SPECTRUM

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Anti-HAV IgM ELISA

REF: 1300 001 96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the qualitative detection of Anti-HAV IgM (IgM antibodies to hepatitis A virus) in human serum or plasma.

Background

Hepatitis A Virus (HAV) is a nonenveloped icosahedral 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 hepatocites. The route of infection is predominantly Feaco-oral with an incubation period of 2-7 weeks during which HAVcan be detected in stools. HAV infection does not originate any chronic hepatitis and complications are uncommon.The infection stimulates a strong immunological response in the patient with elevated titers first of IgM and then of IgG, whose final presence lasts for years after infection.

Assay Principle

This assay is based upon the two-steps capture method. In the first step, the sample and mouse monoclonal Anti-human IgM coated microplate are combined. During the incubation, IgM antibodies present in the sample bind to the Anti-human IgM coated on the wells. After the washing, in the second step, HAV antigens and Enzyme Conjugate are added to the wells. During the incubation, HAV antigens are allowed to react with the Anti-HAV IgM bound to the microplate and enzyme labeled antibodies, then a complex is generated among the solid phase, HAV antigens and enzyme-linked antibodies by immunological reactions. After a second washing, Substrate A and Substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is proportional to the amount of Anti-HAV in the sample.

Reagents

Coated Wells
1 x 96 wells pre-coated with mouse monnoclonal anti-human IgM

Negative Control
1 x 1 ml of a Tris-NaCl buffer solution containing proteins of bovine origin and 0.1% surfactant. 0.1% preservative was added.

Positive Control
1 x 1 ml of purified Anti-HAV IgM antibodies diluted in a Tris-NaCl buffer solution containing proteins of bovine origin and 0.1% surfactant. 0.1% preservative was added.

HAV Antigen Solution
1 x 5.5 ml of a solution containing recombinant HAV antigens and lysed HAV viruses. 0.2% preservative was added.

Enzyme Conjugate
1 x 5.5 ml of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-HAV in a phosphate buffered solution containing proteins of bovine origin and 0.1% surfactant. 0.1% preservative was added.

Substrate A
1 x 7.5 ml of hydrogen peroxide.

Substrate B
1 x 7.5 ml of TMB (3, 3', 5, 5'-tetramethylbenzidine) in a buffer solution.

Wash Solution Concentrate
1 x 50 ml of 20 times working wash solution.

Stop Solution
1 x 7.5 ml of 0.62 mol/l sulfuric acid.

2 Pieces of plate lid and 1 Zip-lock bag.

Reagents Storage and Stability

1. Store all components at 2-8^{O}C. Do not freeze. Avoid strong light.
2. Store unused wells in a sealed zip-lock bag (with desiccant provided) in the aluminum foiled pouch with a plate, as well as the unused reagents at 2-8^{O}C under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Reagent Preparation

1. Bring all reagents to room temperature (18-25 ^{O}C) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
2. Adjust the incubator to 37 ^{O}C.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled water and mix well. Prepare the required volume according to the number of assayed samples.

Specimen Collection and Preservation

1. Serum and plasma are suitable for analysis. The only acceptable anticoagulants are heparin, EDTA and sodium citrate. Specimen should be promptly separated from cells after blood collection.
2. Cap and store the samples at 18-25 ^{O}C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ^{O}C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ^{O}C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. After thawing, bring to room temperature and mix well by gently shaking.

Specimen Dilution

Add 5 µl of each sample to 5 ml of saline solution. Cover and vortex or mix thoroughly by inversion in a clean storage container. The diluted samples must be used within 8 hours.

Precautions and warnings

1. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
2. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
3. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
4. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
5. Do not touch or splash the rim of the well with conjugate.
6. Do not mix or use components from kits with different batch codes.
7. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.
8. It is important that the time of reaction in each well is held constant to achieve reproducible results.
9. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and Stop Solution should be added in the same sequence to eliminate any time deviation during reaction.

Assay Procedure

1. Determine the total number of wells needed for the assay. In addition to specimens, one substrate blank, three negative controls and two positive controls must be included on each plate or partial plate. Unused wells should be stored in the supplied Zip-lock bag with desiccant provided.
2. Prepare a record (plate map) identifying the placement of the blank, controls and specimens in the microwells.
3. To the wells of controls add 100 µl of the negative or positive controls.

4. Add 100 µl of diluted samples into each of the rest of the wells.
5. Shake on a plate shaker for 30 seconds to completely mix the diuted samples in the wells.

6. Cover the plate with a lid and incubate at 37 ^{O}C for 30 minutes.
7. Add 350 µl of Wash Solution to each well, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual Wash Solution onto absorbent paper.

8. Add 50 µl of HAV Antigen Solution, then 50 µl of Enzyme Conjugate into each well except the blank well.
9. Shake on a plate shaker for 30 seconds and incubate at 37 ^{O}C for 30 minutes.
10. Repeat step 7.
11. Add 50 µl of Substrate A, then 50 µl of Substrate B to each well, including the blank well.
12. Shake on a plate shaker for 30 seconds and incubate at 37 ^{O}C in the dark for 10 minutes.
13. Add 50 µl of Stop Solution to each well, including the blank well. Mix thoroughly on a plate shaker. It is very important to make sure that the blue color changes to yellow completely.
14. Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize interference) in a microplate reader. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

Assay Scheme	
Controls / diluted samples	100 µl
Incubation time	30 minutes
Washing step	6 times
HAV Antigen Enzyme Conjugate	50 µl 50 µl
Incubation time	30 minutes
Washing step	6 times
Substrate A Substrate B	50 µl 50 µl
Incubation time in dark	10 minutes
Stop solution	50 µl
Read absorbance	450 nm

Test Validation

The recommended control requirement for this assay is using positive and negative controls to verify assay performance. The result is valid if the following criteria for the controls are both met

Positive Control

Mean absorbance of Positive Control is equal to or higher than 0.7

Negative Control

Mean absorbance of Negative Control is lower than 0.1

Results

Each plate must be considered separately when calculating and interpreting results of the assay.

Cut-off Value

Calculate the mean absorbance of the replicates of the Negative Control.

The cut-off = 2.1 X Mean of the Negative Control replicates

N.B. If the mean absorbance of Negative Control replicates < 0.05, use the following equation:

The cut-off = 2.1 X 0.05

Example

Mean Negative Control	= (0.018 + 0.010+ 0.007)/3 = 0.011
Cut-off Value	= 2.1 × 0.05 = 0.105

Interpretation of Result

Nonreactive Results
Samples giving an absorbance less than the cut-off value are considered nonreactive.

Reactive Results
Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay. Such samples should be retested in duplicate . Samples that are reactive in at least one of the retests are considered positive for Anti-HAV IgM and should be confirmed by using a confirmatory kit. Samples that are nonreactive in both wells on retest should be considered nonreactive.

Limitations
1. This assay is intended as an aid for the clinical diagnosis Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results.Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.

Diagnostic Sensitivity
The diagnostic sensitivity was determined by testing a panel of 350 positive samples. The results obtained show a diagnostic sensitivity of 98.3 %.

Diagnostic Specificity
The diagnostic specificity was determined by testing a panel of 679 samples, in parallel with a test already available on the market. The results obtained show a diagnostic specificity > 98 %.

Performance Charateristics

Measurement pricision

Within run (Repeatability)

With one human serum based panel, using one batch of reagent in replicates of 100 samples data are as following:

Mean = 1.12 **SD =** 0.087 **CV =** 7.12 %

Run to run (Reproducibility)

With one human serum based panel, using one batch of reagent in replicates of 100 samples across 3 separate runs data are as following:

Mean = 1.22 **SD =** 0.12 **CV =** 9.78 %

References

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3. Decker RH, Kosakowski SM, Vanderbilt AS, et al. Diagnosis of acute hepatitis A by HAVAB –M, adirect raioimmunoassay for IgM anti-HAV. AM J clin Pathol 1981;43(8B): 1494-9.
4. Connor BA. Hepatitis A vaccine in the last-minute traveler. *Am. J. Med.* 2005;118 Suppl 10A:58S-62S.

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