

HAV Ab

**Competitive Enzyme ImmunoAssay
(ELISA) for the determination of
antibodies to Hepatitis A Virus
in human plasma and sera**

- for "in vitro" diagnostic use only -



DIA.PRO

**Diagnostic Bioprobes Srl
Via Carducci n° 27
20099 Sesto San Giovanni
(Milano) - Italy**

Phone +39 02 27007161

Fax +39 02 26007726

e-mail: info@diapro.it

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A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera. The kit is used for the follow-up of patients infected by HAV. For "in vitro" diagnostic use only.

B. INTRODUCTION

The Center for Disease Control or CDC, Atlanta, USA, defines Hepatitis A Virus as follows:

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.

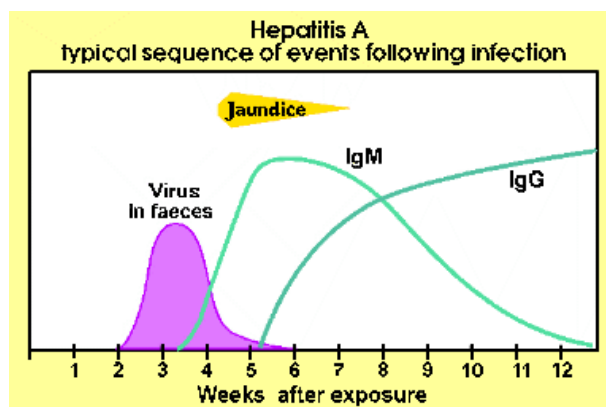
Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most 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).



C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with an anti-HAV specific antibody, labeled with HRP, for a fixed amount of antigen on the solid phase.

A purified and inactivated HAV is coated to the microwells.

The patient's serum/plasma is added to the microwell and antibodies to HAV are captured by the solid phase.

After washing, the enzyme conjugate is added and binds to the free HAV antigen, if still present.

The plate is washed to remove unbound conjugate and then the chromogen/substrate is added.

In the presence of peroxidase the colorless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is inversely proportional to the amount of antibodies to HAV present in the sample.

An additive is added to the sample directly into the well to block interferences able to mask the presence of antibodies, mostly appearing in the follow up of vaccination.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate **MICROPLATE**

8x12 microwell strips coated with purified and inactivated HAV, sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening. Reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: **CONTROL -**

1x2.0ml/vial. Ready to use. Contains bovine serum proteins, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The negative control is color coded pale yellow.

3. Positive Control: **CONTROL +**

1x2.0ml/vial. Ready to use. Contains bovine serum proteins, anti HAV antibodies at a concentration higher than 100 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The positive control is colour coded green.

4. Calibrator: **CAL ...**

n° 1 vial. Lyophilized. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, anti HAV antibodies at a concentration of about 10 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate: **WASHBUF 20X**

1x60ml/bottle. 20x concentrated solution. to be diluted up to 1200ml with distilled water before use.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

6. Enzyme conjugate: **CONJ**

1x16ml/vial. Ready-to-use solution. Contains Horseradish peroxidase conjugated antibody, specific to HAV, in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The reagent is colored with a red dye.

7. Chromogen/Substrate: **SUBS TMB**

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent: **DILSPE**

1x8ml. Buffered solution suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide and 0.1% Kathon GC as preservatives. The reagent is color coded dark green.

9. Sulphuric Acid: **H₂SO₄ 0.3 M**

1x15ml/vial. Contains 0.3 M H₂SO₄ solution.
Attention: Irritant (Xi R36/38; S2/26/30)

10. Plate sealing foils

11. Package insert

Upon request:

Calibration Curve: **CAL N°...**

5x2.0 ml/vial. Ready to use and colour coded standard curve ranging: 0-5-10-50-100 WHO mIU/ml.

(CAL1=0mIU/ml, CAL2=5mIU/ML, CAL3=10mIU/ml,
CAL4=50mIU/ml, CAL5=100mIU/ml).

Contains serum proteins, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Standards are blue colored.

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.1°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
11. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
12. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples

has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..

13. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

14. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water

15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and reading is strongly recommended.

4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.

5. Sera and plasma can be stored at +2°.8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be freeze/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on opened kit has pointed out no relevant loss of performances up to 3 months from first opening.

1. Antigen coated microwells:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in conservation.

In this case, call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°.8°C.

When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use.

4. Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The dissolved calibrator is not stable; store it frozen in aliquots at -20°C.

5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bi-distilled water up to 1200ml and mixed gently end-over-end before use.

Once diluted, the wash solution is stable for 1 week at 2-8°C.

During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Specimen Diluent:

Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (Xi R36/38; S2/26/30)

Legenda: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm 2\%$.

2. The ELISA incubator has to be set at +37°C (tolerance of $\pm 0.5^\circ\text{C}$) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 350µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of $\pm 5\%$.

5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

Legenda: BLK = Blank NC = Negative Control
 CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or Co/S values have been matched in the analysis.
 Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	> 0.750 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 10 mIU/ml (WHO)	Co/S ≥ 1.0
Positive Control	< 0.300 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 0.750 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator Co/S < 1.0	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control > 0.300 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (ex.: dispensation of negative control instead of the positive one); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of a cut-off value determined with the following formula:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 3$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, make sure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the Cut-Off value and the OD450nm of the sample (or Co/S) according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HAV.

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of a past or recent HAV infection and therefore the patient should be treated accordingly.

An example of calculation is reported below.

The following data must not be used instead of real figures obtained by the user.

Negative Control: 1.900 – 2.000 – 2.100 OD450nm
 Mean Value: 2.000 OD450nm
 Higher than 0.750 – Accepted

Positive Control: 0.100 OD450nm
 Lower than 0.300 – Accepted

$$\text{Cut-Off} = (2.000 + 0.100) / 3 = 0.700$$

Calibrator: 0.400-0.360 OD450nm
 Mean value: 0.380 OD450nm
 Co/S > 1 – Accepted

Sample 1: 0.050 OD450nm
 Sample 2: 1.900 OD450nm
 Sample 1 Co/S > 1.1 positive
 Sample 2 Co/S < 0.9 negative

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 2nd International Standard supplied by WHO.

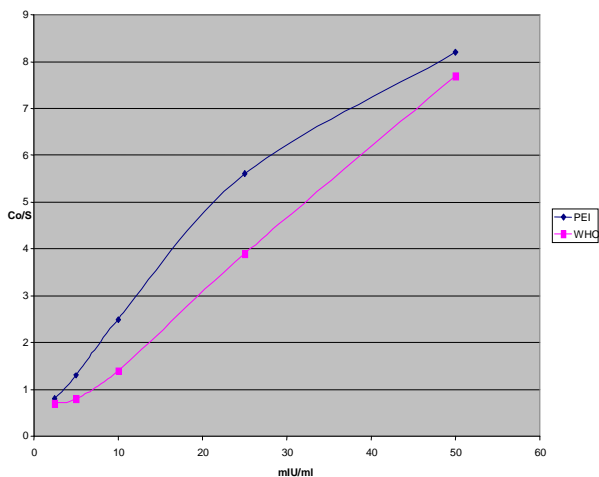
Two control samples, supplied by Boston Biomedica Inc., USA, with code Accurun 52 and 120, were also examined.

The sensitivity shown by the assay is < 10 WHO mIU/ml or < 5 PEI mU/ml.

Results of Quality Control are given in the following table:

WHO mIU/ml	OD450 nm	Co/S	PEI mU/ml	OD450 nm	Co/S
50	0.099	7.7	50	0.093	8.2
25	0.197	3.9	25	0.137	5.6
10	0.543	1.4	10	0.304	2.5
5	0.943	0.8	5	0.587	1.3
2.5	1.015	0.7	2.5	0.949	0.8
Neg. Control	2.217		Neg. Control	2.217	
Accurun 52	0.060	12.7	Accurun 120	0.115	6.6

Curves are reported below:



2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a clinical trial on panels of samples classified positive by a US FDA approved kit.

An overall value of 100% has been found in the study conducted on a total number of more than 200 samples.

Seroconversion and performance panels have also been studied. Results obtained by examining two panels supplied by Boston Biomedica Inc., USA, are reported below.

Seroconversion Panel: PHT 902

Sample	OD450nm	Co/S	DiaSorin
CTRL (-)	1,968	0,3	
CTRL (+)	0,084	8,1	
Calibrator	0,470	1,5	
PHT902			
1	1,878	0,4	neg
2	1,501	0,5	neg
3	0,090	7,6	pos
4	0,123	5,6	pos
5	0,120	5,7	pos

Performance Panel: PHT 201

Sample	OD450nm	Co/S	DiaSorin	Sample	OD450nm	Co/S	DiaSorin
1	0,169	4,0	pos	14	0,139	4,9	pos
2	0,132	5,2	pos	15	0,115	5,9	pos
3	0,143	4,8	pos	16	0,167	4,1	pos
4	0,104	6,6	pos	17	0,086	8,0	pos
5	0,438	1,6	pos	18	0,160	4,3	pos
6	0,121	5,7	pos	19	0,175	3,9	pos
7	0,127	5,4	pos	20	1,772	0,4	neg
8	0,150	4,6	pos	21	0,090	7,6	pos
9	0,115	5,9	pos	22	0,201	3,4	pos
10	0,094	7,3	pos	23	0,281	2,4	pos
11	0,070	9,8	pos	24	0,134	5,1	pos
12	1,814	0,4	neg	25	0,142	4,8	pos
13	0,097	7,1	pos	Neg	1,780	0,4	neg

3. Diagnostic specificity:

The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV, HDV, HBV, HIV) and non viral pathologies of the liver that may interfere with the test were examined.

No cross reaction were observed.

The Performance Evaluation study conducted in the external reference center on more than 1000 samples has provided a value > 98% .

4. Precision

The mean values obtained from a study conducted on two samples of different anti-HAV reactivity, examined in 16 replicates in three separate runs is reported below:

Test # 1

Sample	Negative	Low Pos.
OD450nm	2.425	0.608
Std. Deviation	0.065	0.023
CV %	2.7	3.9

Test # 2

Sample	Negative	Low Pos.
OD450nm	2.373	0.573
Std. Deviation	0.107	0.034
CV %	4.5	6.0

Test # 3

Sample	Negative	Low Pos.
OD450nm	2.478	0.554
Std. Deviation	0.108	0.023
CV %	4.4	4.2

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

REFERENCES

1. CDC. Summary of notifiable diseases, United States, 1997. *MMWR* 1998;46:1-87.
2. CDC. Prevention of hepatitis A through active or passive immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 1996;45(RR-15).
3. Krugman S, Giles JP. Viral hepatitis: new light on an old disease. *JAMA* 1970;212:1019-29.
4. Hadler SC, Webster HM, Erben JJ, Swanson JE, Maynard JE. Hepatitis A in day-care centers: a communitywide assessment. *N Engl J Med* 1980;302:1222-7.
5. Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW. Frequency of illness associated with epidemic hepatitis A virus infection in adults. *Am J Epidemiol* 1985;122:226-33.
6. Glikson M, Galun E, Oren R, Tur-Kaspa R, Shouval D. Relapsing hepatitis A. Review of 14 cases and literature survey. *Medicine* 1992;71:14-23.
7. Skinh j P, Mathiesen LR, Kryger P, M Iler AM. Faecal excretion of hepatitis A virus in patients with symptomatic hepatitis A infection. *Scand J Gastroenterol* 1981;16:1057-9.
8. Tassopoulos NC, Papaevangelou GJ, Ticehurst JR, Purcell RH. Fecal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees. *J Infect Dis* 1986;154:231-7.
9. Rosenblum LS, Villarino ME, Nainan OV, et al. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. *J Infect Dis* 1991;164:476-82.
10. Sjogren MH, Tanno H, Fay O, et al. Hepatitis A virus in stool during clinical relapse. *Ann Intern Med* 1987;106:221-6.
11. Lemon SM. The natural history of hepatitis A: the potential for transmission by transfusion of blood or blood products. *Vox Sang* 1994;67(suppl 4):19-23.
12. Bower WA, Nainan OV, Margolis HS. Duration of viremia in naturally-acquired hepatitis A viral infections. [Abstract 103] In: Abstracts of the Infectious Diseases Society of America 35th Annual Meeting. Alexandria, VA: Infectious Diseases Society of America, 1997.
13. Liaw YF, Yang CY, Chu CM, Huang MJ. Appearance and persistence of hepatitis A IgM antibody in acute clinical hepatitis A observed in an outbreak. *Infection* 1986;14:156-8.
14. Stapleton JT. Host immune response to hepatitis A virus. *J Infect Dis* 1995;171(suppl 1):S9-14.

15. Hutin YJF, Pool V, Cramer EH, et al. A multistate, foodborne outbreak of hepatitis A. *N Engl J Med* 1999;340:595-602.
16. Soucie JM, Robertson BH, Bell BP, McCaustland KA, Evatt BL. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion* 1998;38:573-9.
17. Cohen JI, Feinstone S, Purcell RH. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. *J Infect Dis* 1989;160:887-90.
18. McCaustland KA, Bond WW, Bradley DW, Ebert JW, Maynard JE. Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol* 1982;16:957-8.
19. Favero MS, Bond WW. Disinfection and sterilization. In: Zuckerman AJ, Thomas HC, eds. *Viral hepatitis, scientific basis and clinical management*. New York, NY: Churchill Livingstone, 1993:565-75.
20. Staes C, Schlenker T, Risk I, et. al. Source of infection among persons with acute hepatitis A and no identified risk factors, Salt Lake County, Utah, 1996 [Abstract 302]. *Clin Infect Dis* 1997;25:411.
21. Smith PF, Grabau JC, Werzberger A, et al. The role of young children in a community-wide outbreak of hepatitis A. *Epidemiol Infect* 1997;118:243-52.
22. Williams I, Bell B, Kaluba J, Shapiro C. Association between chronic liver disease and death from hepatitis A, United States, 1989-92 [Abstract A39]. IX Triennial International Symposium on Viral Hepatitis and Liver Disease. Rome, Italy, April 1996.
23. Akriviadis EA, Redeker AG. Fulminant hepatitis A in intravenous drug users with chronic liver disease. *Ann Intern Med* 1989;110:838-9.
24. Willner IR, Uhl MD, Howard SC, Williams EQ, Riely CA, Waters B. Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. *Ann Intern Med* 1998;128:111-4.

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Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n°27 – Sesto San Giovanni (MI) – Italy

