DRG® Human Delta Virus (HDV) Ab ELISA (EIA-4269)

Revised 16 Sept. 2010 rm (Vers. 3.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

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

For Research Use Only.

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 polyclonal 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

COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8 x 12 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 -
1 x 2.0 ml/vial. Ready to use.
Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.1% Kathon GC as preservatives.
The negative control is colour coded pale yellow

3. Positive Control: CONTROL +
1 x 2.0 ml/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 0.1% Kathon GC 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.

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Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label

5. Wash buffer concentrate: WASHBUF 20X
1 x 60 ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme conjugate: CONJ
1 x 16 ml/vial. Ready-to-use solution.
Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated polyclonal antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. The component is colour coded

7. Chromogen/Substrate: SUBS TMB
1 x 16 ml/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_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H_2SO_4 O.3 M
1 x 15 ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (Xi R36/38; S2/26/30)

Plate sealers n° 2

Instructions for Use n° 1

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 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.
6. Calibrated ELISA microwell reader with 450nm (reading) and if possible with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

SPECIMEN: PREPARATION AND RECOMMENDATIONS

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 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°C - 8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be 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 0.2-0.8 µ filters to clean up the sample for testing.

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 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 lyophilised 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.*

5. **Wash buffer concentrate:**
   The whole content of the 20x concentrated solution has to be diluted with bidistilled 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 ashing 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 (Xi R36/38; S2/26/30)
   Legenda: R 36/38 = Irritating to eyes and skin. S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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 +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 and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of +5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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.

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°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 found in the validation of the instrument for its use with the kit.
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.

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. Pipette 100 µl of Negative Control in triplicate, 100 µl of Calibrator in duplicate, 100 µl Positive Control in single and then 100 µl of samples.
   Check that controls/calibrator and samples have been correctly added.
   Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section 9.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate.
   Check that the reagent has been correctly added.
   Then incubate the microplate at **+37°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_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 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 9.5 using a 450nm filter (reading) and if possible a 620-630nm filter (background subtraction), blanking the instrument on A1.

   Important notes:
   1. *If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. 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.*

ASSAY SCHEME

Controls/Calibrator Samples	100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Washing step	4-5 cycles
Enzyme Conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Washing step	4-5 cycles
TMB/H2O2 mix	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm & 620nm

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 PC = Positive Control S = Sample

RESULTS

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

Cut-Off = (NC + 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.*

REFERENCES / Literature

1. Engvall E. and Perlmann P.. J.Immunochemistry 8: 871-874, 1971
2. Engvall E. and Perlmann P.. J.Immunol.. 109: 129-135, 1971
3. Chaggar K. Et al.. Journal of Virological Methods. 32: 193-199, 1991
4. Lazinski D.W. et al.. Journal of Virol.. 67: 2672-2680, 1993
5. Govindarajan S. et al.. Microbiol. And Immunol.. 95: 140-141, 1990
6. Shattock A.G. et al.. J.Clin.Microbiol.. 29: 1873-1876, 1991
7. Forbes B.A. et al.. Clin.Microbiol.News.. 13: 52-54, 1991
8. Bergmann, K. et al. J.Immunol. 143:3714-3721, 1989
9. Bergmann, K. et al. J.Infect.Dis. 154:702-706, 1986
10. Buti, M. et al. Hepatology 8:1125-1129, 1988
11. Rizzetto, M. Hepatology 3729-737, 1983
12. Rizzetto, M. et al. Proc.Natl.Acad.Sci. USA 77:6124-6128, 1980
13. Dubois, F. et al. J.Clin.Microbiol. 26:1339-1342, 1988
14. Wang, K. et al. Nature 323:508-514, 1986