DRG® HDV Ag (EIA-4268)

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

Third generation Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis Delta Virus or HDV in human plasma and sera.

For Research Use only.

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.

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 -**
   1 x 2.0 ml/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 0.1% Kathon GC as preservatives.
   The negative control is color coded pale yellow.

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

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 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, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.
   The component is red color coded.

7. **Chromogen/Substrate**: **SUBS TMB**
   1 x 16 ml/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 H2O2.
   Note: To be stored protected from light as sensitive to strong illumination.

8. **Specimen Diluent**: **DILSPE**
   1 x 16 ml.
   Contains a solution of 6% NP40, 0.1% Kathon GC 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**
   1 x 15 ml/vial.
   It contains 0.3 M H_2SO_4 solution.
   Attention: Irritant (Xi R36/38; S2/26/30)

10. **Plate sealers**: n° 2

11. **Instructions for Use**: n° 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150 µl, 100 µl and 50 µl) 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 possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

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°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. **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 customer service.
   Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°C - 8°C. After first opening, remaining 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:**
   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.*
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. To be stored frozen in aliquots at –20°C.*
5. **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 C- 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. **Specimen Diluent**
   Ready to use reagent. Mix gently and avoid foaming.
9. **Sulphuric Acid:**
   Ready to use. Mix well on vortex before use.
   Attention: Irritant (Xi R36/38; S2/26/30)
   Legenda: R 36/38 = Irritating to eyes and skin. S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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 EL ISA 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 EL ISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 350 µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ± 5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620 - 630 nm) for blanking purposes Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

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 "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 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.

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 found in the validation of the instrument for its use with the kit.
7. Check that the EL ISA 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 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 µl of the Calibrator in duplicate and then
   100 µl of the Positive Control in single followed by
   100 µl 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 9.3).
8. Dispense 100 µl 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 9.3).
11. Pipette then 100 µl 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 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm 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 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.*

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ASSAY SCHEME

• **Controls & Calibrator** • **Samples**	• **100 µl** • **100 µl**
• Specimen Diluent	• 100 µl
• **1st incubation**	• 120 min
• Temperature	• + 37 °C
• Washing steps	• n° 4-5
• Enzyme Conjugate	• 100 µl
• **2nd incubation**	• 60 min
• Temperature	• + 37 °C
• Washing steps	• n° 4-5
• Chromogen/Substrate	• 100 µl
• **3rd incubation**	• 20 min
• Temperature	• room
• Sulphuric Acid	• 100 µl
• Reading OD	• 450nm

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

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 OD_{450nm} + 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.*

The Performance Evaluation study conducted in a qualified external reference center on more than 300 samples has

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