INTENDED USE

"Bio Tech Assay's HAV IgM is a Qualitative detection of human serum/ plasma of hepatitis A virus IgM."

SUMMARY AND EXPLANATION

Hepatitis A is a highly contagious liver disease caused by the hepatitis A virus (HAV). Many cases are asymptomatic, especially among young individuals, but when symptoms occur, they typically appear 2-6 weeks after infection and last around 8 weeks. Common symptoms include nausea, vomiting, diarrhea, jaundice (yellowing of skin and eyes), fever, and abdominal pain. Approximately 10-15% of individuals experience symptom recurrence within six months, and rare cases may lead to acute liver failure, primarily among the elderly.

PRINCIPLE OF THE TEST

Capture method. Total duration of assay: 70 mins.

The ELISA (Enzyme-Linked Immunosorbent Assay) procedure is a precise method for detecting IgG antibodies in patient serum. It begins with the addition of diluted patient serum to wells pre-coated with purified antigen. If IgG antibodies are present, they bind to the antigen, forming an antibody-antigen complex. Next, unbound materials are washed away, and an enzyme conjugate is added, which selectively binds to the antibody-antigen complex. Excess conjugate is then removed through washing. Subsequently, a substrate is added to the wells, and the plate is incubated, allowing the enzyme to hydrolyze the substrate. This enzymatic reaction produces a color change that is directly proportional to the concentration of IgG-specific antibodies in the sample. The resulting color intensity serves as a quantitative measurement of the patient's immune response, providing valuable insights into their antibody levels.

Sr. No	**MATERIALS PROVIDED**	**96 Tests**
1	Positive Control: 1 vial (ready to use)	0.2 ml
2	Negative Control: 1 vial (ready to 6use)	0.2 ml
3	Enzyme conjugate: 1 bottle (ready to use)	6 ml
4	Sample Diluent: 2 bottles (ready to use)	11 ml
5	Ag Solution: 1 Vial (ready to use)	6 ml
6	Substrate: 1 bottle (ready to use)	11ml
7	Stop Solution: 1 bottle (ready to use)	6 ml
8	Wash concentrate 40X: 1 bottle	25ml
9	Microwells coated	12x8x1

Additional Resources Needed

- ELISA reader (450nm absorbance capability)
- Micro pipettes
- Disposable pipette tips
- Absorbent paper (or paper towel)
- Graph paper
- Distilled or deionized water

Storage and Stability:

- Keep microwells sealed in a dry bag with desiccants.
- Reagents stable until kit expiration.
- Store kit at 2-8 °C.
- Avoid exposure to heat, direct sunlight, and strong light.

Warnings and Precautions

1. Research Use Only: Not intended for diagnostic procedures.
2. Handle as Biosafety Level 2 material.
3. Components contain human source materials, tested non-reactive for HIV, Hepatitis B, but handle with caution.
4. Follow test protocol strictly for optimal results.
5. Use pipettes, avoid mouth pipetting.
6. Refrain from smoking, eating, or drinking near specimens or reagents.
7. Use components from the same lot; avoid mixing.
8. Sodium azide in control sera and sample diluent may react with lead/copper plumbing; flush with water upon disposal.

SPECIMEN COLLECTION AND HANDLING

1. Separate serum from collected blood specimens.
2. Store specimens refrigerated at 2-8°C for up to one week, or frozen at -20°C or below for upto six months. To maintain sample quality, avoid multiple freeze-thaw cycles.

REAGENT PREPARATION

Dilute 25ml of 40X Wash Solution concentrate with 975ml of deionized water to create a 1000ml working solution, stable for 2 months at room temperature.

Assay Procedure

Preparation

Mark wells for Negative Control (e.g., B1, C1), Positive Control (e.g., D1, E1), and Blank (e.g., A1). Omit the Blank well if using a dual-wavelength plate reader. Use only required strips.

Assay Procedure
1. Add 100 µL Sample Diluent to respective wells (except Blank).
2. Add 10 µL Positive Control, Negative Control, and specimen to respective wells (except Blank). Mix gently.

Incubation and Washing
1. Incubate for 30 minutes at 37°C.
2. Wash each well 5 times with diluted Wash Buffer, soaking for 30-60 seconds.
3. Remove excess liquid.

Conjugate Addition
1. Add 100 µL Conjugate to each well (except Blank).
2. Incubate for 30 minutes at 37°C.
3. Wash each well 5 times.

Color Development
1. Add 100 µL Substrate to each well (including Blank).
2. Incubate at room temperature for 10 minutes, avoiding light.

Stopping Reaction
1. Add 50 µL Stop Solution to each well and mix gently.

Measurement
1. Calibrate plate reader with Blank well.
2. Read absorbance at 450 nm (reference wavelength: 630 nm if using dual filter).
3. Calculate cut-off value and evaluate results within 10 minutes.

Note: Handle samples with separate pipette tips to avoid cross-contamination.

CALCULATION OF RESULTS

1. To interpret the results, begin by reading the sample's optical density (OD) at 450 nm using a microplate reader. For the test to be considered valid, the mean negative control OD value must be less than or equal to 0.8, and the positive control OD value must be greater than or equal to 0.8. If these conditions are not met, the test is invalid.
2. Next, calculate the cut-off value (C.O.) by adding 0.13 to the mean negative control OD value. However, if the mean negative control OD value is less than 0.05, use 0.05 as the adjustment; otherwise, use the actual value.
3. This calculation determines the cut-off value, which serves as the threshold for result interpretation.

POSITIVE RESULT

A positive result is determined when the sample's OD value is ≥ C.O., indicating probable HAV

IgM detection. However, all initially reactive specimens must be retested in duplicate. Only repeatedly reactive specimens are considered positive for HAV IgM.

Negative Result

Specimens with an absorbance less than the calculated cut-off (C.O.) value are considered negative for this assay. This indicates that HAV IgM has not been detected using HAV ELISA, suggesting that the patient is likely not infected with Hepatitis A virus (HAV) and the blood unit does not contain HAV IgM.

Limitations

- Positive results must be confirmed with other available method and interpreted in conjunction with the patient clinical information.
- The reagent is qualitative, and cannot be used as a quantitative reagent.
- This reagent is only used for the detection of human serum /plasma samples.

Performance Characteristics

Negative Specificity: The amount of false positive should not exceed 1 when detecting 15 national negative quality control samples with ELISA kits of anti-HAV IgM.

Positive Specificity The amount of false negative should not exceed 1 when detecting 15 national negative quality control samples with ELISA kits of anti-HAV IgM.

Limit of Detection: When detecting anti-HAV IgM national limit quality control samples at the ration 1:8 with the ELISA Kits of anti-HAV IgM all results should be in positive.

Precision: The CV Should not over 15% with correspond reference material after testing 10 wells.

Test 1280 clinical samples with this test kit and listed test kit. And result shows, the final negative coincidence rate is 99.5%, the final positive coincidence rate is 98.0%, the total coincidence rate is 98.9%.

References

1. Matheny , SC;Kingery , JE (1 December 2012).Hepatitis A. Am Fam Physician.86(11):1027-34, quiz 1010-1012. PMID 23198670. Archived from the original on 9 March 2014.

2. Connor BA (2005). Hepatitis A vaccine in the last –minute traveler . Am .J. PMID 16271543.

3. GBD 2015 Disease and injury Incidence and Prevalence , Collaborators.
(8 October 2016). Global, regional, and national incidence , prevalence , and years lived with disability for 310 diseases and injuries,1990-2015: a systematic analysis for the Global Burden of Disease study 2015. The Lancet.388 (10053):1545-1602.doi:10.1016/SO140-6736(16)31678-6.
PMC 5055577.PMID 27733282.

4. Ryan KJ , Ray CG (editors) (2004) .Sherris Medical Microbiology (4th ed.).McGraw Hill.pp.541-4.ISBN 978-0-8385-8529-0

5. Hepatitis A Fact sheet N 328.World Health Organization. July 2013.Archived from the original on 21 February 2014. Retrieved 20 February 2014