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 -

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REF AVAB.CE
96 Tests

HAV Ab

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:

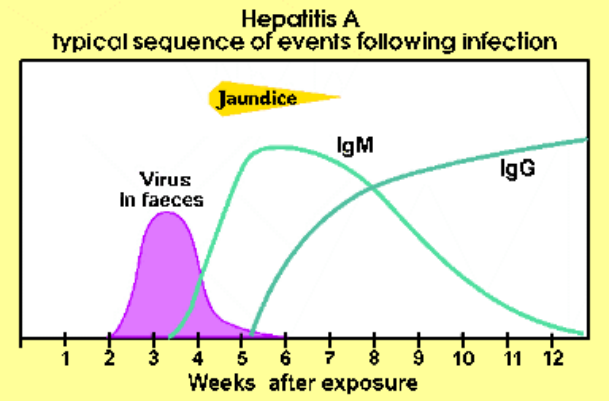
Hepatitis A continues to be one of the most frequently reported vaccine-preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially eliminate indigenous 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 -
1x4.0ml/vial. Ready to use. Contains bovine serum proteins, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The negative control is color coded pale yellow.

3. Positive Control: CONTROL +
1x4.0ml/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 0.045% ProClin 300 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 0.045% ProClin 300 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 0.045% ProClin 300.

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, 0.045% ProClin 300 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 H_2O_2.
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 0.045% ProClin 300 as preservatives. The reagent is color coded dark green.

9. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils **n° 2**

11. Package insert **n° 1**

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 0.045% ProClin 300 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. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
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 RECOMMANDATIONS
1. Blood is drawn aseptically by venipuncture 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 in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/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 0.2-0.8u 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 (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

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 ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°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 in advance, checked for the delivery of the right

dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.

4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; (d) repeatability $\geq$ 1%. 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 that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at +2..8°C, sealed.

2. Dispense 50 ul Specimen Diluent in all the wells identified for samples and controls/calibrator, except for A1.
Then pipette 100 µl of Negative Control in triplicate, 100 µl of Calibrator in duplicate, 100 µl Positive Control in single and then 100 ul of samples.
Check that controls/calibrator and samples have been correctly added. Incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.
Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Pipette 100 µl Sulphuric Acid into each well to stop the enzymatic reaction using the same pipetting sequence as in step 6. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 well (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Specimen Diluent Controls/Calibrator(*) Samples	50 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL(*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator - Not mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls 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%
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.
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.

If Calibrator has used, verify the following data:

Parameter	Requirements
Calibrator 10 mIU/ml (WHO)	Co/S $\geq$ 1.0

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
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.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = (NC+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 or 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

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/ < 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.*

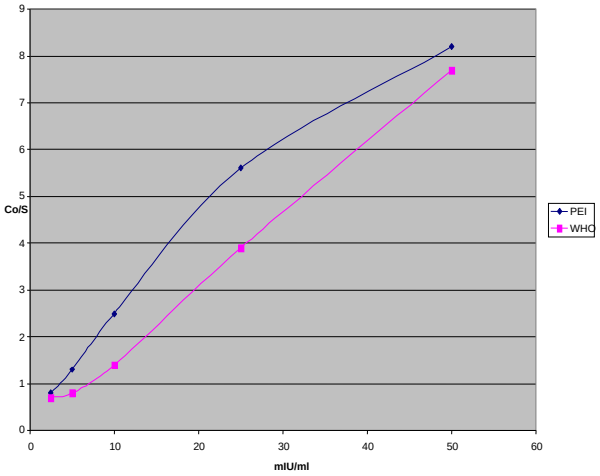
R. PERFORMANCE CHARACTERISTICS

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.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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