



## EC DECLARATION OF CONFORMITY

<b>MANUFACTURER</b>	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
<b>PRODUCT</b>	<b>HDV Ab</b> CODE: <b>DAB.CE</b> (96 tests)
<b>CLASSIFICATION</b>	ANNEX II – LIST A
<b>CONFORMITY ASSESSMENT ROUTE</b>	ANNEX IV

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL **DIRECTIVE 98/79/EC** FOR IN VITRO DIAGNOSTIC DEVICES, BY VIRTUE OF THE ARTICLE 110(3) OF **REGULATION 2017/746 (IVDR)** AS AMENDED BY REGULATION (EU) 2022/112

<b>NOTIFIED BODY</b>	<b>AEMPS – n° 0318</b>
<b>(EC) CERTIFICATE(S)</b> <i>valid from: 20-05-2022</i> <i>to: 26-05-2025</i>	<ul style="list-style-type: none"><li>• FULL QUALITY ASSURANCE SYSTEM N° 2003 12 0388 CT (in accordance with Annex IV – except Section IV) of the Directive 98/79/EC)</li><li>• DESIGN CERTIFICATE N° 2003 12 0393 ED</li></ul>
<b>ISO CERTIFICATE</b>	<ul style="list-style-type: none"><li>• UNE EN ISO 13485 N° 2013 11 0039 EN</li></ul>

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<b>SIGNATURE</b> Legal Representative Dr.ssa Fiorenza Scozzesi	 Dott.ssa Fiorenza Scozzesi Legale Rappresentante 

Rev: 0522

# HDV Ab

**Competitive Enzyme Immunoassay  
for the qualitative determination of  
antibodies to Hepatitis Delta Virus  
in human serum and plasma**

- for "in vitro" diagnostic use only -



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REF DAB.CE  
96 Tests

29. Dia.PRO HDV Ab yra reagentų rinkinys, skirtas hepatito D antikūnų nustatymui IFA metodu. Plokštelės laužomos po 8 šulinėlius.

## HDV Ab

### A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology.

The kit is used for the follow-up of patients infected by HDV. 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 Ab, 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 total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

### C. PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

### D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

#### 1. Microplate: MICROPLATE

8x12 microwell strips coated with recombinant HDV-specific antigen and 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 4°C.

#### 2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The negative control is colour coded pale yellow.

#### 3. Positive Control: CONTROL +

1x2.0ml/vial. Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The positive control is colour coded green.

#### 4. Calibrator: CAL ....

n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml 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 solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The component is colour coded red.

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

1x16ml/vial. 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<sub>2</sub>O<sub>2</sub>.

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

#### 8. Sulphuric Acid: H<sub>2</sub>SO<sub>4</sub> 0.3 M

1x15ml/vial. Contains 0.3 M H<sub>2</sub>SO<sub>4</sub> solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Plate sealers n° 2

Instructions for Use n° 1

### E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionized, 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.
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-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H<sub>2</sub>O<sub>2</sub>) 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.
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 labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid 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. 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 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 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 to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
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 electronic reading is strongly recommended.
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 0.2-0.8µ 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.

##### 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 manufacturing.

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

Unused strips have to be placed back into the aluminium 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:

Low positive control. Add precisely the volume of EIA grade water, reported on its label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

**Note:** *The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C. When thawed do not freeze again; discard it.*

##### 5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml 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.*

##### 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. 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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. 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  $+37^{\circ}\text{C}$  (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 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, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". 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 samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and 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 full 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 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  $+37^{\circ}\text{C}$  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  $+2.8^{\circ}\text{C}$ , sealed.
2. Pipette 100  $\mu\text{l}$  of Negative Control in triplicate, 100  $\mu\text{l}$  Positive Control in single and then 100  $\mu\text{l}$  of samples. Check that controls and samples have been correctly added. Then incubate the microplate at  **$+37^{\circ}\text{C}$  for 60 min.**
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100  $\mu\text{l}$  Enzyme Conjugate. Check that the reagent has been correctly added. Then incubate the microplate at  **$+37^{\circ}\text{C}$  for 60 min.**

**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<sub>2</sub>O<sub>2</sub> 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 min**.

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

7. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. 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 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.
3. The use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL does not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 ul of it, possibly in duplicate.

**N. ASSAY SCHEME**

Controls/Calibrator	100 ul
Samples	100 ul
<b>1<sup>st</sup> incubation</b>	<b>60 min</b>
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
<b>2<sup>nd</sup> incubation</b>	<b>60 min</b>
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H <sub>2</sub> O <sub>2</sub> mix	100 ul
<b>3<sup>rd</sup> incubation</b>	<b>20 min</b>
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme (including CAL) 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 PC = Positive Control S = Sample

**O. INTERNAL QUALITY CONTROL**

A check is performed on the negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first time, in order to verify whether the expected OD<sub>450nm</sub> / 620-630nm or Co/S values have been matched in the analysis. Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD <sub>450nm</sub> value
Negative Control (NC)	> 1.000 OD <sub>450nm</sub> after blanking If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD <sub>450 nm</sub> < NC/10
Calibrator (CAL)	PC ≤ OD <sub>450nm</sub> < (NC+PC)/5

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

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

Problem	Check
Blank well > 0.100 OD <sub>450nm</sub>	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD <sub>450nm</sub> 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.

<b>Calibrator</b> OD450nm Outside the range	<ol style="list-style-type: none"> <li>1. that the procedure has been correctly performed;</li> <li>2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of Calibrator);</li> <li>3. that the washing procedure and the washer settings are as validated in the pre qualification study;</li> <li>4. that no external contamination of the calibrator has occurred.</li> </ol>
<b>Positive Control</b> OD450nm > NC/10	<ol style="list-style-type: none"> <li>1. that the procedure has been correctly performed;</li> <li>2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control).</li> <li>3. that the washing procedure and the washer settings are as validated in the pre qualification study;</li> <li>4. that no external contamination of the positive control has occurred.</li> </ol>

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 8.*

**P. RESULTS**

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

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

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

**Q. INTERPRETATION OF RESULTS**

Results are interpreted as ratio between the cut-off value and the sample OD450nm / 620-630nm or Co/S. Results are interpreted 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 HDV.

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

A positive result is indicative of 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. *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.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 8).

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

*Negative Control: 2.100 – 2.200 – 2.000 OD450nm*

*Mean Value: 2.100 OD450nm*

*Higher than 1.000 – Accepted*

*Positive Control: 0.100 OD450nm*

*Lower than NC/10 – Accepted*

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

*Calibrator: 0.300-0.260 OD450nm*

*Mean value: 0.280 OD450nm*

*Within the range PC ≤ OD450nm < (NC+PC)/5 – Accepted*

*Sample 1: 0.020 OD450nm*

*Sample 2: 1.900 OD450nm*

*Sample 1 Co/S > 1.1                      positive*

*Sample 2 Co/S < 0.9                      negative*

**R. PERFORMANCES**

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:**

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA .

The table below reports the OD450nm shown by this preparation when diluted in Fetal Calf Serum to prepare a limiting dilution curve, in three different lots.

**Co/S values**

Accurun # 127	DAB.CE	Lot # 1102	DAB.CE	Lot # 0103	DAB.CE	Lot # 0403
	OD450 nm	Co/S value	OD450 nm	Co/S value	OD450 nm	Co/S value
1x	0.171	3.0	0.163	2.9	0.156	2.8
2x	0.187	2.7	0.176	2.6	0.179	2.5
4x	0.230	2.2	0.220	2.1	0.202	2.2
8x	0.298	1.7	0.285	1.6	0.271	1.6
16x	0.417	1.2	0.405	1.1	0.402	1.1
32x	0.514	1.0	0.490	0.9	0.482	0.9
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	////////	2.261	////////	2.114	////////

**2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY**

The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit.

Negative, positive and potentially interfering samples were examined in the trial.

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.

Results are briefly reported in the tables below:

Sensitivity	> 98 %
Specificity	> 98 %

### 3. PRECISION

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

#### DAB.CE: lot #1102

##### Negative Control (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	2.342	2.428	2.433	2.401
Std.Deviation	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

##### Calibrator (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	0.298	0.289	0.286	0.291
Std.Deviation	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/S	1.6	1.7	1.7	1.7

#### DAB.CE: lot #0103

##### Negative Control (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	2.208	2.237	2.246	2.230
Std.Deviation	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

##### Calibrator (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	0.269	0.277	0.266	0.271
Std.Deviation	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/S	1.7	1.7	1.7	1.7

#### DAB.CE: lot # 0403

##### Negative Control (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

##### Calibrator (N = 16)

Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average value
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/S	1.6	1.7	1.6	1.6

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 8.*

### S. LIMITATIONS

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 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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