. Preservatives: 0.003% gentamycin and 0.01% thimerosal.</td>
<td>5 ml</td>
</tr>
<tr>
<td>Anti-HAV-IgM Positive Control</td>
<td>Serum containing diluted Anti-HAV IgM in buffer with protein stabilizers. Preservatives: 0.003% gentamycin and 0.01% thimerosal.</td>
<td>2 ml</td>
</tr>
<tr>
<td>Anti-HAV IgM Negative Control</td>
<td>Protein stabilizer in buffer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.</td>
<td>2 ml</td>
</tr>
<tr>
<td>Specimen Diluent</td>
<td>Protein stabilizer in buffer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.</td>
<td>10 ml</td>
</tr>
<tr>
<td>Hepatitis A Virus Antigen Solution</td>
<td>Hepatitis A virus antigen in buffer and protein stabilizer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.</td>
<td>5 ml</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>3,3',5,5'-tetramethylbenzidine (TMB) in an organic base (0.6 mg/ml).</td>
<td>10 ml</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>Citric acid buffer containing 0.03% H₂O₂.</td>
<td>10 ml</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20x)</td>
<td>Phosphate buffer with tween-20.</td>
<td>50 ml</td>
</tr>
<tr>
<td>2N H₂SO₄</td>
<td>2 N sulfuric acid</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

Accessories: (provided as needed)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive slips</td>
<td>1</td>
</tr>
<tr>
<td>Black cover</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage Instruction

- The kit must be stored at +2 to +8°C. Do not freeze.
- Strips of the plate should be used within 1 month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
- Return the reagents to +2 to +8°C immediately after use.
- Washing Solution D (20x) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a +37°C water bath till the crystal is dissolved.
**Materials Required but Not Supplied**

- 10µl, 100µl and 1 ml micropipettes and tips are needed
- Incubator or water bath with temperature control at 37±1°C
- Plate washing equipment.
- ELISA microwell reader: Wavelength 450/650nm
- Purified water: distilled or deionized water
- 100 ml of 0.15M Normal Saline
- Tubes for specimen dilution

**Precautions for Use**

- For professional use only.
- Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix gently before use.
- Do not use reagent beyond its expiration date.
- Do not interchange reagents between different lots.
- Do not pipette in the mouth.
- Do not smoke or eat in areas where specimens or reagents are handled.
- The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user’s laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
- Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory’s practice for potential bio-hazard control.
- Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:
  - Both liquid and solid waste should be autoclaved maintaining +121°C for at least 30 minutes.
  - Solid waste can also be incinerated.
  - Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.
  - Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
- 2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
- TMB substrate solution A contains an organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
Assay Protocol

Reagent Preparation

✓ Washing solution
  Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.

Sample Preparation

✓ Specimen Collection and Preparation for Analysis
  • Either serum or plasma can be used with this kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
  • The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.
  • Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.
  • No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.

✓ Stability and storage
  • Specimens must be stored at 2-8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
  • Frozen specimens must be thoroughly thawed and mixed homogenously before test.
  • Avoid multiple freeze-thaw procedures

Assay Procedure

Assay process can be performed by an automatic EIA micro-plate immunoanalyzer. Please set up the program according to the following test procedure.

1. Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to +37±1°C.
2. Make 1:200 dilution of each specimen:
   Prepare the tubes for dilution as number of specimens. Add 1.0 ml of 0.15M Saline Solution and 5 μl of each specimen to each tube, respectively and shake to mix.
3. Prepare the needed number of wells, including two wells for blanks, three wells for Negative Control, two wells for Positive Control, and one well for each specimen.
4. Reserve 2 wells for Blanks. Add 100 μl of Negative Control to each of three wells, 100 μl of Positive Control to each of the two wells, and 100 μl of Specimen Diluent to each of the other reaction wells for the
test specimens.

5. Add 5 μl of each diluted specimen to each well containing Specimen Diluent, respectively.

6. Gently tap the plate.

7. Seal the plate with an adhesive slip.

8. Incubate the plate in incubator or water bath at +37 ± 1°C for one hour.

9. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate.

10. Add 50 μl of Hepatitis A Virus Antigen Solution and 50 μl of Anti-HAV-Peroxidase Conjugate Solution into each reaction well, except the Blanks. Apply new adhesive slip.

11. Incubate the plate in incubator or water bath at +37 ± 1°C for one hour.

12. At the end of the incubation period, remove and discard the adhesive slip, wash the plate.

13. Choice one of the following two methods for color development:

   A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 μl of the mixture solution to each well including the two blank wells.

   B. Add 50 μl of TMB Substrate Solution A first, and then add 50 μl of TMB Substrate Solution B into each well including the two blanks. Mix well gently.

   Note: TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be avoided from intense light and used within 30 minutes after mixing.

14. Cover the plate with black cover and incubate at room temperature (20-30°C) for 30 minutes.

15. Stop the reaction by adding 100 μl of 2N H₂SO₄ to each well including the blank.

16. Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450 nm with a selected reference wavelength at 650 nm.

   Note: The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated.

✓ Plate Wash Procedure

- Automatic or Semi-automatic Plate Washer
  Any commercial automatic microplate washer or other liquid aspirating/dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. We suggested 6 wash cycles with at least 350 μl per well per wash and soaking for 10 seconds is necessary.

- Manual Plate Wash
  Cover the reaction plate with an absorbent paper. Invert the plate and allow the liquid absorb onto the absorbent paper, then return the plate back to upright position. Fill each well with 350 μl of washing buffer. Aspirate the water after soaking 10 seconds. Repeat this procedure 6 times. Blot dry by inverting the plate and tapping firmly onto absorbent paper. All residual washing buffer should be blotted dry.

   WARNING: Improper washing can cause false
Data Analysis

Calculation of Results

- Calculation of the NCx (Mean Absorbance of Negative Control)

  Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
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<tbody>
<tr>
<td>1</td>
<td>0.080</td>
</tr>
<tr>
<td>2</td>
<td>0.085</td>
</tr>
<tr>
<td>3</td>
<td>0.079</td>
</tr>
</tbody>
</table>

  $\text{NCx} = \frac{(0.080 + 0.085 + 0.079)}{3} = 0.081$

  NCx must be $\leq 0.2$, otherwise, the test is invalid.

- Calculation of PCx (Mean Absorbance of Positive Control)

  Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.223</td>
</tr>
<tr>
<td>2</td>
<td>1.205</td>
</tr>
</tbody>
</table>

  $\text{PCx} = \frac{(1.223 + 1.205)}{2} = 1.214$

  PCx must be $\geq 0.5$, otherwise, the test is invalid.

- Calculation of the P-N Value

  $\text{N-P} = \text{NCx} - \text{PCx}$

  Example:

  $\text{P} - \text{N} = 1.214 - 0.081 = 1.131$

  P - N Value must be $\geq 0.30$, otherwise, the test is invalid.

- Calculation of the Cutoff Value

  $\text{Cutoff Value} = (\text{NCx} + \text{PCx})/4$

  Example:

  Cutoff Value = $0.081 + (1.214/4) = 0.385$

- Calculation of the Retest Range

  $\text{Retest Range} = \pm 10\%$ of Cutoff Value

  Example: Cutoff Value = 0.385

  Retest Range = (0.385 - 0.039) to (0.385 + 0.039) = 0.346 to 0.424
Note:

✓ Specimens with absorbance values LOWER than the Cutoff Value are considered non-reactive for Anti-HAV IgM

✓ Specimen with absorbance value GREATER than or EQUAL TO the Cutoff Value is considered reactive for Anti-HAV IgM.

✓ If the data is within the Retest Range, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.

✓ Flow chart of the test procedure

- Make 1: 200 dilution of each specimen.
  - Add 5 μl specimen into 1 ml Saline Solution, mix.
  - Do not dilute controls.

- Add 100 μl controls (3x NC, 2x PC).
  - Reserve 2 wells for blank.

- Add each Specimen Samples 5 μl into each well contain 100 μl Specimen diluent, respectively

- Incubate 37°C for 1 hr
  - Wash the plate

- Add 50 μl HAV Antigen solution and 50 μl of Anti-HAV Peroxidase.
  - Except 2 blanks.

- Incubate 37°C for 1 hr
  - Wash the plate

(Choose one of the following two methods for color development)

- Mix equal volumes of TMB Substrate Solution A and B. Add 100 μl of the mixed solution to wells.
  - Incubate at R.T. for 30 minutes.
  - Add 100 μl of 2N H₂SO₄ into each well.
  - Determine absorbance at 450nm or 450/650nm.

- Add 50 μl of TMB Substrate Solution A to wells and then add 50 μl of TMB Substrate Solution B. Mix well, gently.
## Resources

### Plate Layout

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<thead>
<tr>
<th>A</th>
<th>B</th>
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