HAV IgM

Enzyme Immunoassay for the qualitative determination of IgM Class antibodies to Hepatitis A Virus or HAV IgM in human serum or plasma

REF GD6250 00

IVD

INDICATION

Hepatitis A Virus (HAV) is non-enveloped icosahedra RNA virus with a linear single stranded 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 oral-foecal with an incubation period of 2-7 weeks, during which HAV can 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.
The determination of both IgG and IgM can distinguish the infective nature of the illness, the classification of the virus involved and the phase of the infection cycle, as well.

PRINCIPLE OF THE ASSAY

Microplates are coated with a mix of purified recombinant hepatitis A virus antigens that, in the first incubation, capture the anti HAV antibodies, eventually present in the sample. After washing out all the other components of the sample, in the second incubation bound anti HAV IgM specific antibodies are detected by the addition of anti human IgM antibody conjugated with horseradish peroxidase (HRP). The enzyme captured on the solid phase, acting on the TMB-Substrate, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample and can be detected by an ELISA reader.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with a mix of purified recombinant hepatitis A virus antigens. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B1 – Conjugate 11x**
   1 vial of 1.5 ml.
   Concentrated monoclonal anti-human IgM antibodies conjugated with Horseradish peroxidase (HRP). Transparent or slightly opalescent liquid, colorless or light yellow colored. Preserving agent: 0.04% ProClin300, 0.001% Gentamycin sulphate.

3. **Reagent B2 – Conjugate Diluent**
   1 vial of 12.5 ml.
   Buffer that is used to dilute the Conjugate concentrated before analysis. Yellow coloured liquid, at 18-24 °C it becomes opalescent. Preserving agent: 0.04% ProClin300, 0.008% Gentamycin sulphate and 0.01% Thimerosal.

4. **Reagent C – Washing Solution 25x**
   1 bottle of 50 ml.
   Concentrated solution to be diluted 1:25 with distilled water. Transparent or slightly opalescent liquid, colorless or pale yellow.

5. **Reagent D – Chromogen 21x**
   1 vial of 1.5 ml.
   Transparent colourless liquid containing Tetramethylbenzidine (TMB).
   Avoid light exposure.

6. **Reagent E – Substrate Buffer**
   1 vial of 15 ml.
   Transparent colourless liquid containing, in Citric acid and Sodium acetate solution pH 4.1-4.3, H_2O_2. Preserving agent: 0.05% ProClin 300.

7. **Reagent F – Stop Solution**
   1 vial of 25 ml.
   It contains 0.75 mol/l Sulphuric acid solution. Transparent colourless liquid.

8. **Reagent G – Sample Diluent**
   1 vial of 12.5 ml.
   Violet-blue transparent or slightly opalescent fluid. Preserving agent: 0.04% ProClin 300, 0.004% Gentamycin sulphate.

9. **Negative Control**
   1 vial of 3 ml.
   Heat inactivated human plasma, negative for anti HAV IgM antibodies, HBsAg, antigen p24 HIV 1, anti HIV 1/2 and anti HCV. Green transparent or slightly opalescent fluid. Preserving agent: 0.04% ProClin 300, 0.05% Sodium azide.

10. **Positive Control**
    1 vial of 1.5 ml.
    Heat inactivated human plasma, containing anti HAV IgM antibodies, negative for HBsAg, antigen p24 HIV 1, anti HIV 1/2 and anti HCV. Red transparent or slightly opalescent fluid. Preserving agent: 0.04% ProClin 300, 0.05% Sodium azide.

11. **Cardboard sealers**
    2 cardboard sealers to be used to cover the plate during the incubations.

12. **Package insert:** instruction for use GD6250 00.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples.
2. Avoid any contact with skin and mucous membrane, in particular for Substrate Buffer, Chromogen and Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported.
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.
7. Do not forget to neutralize and/or autoclave the solution or washing wastes or any fluids containing biological samples before discarding them into the sink. Solid waste (used plates, tips, vials, glassware, etc.) should be disinfected by 6% peroxide of hydrogen with 0,5 % synthetic washing-up liquid or 3% of chloramin B solutions. Total time of deactivation should be no less than one hour. Another resolved to application disinfectant is possible to use. Also solid waste should be disinfected by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1,5 kHz/sm2 (0,15 mPa). Liquid waste (washing water) should be disinfected by dry chloramin B adding in concentration 30g/l (deactivation time – no less than 2 hours). Also liquid waste can be disinfected by boiling treatment for 30 min or by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1,5 kHz/sm2 (0,15 mPa). Tools and equipment should be wiped 2 times by 70 % ethanol before and after work.
8. Some reagents contain 0.04 and 0.05% ProClin 300.

Xi

ProClin 300: **Irritant**. May cause sensitization by skin contact. After contact with skin, wash immediately with plenty of soap and water.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. The diluted washing solution can be stored 14 days at 18-24 °C when stored in a tightly sealed vial.
4. Working solution of conjugate may be stored for not more than 12 hours at 18-24 °C in a dark place.
5. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes able to dispense of 10, 50, 90, and 100µl and disposable pipette tips
- Graduated cylinders of 25, 100, 1000 ml.
- Disposable gloves
- Chronometer
- Purified water
- Microplate reader equipped with 450 nm and 620-680 nm filters.
- Microplate incubator set at 37 (±0.5) °C.
- Automatic microplate washer

SAMPLES

Collect a blood sample according to the current practices. The test should be performed on serum or plasma. Separate the serum or plasma from the clot or red cells as soon as possible to avoid any haemolysis.
Extensive haemolysis may affect test performance.
Specimens with observable particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. The specimens can be stored at 2-8 ºC if screening is performed within 3 days or they may be deep-frozen at -20 ºC. The plasma must be quickly thawed by warming for a few minutes in a water bath at 40 ºC (to avoid fibrin precipitation). Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Do not use contaminated, hyperlipaemic or hyperhemolysed sera or plasma.

REAGENTS PREPARATION

- WORKING WASHING SOLUTION
  The concentrated solution may present a sediment that can be dissolved at 35-39 °C and shaking.
  Dilute the required volume of concentrated solution with the corresponding volume of purified water (see table below) and mix carefully before use.
- WORKING CONJUGATE SOLUTION
  Working conjugate solution must be prepared before use. Thoroughly shake conjugate concentrate. To make working conjugate solution, take required amount of concentrate and mix with conjugate diluent in a separate vial (see table below). Thoroughly mix the buffer. Do not apply intensive mixing.
- CHROMOGEN/SUBSTRATE
  Dilute the required volume of Substrate Buffer with the corresponding volume of Chromogen (see table below) and mix carefully before use.
  Substrate/Chromogen mixture should be colourless.

Consumption of reagents for the manual test procedure

n° of strips	Working Sol. Washing		Working Sol. Conjugate		Working Sol. Chromogen/Substrate	
	Washing Sol. 25x (ml)	Purified water (ml)	Conjugate 11x (ml)	Conjugate Diluent (ml)	TMB 21x (ml)	Substrate Buffer (ml)
1	4.0	96.0	0.1	1.0	0.05	1.0
2	8.0	192.0	0.2	2.0	0.10	2.0
3	12.0	288.0	0.3	3.0	0.15	3.0
4	16.0	384.0	0.4	4.0	0.20	4.0
5	20.0	480.0	0.5	5.0	0.25	5.0
6	24.0	576.0	0.6	6.0	0.30	6.0
7	28.0	672.0	0.7	7.0	0.35	7.0
8	32.0	768.0	0.8	8.0	0.40	8.0
9	36.0	864.0	0.9	9.0	0.45	9.0
10	40.0	960.0	1.0	10.0	0.50	10.0
11	44.0	1056.0	1.1	11.0	0.55	11.0
12	48.0	1152.0	1.2	12.0	0.65	13.0

ASSAY PROCEDURE

1. At least 30 minutes before use, bring all reagents, controls and samples to room temperature (18-24 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute the controls in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-680 nm and to read the absorbance of samples and controls at 450 nm. Reading the absorbance at 450 nm only is possible.

ASSAY SCHEME

1. Put the desired number of microstrips into the frame.
2. Follow the scheme:

First incubation		Recombinan HAV antigens coated wells	
	REAGENTS	**Controls**	**Samples**
	Sample Diluent	-	90 µl
	Controls	100 µl	-
	Samples	-	10 µl
	- Carefully mix fluids in wells - Cover the strips with cardboard sealer - Incubate **30 minutes** at **37 (± 0.5) °C**		
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Add into each well 380-400 µl of working washing solution. Allow a soak time at least 40 seconds and aspirate. Repeat this procedure 6 times. Incomplete washing will adversely affect assay precision.		
Second incubation	Working conjugate solution	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **30 minutes** at **37 (± 0.5) °C**		
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Add into each well 380-400 µl of working washing solution. Allow a soak time at least 40 seconds and aspirate. Repeat this procedure 5 times. Incomplete washing will adversely affect assay precision.		
Colorimetric reaction	Chromogen-Substrate mixture	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **20 minutes** at **room temperature (18-24 °C)**, **avoiding light exposure**		
	Stop Solution	50 µl	50 µl
	Read the optical density at least 3-4 min after stopping of the reaction. Read the optical density at 450 nm with 620-680 nm as reference.		

VALIDITY OF THE ASSAY

The assay is to be considered valid if:

1. The mean OD value for the Negative Control is lower than 0.200.
2. The mean OD value of the Positive Control is higher than 1.000.

If these criteria are not met the test should be considered invalid and should be repeated.

CALCULATION OF RESULTS

The presence of detectable HAV IgM antibodies is determined by comparing the absorbance measured for each sample to the cut-off value calculated according to the following formula:

Cut-off = mean OD value of Negative Control + 0.150

0.150 is a coefficient defined by manufacturer during statistical processing.

INTERPRETATION OF RESULTS

Positive: if the OD value is > Cut-off
Negative: if the OD value is ≤ Cut-off

LIMITS OF THE TEST

- A non-reactive test result does not exclude the possibility of exposure to Hepatitis A virus.
- Results from immunosuppressed patients should be interpreted with caution.
- Bacterial contamination, heat inactivation may influence on the samples OD value.
- The diagnosis should not be based on the results of one testing. Patient's history, symptoms of the diseases and serological study should be taken into consideration during the diagnosis.

PRECAUTIONS IN USE

Refere to Safety Data Sheet.
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

AMS S.p.A. – Registered Office and Plant
Via E. Barsanti 17/A
00012 Guidonia (Rome) – Italy
Ph. + 39 0774 354441 r.a.
Fax + 39 0774 578035
www.ams-analyzers.com
info@ams-analyzers.com

Diagnostics Manufacturing
Via Galileo Galilei, 38
Seggiano di Pioltello (MI) - Italy
Ph. + 39 02 929189.1
Fax + 39 02 929189.39
www.gdsrl.com
infodiagnostics@ams-analyzers.com