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IVD | See external label | 2°C – 8°C | 96 tests | REF 1868-12

ENZYME-LINKED IMMUNOASSAYS FOR

AccuDiag™ HDV-Ag

HEPATITIS D VIRUS ANTIGEN ELISA KIT
Two-step Incubation, Double Antibody Sandwich Principle

Cat # 1868-12

Test	**HDV Ag ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Indirect ELISA : Antigen Coated Plate**
Detection Range	**Qualitative: Positive & Negative Control**
Sample	**50ul**
Specificity	**100%**
Sensitivity	**100%**
Total Time	**~ 75 min**
Shelf Life	**12-14 Months from the manufacturing date**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The purpose of the HDV Ag ELISA Test is for clinical lab diagnosis of patients who are suspected of having a hepatitis D (HDV-Ag) virus infection, and for epidemic analysis. The test has 100% sensitivity and specificity. The HDV Ag ELISA is an enzyme-linked immunosorbent assay for the qualitative determination of hepatitis D antigens in human serum/plasma.

SUMMARY

What causes Hepatitis D virus is an agent known as Delta agent (a defective 36nm-43nm RNA virus) which puts it into the classification of Hepatitis delta virus. Hepatitis D can only reproduce when it is linked with Hepatitis B virus (HBV). The disease is transmitted through the skin or through sexual contact with infected blood. Generally, the relationship of HDV and HBV involves some of the most acute and chronic HBsAg carriers. Because of this co-infection, it is important to know whether the HDV and HBV developed at the same time, or if the patient was already a carrier of HBsAg. The co-infection, especially super-infected HDV, can develop into severe acute hepatitis disease. Chronically infected HBV patients infected with HDV have a 70-80% chance of developing cirrhosis of the liver.

Serologically, it is important to know the specific HDV antibodies (anti-HDV) or antigens (HDV-Ag) to give an accurate diagnosis. Six to eight weeks after exposure and co-infection, identifiable concentrations of HDV specific antigens appear. They clear up during the convalescence stage and, together with heightened levels of HDV antibodies, indicate recovery. However, circulating HDV-Ag is a signal that an acute infection is present, and it is present only transiently at very low levels. During super infection with HDV, identifiable levels of HDV antigens are present two weeks after exposure. Chronic, long-term hepatitis develops if, after this two-week period, the infection fails to clear up.

PRINCIPLE OF THE ASSAY

The HDV-Ag ELISA employs the solid phase, two-step incubation double antibody sandwich method. The patient's serum/plasma is added together with extraction solution after the polystyrene microwell strips are pre-coated with purified antibodies specific to HDV. If the HDV virus is present, the HDV particles are disrupted and what's captured in the wells is the specific HDV antigens. At this stage, unbound serum proteins must be washed off the microwells. What is added next is Horseradish Peroxidase (HRP) which is conjugated with a secondary antibody. Again, after washing, unbound conjugates are removed. Added to the wells after this are both the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. During this stage of a combined presence of antibody-antigen-antibody (HRP) sandwich immunocomplex, a blue-colored product appears, which is the result of colorless chromogens hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antigen in the sample. Colorless wells appear when samples are negative for HDV antigens.

Assay principle scheme:
Double antibody sandwich ELISA

Ab(p)+Ag(s)→[Ab(p)–Ag(s)+ENZ]→[Ab(p)–Ag(s)–ENZ]→blue→yellow (+)
Ab(p) →[Ab(p) +ENZ]→[Ab(p)]→ No Color (-)

Incubation 1	Incubation 2	Immobilized Complex	Coloring	Results
30 min	30 min.		10min.	

Ab(p)–pre-coated anti–HDV antibodies;

Ag(s)–HDV antigens in sample;

ENZ–HRP conjugated anti-HDV

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1plate

Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant.
8×12/12×8-well strips per plate. Each well contains purified antibodies reactive to HDV.
The microwell strips can be broken to be used separately.
Place unused wells in the plastic sealable storage bag together with the desiccant and return to 2~8°C.

- **NEGATIVE CONTROL** 1vial

Blue liquid filled in a vial with green screw cap.
0.5 ml per vial
Protein-stabilized buffer tested non-reactive for HDV-Ag.
Preservatives: 0.1% ProClin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **POSITIVE CONTROL** 1vial

Red liquid filled in a vial with red screw cap.
0.5 ml per vial
Purified HDV-Ag diluted in protein-stabilized buffer.
Preservatives: 0.1% ProClin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **HRP-CONJUGATE REAGENT** 1vial

Red liquid in a white vial with red / orange screw cap.
12 ml per vial
Horseradish peroxidase-conjugated anti-HDV antibodies.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **EXTRACTION SOLUTION** 1vial

Blue solution filled in a white vial with black/brown screw cap.
6ml per vial
Chaotropic agent for disruption of the viruses during incubation.
Once open, stable for one month at 2-8°C.

- **STOCK WASH BUFFER** 1bottle

Colorless liquid.
50ml per bottle
PH 7.4 20× PBS (Containing Tween-20 as a detergent).
DILUTE BEFORE USE The concentrate must be diluted

1 to 20 with distilled/deionized water before use. Once diluted ,stable for one week at room temperature or for two weeks when stored at 2-8°C.

- **CHROMOGEN SOLUTION A** 1vial

Colorless liquid filled in a white vial with green screw cap.
7 ml per vial
Urea peroxide solution. Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **CHROMOGEN SOLUTION B** 1vial

Colorless liquid filled in a brown vial with brown/black screw cap.
TMB solution. Tetramethylbenzidine dissolved in citric acid.
7 ml per vial
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **STOP SOLUTION** 1vial

Colorless liquid filled in a white vial with white / yellow screw cap.
7 ml per vial
Diluted sulfuric acid solution (2.0M H_2SO_4).
Ready to use as supplied.

- **PLATIC SEALABLE BAG** 1unit

For enclosing the strips not in use.

- CARDBOARD PLATE COVER 2sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

- **PACKAGE INSERTS** 1copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

SPECIMEN COLLECTION TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they can give false results in the assay. Do not heat inactivated samples. This can cause sample deterioraration.

2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Avoid multiple freeze-thaw cycles.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution..

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8℃ , **do not freeze**. To assure maximum performance of this HDV-Ag ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** IVD

FOR PROFESSIONAL USE ONLY

The ELISA assay is time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and samples to reach room temperature(18-30℃) before use. Shake reagent gently before use. Return at 2-8℃ immediately after use.
4. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
5. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside

the wells.

6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all the wells.
8. Calibrate the pipette frequently to assure the accuracy. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations. Never pipette solutions by mouth.
9. The use of automatic pipettes and disposable tips is recommended.
10. Assure that the incubation temperature is 37℃ inside the incubator.
11. When adding samples avoid touching the well's bottom with the pipette tip.
12. When measuring with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
13. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
14. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium h ypochlorite for 30 minutes to decontaminate before any further steps for disposal.
15. The Stop Solution contains 2M H_2SO_4. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
16. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

ASSAY PROCEDURE

Step1 Reagents preparation: Allow the reagents to reach room temperature **(18-30℃)** . Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither samples nor HRP-Conjugate should be added into the Blank well). Use only number of strips required for the test.

Step3 Adding Sample: Add **50 µl** of Positive control, Negative control, and specimen into their respective wells. Add 50µl of Extraction Solution supplied with the kit to each well. Then mix slightly. Note: **Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control and Extraction Solution as to avoid cross-contamination. Never add Extraction Solution to the Blank Well.**

Step4 Incubating: Cover the plate with the plate cover and incubate for **30 minutes at 37℃** . It is recommended to use water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step5 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the strips plate onto blotting paper or clean towel, and tap out any remainders.

Step6 Adding HRP-Conjugate: Add **100 µl** of HRP-Conjugate Reagent into each well except the blank, and mix gently. **Never add HRP-Conjugate to the Blank Well.**

Step7 Incubating: Cover the plate with the plate cover and incubate the plate for **30 minutes at 37℃.**

Step8 Washing: Wash the plate **5 times** with wash buffer as **step 5.**

Step9 Coloring: Dispense **50 µl** of Chromogen A and **50 µl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Incubate the plate at **37℃ for 10 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the

HRP-Conjugate produces blue color in Positive control and HDV-Ag Positive sample wells.

Step10 Stopping Reaction: Using a multichannel pipette or manually add **50 µl** Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HDV-Ag Positive sample wells.

Step11 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **5** minutes after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on Dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of Cut-off value (C.O.) = *Nc × 2.1

***Nc** = the mean absorbance value for three negative controls.)

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

Example:

1. Calculation of Nc:

Well No	B1	C1	D1
Negative control OD values	0.020	0.012	0.016

Nc=0.016 (Nc is lower than take 0.05 so take it as 0.05)

2. Calculation of Cut-off value (C.O.) =0.05x2.1=0.105

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded, and the mean value is calculated again using the remaining two values. If more than one control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range

1. The absorbance of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The absorbance value OD of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The absorbance value OD of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1)： Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis D virus specific antigens have been detected with this HDV–Ag ELISA kit. Therefore, there are no indications for possible current infection with HDV.

Positive Results:(S/C.O.≥1)： Samples giving an absorbance greater than or equal to the Cut-off

value are considered initialy reactive ,which indicates that HDV virus specific antigens have been detected using this HDV–Ag ELISA kit. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples can be considered positive for antigens to HDV and therefore, there are indications for possible current infection with hepatitis D virus.

Borderline (S/CO =0.9-1.1) : Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Retesting of these samples in duplicates is recommended. Repeatedly reactive samples could be considered positive for antigens to HDV. Follow up and supplementary testing any positive with other HDV tests or analytical systems (e.g. PCR, WB) is required to establish the infection state.

TEST PERFORMANCE AND EXPECTED RESULTS

Sensitivity: The clinical sensitivity of this assay has been calculated by a panel of samples obtained from 2500 acute and chronic hepatitis B patients in which 2400 samples were found HBsAg positive. After testing with HDV RT-PCR, 150 individuals were diagnosed infected with HDV. During testing with this HDV-Ag ELISA kit, 129 of the RT-PCR confirmed HDV positive samples were found positive for HDV-Ag and 129 samples were confirmed HDV-Ag positive when tested another commercially available HDV-Ag ELISA kit. Sensitivity was determinate to be 100%.

Specificity: The clinical specificity of this assay has been evaluated by a panel of samples obtained from 500 healthy individuals. No false positive results observed, which indicates 100% specificity of the kit.

Analytical Specificity: No cross reactivity with HAV, HCV, HIV, CMV, TP and HBV were observed during clinical testing. No interference from elevated levels of rheumatoid factors up to 2000U/ml and high dose hook effect observed during clinical testing. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein. Frozen specimens have been tested to check for interferences due to collection and storage.

Reproducibility		**Within Run**		**Between Run**	
Specimen Type	No	Mean OD	CV%	Mean OD	CV%
Weak positive	10	0.257	9.0%	0.205	9.5%
Moderate positive	10	1.09	7.0%	9.531	7.5%
Strong positive	10	2.3	4.2%	2.264	4.4%
Positive control	10	2.157	4.0%	2.012	4.1%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HDV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay's predictive values.

VALIDITY

Please do not use this kit beyond the expiration indicated on the kit box and reagent labels

REFERENCES:

1. Purcell RH and Gerin JL, Hepatitis Delta virus. In: Fields Virology,3rd ed. Philadelphia,Lippincott-Raven,1996.
2. Hadziyannis SJ. Hepatitis delta: an overview. In: Rizzetto M, Purcel RH, Gerin JL, and Verme G,eds. Viral hepatitis and liver disease, Turin, Edizoni Minerva medica, 1997
3. Lai MCC. The molecular biology of hepatitis Delta virus. Annual Review of Biochemistry, 1995 64:259-286
4. Centers for Disease Control and Prevention. Epidemiology and Prevention of Viral Hepatitis A to E: An Overview 2000.
5. Hepatitis Delta: WHO/CDS/CSR/NCS 2001.1

SUMMARY OF THE ASSAY PROCEDURE:	
Add sample	**50µl**
Add Extraction solution	**50µl**
Incubate	**30minutes**
Wash	**5times**
Add HRP-Conjugate	**100µl**
Incubate	**30minutes**
Wash	**5times**
Coloring	**50µl A + 50µl B**
Stop the reaction	**50µl stop solution**
Read the absorbance	**450nm or 450/630 nm**

Date Adopted	Reference No.
2008-01-05	DA-HDV-Ag-2009

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ISO 13485-2003

Revision B Date: 08-27-2013