

# HAV IgM ELISA Kit

Enzyme-linked immunosorbent assay for the detection IgM antibody against HAV serum or plasma

## INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera with the "capture" system. The kit may be used for the identification of the viral agent causing hepatitis in the patient and the follow up of the acute phase of the infection.

## SUMMARY AND PRINCIPLE OF THE TEST

Hepatitis A continues to be one of the most frequently reported vaccine-preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially eliminate indigenous transmission of hepatitis A virus (HAV) infection. HAV, a 27-nm RNA agent classified as a picornavirus, can produce either asymptomatic or symptomatic infection 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. The likelihood of having symptoms with HAV infection is related to the person's age. In children less than 6 years of age, most (70%) infections are asymptomatic; if illness does occur, it is not usually accompanied by jaundice. Among older children and adults, infection is usually symptomatic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic persons have prolonged or relapsing disease lasting up to 6 months. In infected persons, HAV replicates in the liver, is excreted in bile, and is shed in the stool. Peak infectivity of infected persons occurs during the 2-week period before onset of jaundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool declines after jaundice appears. Children and infants can shed HAV for longer periods than adults, up to several months after the onset of clinical illness. Chronic shedding of HAV in feces does not occur; however, shedding can occur in persons who have relapsing illness. Viremia occurs soon after infection and persists through the period of liver enzyme elevation. Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most persons, IgM anti-HAV becomes detectable 5-10 days before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV, which appears early in the course of infection, remains detectable for the person's lifetime and confers lifelong protection against the disease. Commercial diagnostic tests are available for the detection of IgM and total (IgM and IgG) anti-HAV in serum. HAV RNA can be detected in the blood and stool of most persons during the acute phase of infection by using nucleic acid amplification methods, and nucleic acid sequencing has been used to determine the relatedness of HAV isolates.

HAV infection is acquired primarily by the fecal-oral route by either person-to-person contact or ingestion of contaminated food or water. On rare occasions, HAV infection has been transmitted by transfusion of blood or blood products collected from donors during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been detected in saliva during the incubation period; however, transmission by saliva has not been demonstrated. Depending on conditions, HAV can be stable in the environment for months. Heating foods at temperatures greater than 185 F (85°C) for 1 minute or disinfecting surfaces with a 1:100 dilution of sodium hypochlorite (i.e., household bleach) in tap water is necessary to inactivate HAV. Because most children have asymptomatic or unrecognized infections, they play an important role in HAV transmission and serve as a source of infection for others. In one study of adults without an identified source of infection, 52% of their households included a child less than 6 years old, and the presence of a young child was associated with HAV transmission within the household. In studies where serologic testing of the household contacts of adults without an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had serologic evidence of acute HAV infection (IgM anti-HAV).

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody. After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of HAV Ag, labelled with a monoclonal antibody conjugated with peroxidase (HRP). After incubation, microwells are

washed to remove unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HAV present in the sample.

## REAGENTS

### Materials provided with the kits:

1. Microtiter Well: coated with goat anti-human IgM.
2. Negative Control: 0.25ml normal serum.
3. Positive Control: 0.25ml anti-HAV IgM positive serum.
4. Enzyme Conjugate: 6 ml, monoclonal anti-HAV-HRP with HAV antigen immunocomplex.
5. Wash Buffer Concentrate (20x): 40 ml, The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6 ml Urea Peroxide
7. Substrate Solution B: 6 ml TMB solution
8. Stop Solution: 6 ml 2N Sulfuric Acid

### Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

## SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

## PRECAUTIONS

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

## STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

## WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate.

## WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

#### STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of the plates to humidity drastically reduces the shelf life.

#### ASSAY PROCEDURE:

1. Dispense one drop (50 ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one black well as background control.
2. Dispense 50 ul of undiluted specimen into each well. Then incubate for 30 minutes at 37°C
3. Remove the incubation mixture by flicking plate content into a waste container.
4. Wash each well 4 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
5. Add one drop of HAV -Peroxidase Conjugate into each of the wells. Do not add Antigen and Enzyme Conjugate to the blank well. Please add 100ul washing buffer to the blank well
6. Mix thoroughly the liquid in the wells. Incubate for 30 minutes at 37°C.
7. Wash the plate 4 times as step 4.
8. Add one drop (50µl) of Substrate Solution A and one drop (50µl) of Substrate Solution B (TMB) to each well and mix well.
9. Incubate for 10 minutes at 37°C.
10. Add one drop (50µl) of Stop Solution into each well. Measure OD with an EIA plate reader at the wavelength of 450nm.

#### INTERPRETATION OF RESULTS

##### EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Positive: P/N value is equal to or greater than 2.1

Negative: P/N value is less than 2.1

$$P/N \text{ value} = \frac{\text{OD value of specimen}}{\text{Average OD value of Negative Control}}$$

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

#### LIMITATIONS OF THE ASSAY

1. HAV IgM EIA is limited to the detection of IgM antibody against HAV in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells completely before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.
4. Do not use reagents from different tests that will cause incorrect results.
5. Following the procedure instruction closely, especially the incubation time and temperature.

#### RELATED READING MATERIALS

1. Dienstag J.L.. "Hepatitis A Virus : identification, characterization and epidemiologic investigations". Progress in liver disease VI, Popper E., Schaffner F.(eds), pp 343-370, New York, Gruner and Stratton, 1979.
2. Duermeyer W., Van der Veen J., Koster B. "ELISA in Hepatitis A". Lancet. I.: 823-824, 1978
3. Parry J.V., (1981) "Hepatitis A infection: guidelines for the development of satisfactory assays for laboratory diagnosis". The Institute of Medical Laboratory Sciences, 38, 303-311.
4. J., Frosner G., Hansson B.G. et al. "Serologic markers of hepatitis A and B in chronic active hepatitis". Scandinavian Journal of Gastroenterology, 13:525-527, 1978.

5. Barbara J.A., Howell D.R., Briggs M., Parry J.V.. "Post transfusion hepatitis A". Lancet (1982), 1-738.
6. Zchoval R., Dienstag J.L., Purcell R.H. "Tests for hepatitis A virus antigen and antibody" in "Hepatitis A". Gerety R.J. (Ed), pp 33-46, Orlando, Academic Press, Inc. 1984

#### Manufacturer:

Biocare Diagnostics Ltd.  
6F, Building B, 108 Xinghua Road  
Xiangzhou, Zhuhai, China 519000  
Tel: +86-756-8238560  
E-mail: [info@ivdbiocare.com](mailto:info@ivdbiocare.com)  
Website: [www.ivdbiocare.com](http://www.ivdbiocare.com)

April 10, 2008 Revision: 03



*In vitro* diagnostic device



Consult instructions for use



Keep dry



Temperature limitation



Use by



Lot code



Catalogue number



Contains sufficient for <n> tests



Manufacturer



Do not use if package damaged