HDV Ag

Enzyme Immunoassay for the determination of Hepatitis Delta Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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REF DAG.CE
96 Tests

HDV Ag

A. INTENDED USE
Third generation Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis Delta Virus or HDV in human plasma and sera. The kit is intended for the follow-up of HDV infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION
The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.
Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone.
In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.
The determination of HDV specific serological markers (HDV Ag, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment.
The detection of HDV antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST
HDV Ag, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation. A detergent is added to the sample in order to dissolve the specific antigen from HDV particles.
In the 2nd incubation, after washing, a tracer, composed of a second anti HDV Ag antibody, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HDV Ag.
The concentration of the bound enzyme on the solid phase is proportional to the amount of HDV Ag in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.
The presence of HDV Ag in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of the antigen.

D. COMPONENTS
It contains reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1 microplate
12 strips x 8 breakable wells coated with a mouse monoclonal antibody specific to HDV antigen and sealed into a bag with desiccant.

2. Negative Control: CONTROL -
1x2.0ml/vial. Ready to use control. It contains 5% goat serum albumin, 100 mM Tris-HCl buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.
The negative control is color coded pale yellow.

3. Positive Control: CONTROL +
1x2.0ml/vial. Lyophilized control to be dissolved with 2 ml bidistilled water. It contains 5% goat serum albumin, high titer non infectious recombinant HDV antigen, 25 mM Tris-HCl buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

4. Calibrator: CAL ….
n° 1 vial. Lyophilised calibrator. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, non infectious recombinant low titer HDV antigen, 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.
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 and color coded regent. Contains Horseradish peroxidase conjugated polyclonal antibody to HDV antigen, 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 1% normal mouse serum, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The component is red color coded.

7. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 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
1x16ml. Contains a solution of 6% NP40, 0.045% ProClin 300 and 0.09% sodium azide as preservatives in 10 mM phosphate buffer pH 7.4+/-0.1.

9. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial. It contains 0.3 M H2SO4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Plate sealers: n° 2

11. Instructions for Use: n° 1

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), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
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/Substrate (TMB/H2O2) 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 for kit replacement.
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 the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 3 months.
11. 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.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. 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 before waste. 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..
14. Accidental spills from samples and operations 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.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. 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 WARNINGS

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 to samples; 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.
4. Haemolysed (red) 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 at least 12 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 an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplates:
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 manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Dissolve with 2 ml ELISA grade water and mix well on vortex before use. The positive control does not contain any infective HDV as it is composed of recombinant synthetic HDV.
Note: *The dissolved control is not stable. To be stored frozen in aliquots at –20°C.*

Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved Calibrator is not stable. To be stored frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note*: Once diluted, the wash solution is stable for 1 week at +2..8° C.*

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.

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

Specimen Diluent
Ready to use reagent. Mix gently and avoid foaming.

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.
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; 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 sections "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 is colourless 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 aluminium 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 Positive Control and 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 performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

1. Place the required number of strips in the plastic holder. Carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.

3. Pipette 100 µl of the Negative Control in triplicate, 100 ul of the Calibrator in duplicate and then 100 ul of the Positive Control in single followed by 100 ul of samples.
4. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
5. Then add 100 µl Specimen Diluent to all the wells, except for A1.
6. Finally incubate the microplate for **120 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

7. When the first incubation is over, wash the microwells as previously described (section I.3)
8. Dispense 100 ul Enzyme Conjugate in all wells, except for A1, used for blanking operations.

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

9. Following addition of the conjugate, check that the colour of wells have changed to red and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette then 100µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11 to stop the enzymatic reaction. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

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 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.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Controls & Calibrator Samples	100ul 100ul
Specimen Diluent	100ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported 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 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 / 620-630nm or S/Co values have been matched in the analysis.
Ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.100 OD450nm value after blanking coefficient of variation < 30%
Calibrator	S/Co > 1
Positive Control	> 1.000 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) > 0.100 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 S/Co < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 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 < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 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.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 14.

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 mean OD450nm/620-630nm + 0.100

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, en 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 sample OD450nm / 620-630nm and the Cut-Off value (or S/Co) according to the following table:

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

A negative result indicates that the patient is not infected by HDV (acute phase).
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 an ongoing HDV infection and therefore the patient should be treated accordingly.

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. *Any positive result should be confirmed by testing the patient for the other HDV markers, before a diagnosis of viral hepatitis is confirmed.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 14).

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

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.100 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.025+0.100 = 0.125

Calibrator: 0.280 - 0.290 OD450nm
Mean value: 0.285 OD450nm S/Co = 2.3
S/Co higher than 1 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. Limit of detection
No international standard for HDV antigen detection is available at the moment. An Internal Gold Standard (or IGS), derived from a patient in the very early acute phase of the infection, has been defined in order to provide the device with a constant and optimal sensitivity.
The limit of detection of the assay has been therefore calculated by comparison with a commercial European kit.
A limiting dilution curve was prepared in negative plasma.
Results of Quality Control are given in the following table:

Internal Gold Standard (IGS)

IGS	Lot #	1102	Lot #	0103	Lot #	0403	DiaSorin
dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co	S/Co
2 K	0.486	**4.4**	0.539	**4.7**	0.504	**4.7**	**4.5**
4 K	0.266	**2.4**	0.289	**2.5**	0.304	**2.8**	**2.6**
8 K	0.151	**1.4**	0.144	**1.3**	0.163	**1.5**	**1.3**
16 K	0.071	0.6	0.078	0.7	0.081	0.8	0.7
Neg.Control	0.011	/////	0.015	/////	0.008	/////	/////

2. Diagnostic Sensitivity and Specificity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a reference European kit.
Positive samples were collected from patients undergoing very early acute HDV infection.

The diagnostic specificity has been determined on panels of more than 250 negative samples from normal individuals and blood donors, classified negative with the reference 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 samples freezing 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, HAV) 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 a qualified external reference center on more than 300 samples has provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Precision:
It has been calculated on three samples examined in replicates in different runs.
The mean values obtained from a study conducted on three samples of different HDV Antigen reactivity, examined in 16 replicates in three separate runs is reported below:

DAG.CE: lot # 1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.021	0.020	0.019	0.020
Std.Deviation	0.004	0.005	0.004	0.004
CV %	17.7	22.7	19.3	19.9

IGS at 8 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.167	0.166	0.169	0.167
Std.Deviation	0.006	0.008	0.005	0.006
CV %	3.9	4.6	3.1	3.9
S/Co	1.3	1.4	1.4	1.4

IGS at 0.5 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.416	2.404	2.372	2.397
Std.Deviation	0.150	0.143	0.130	0.141
CV %	6.2	5.9	5.5	5.9
S/Co	19.9	20.0	19.9	19.9

DAG.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.019	0.020	0.018	0.019
Std.Deviation	0.003	0.004	0.003	0.003
CV %	17.6	19.4	17.7	18.2

IGS at 8 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.180	0.180	0.181	0.180
Std.Deviation	0.007	0.008	0.008	0.008
CV %	3.8	4.5	4.4	4.3
S/Co	1.5	1.5	1.5	1.5

IGS at 0.5 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.415	2.453	2.437	2.435
Std.Deviation	0.135	0.131	0.153	0.140
CV %	5.6	5.3	6.3	5.7
S/Co	20.3	20.4	20.7	20.5

DAG.CE: lot #0403

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.015	0.016	0.015	0.015
Std.Deviation	0.003	0.003	0.003	0.003
CV %	17.7	18.8	19.3	18.6

IGS at 8 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.170	0.169	0.172	0.170
Std.Deviation	0.008	0.008	0.008	0.008
CV %	4.8	4.7	4.5	4.7
S/Co	1.5	1.5	1.5	1.5

IGS at 0.5 K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.378	2.370	2.393	2.380
Std.Deviation	0.103	0.094	0.099	0.099
CV %	4.4	4.0	4.1	4.2
S/Co	20.9	20.6	21.0	20.8

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

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS
The detection of HDV antigen in ELISA is strongly influenced by the presence of antibodies. Once anti HDV antibodies are raised by the patient HDV Ag becomes undetectable in ELISA, while HDV RNA remains still detectable with PCR techniques.
False positivity has been assessed as less than 2 % of the normal population, mostly due to high titers of Heterophilic Anti Mouse Antibodies (HAMA).
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. 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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