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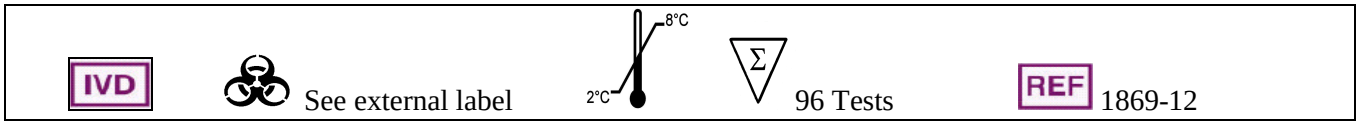

AccuDiag™ HDV IgM ELISA

Cat # 1869-12

Test	HDV IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA: Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	100ul Serum
Specificity	100%
Sensitivity	100%
Total Time	~ 75 min
Shelf Life	12 -18 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 IgM ELISA Test is for clinical lab diagnosis and treatment of patients who are suspected of having a hepatitis D virus infection. The HDV IgM ELISA test is an enzyme-linked immunosorbent assay for qualitative identification of IgM-class antibodies to hepatitis D virus 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. The first antibody to appear at the acute stage is IgM anti-HDV, which is transient and quickly replaced by IgG anti-HDV. If the IgM anti-HDV infection persists, a chronic HDV infection develops and continues to reproduce. Through the course of HBV-HDV co-infection, and after the tenth week of exposure, identifiable concentrations of HDV antibodies appear. If cleared up during convalescence, then evidence of recovery is shown. An important marker for diagnosis and post treatment of patients during early infection stage is the serological detection of IgM class antibodies. Early recovery during HDV co-and-acute super infection is shown by decreasing or low titers of IgM; chronic carrier stage is signaled by elevated levels of IgM.

PRINCIPLE OF THE ASSAY

The HDV IgM ELISA employs the solid phase, two-step incubation, antibody capture assay. Antibodies directed to human IgM (anti-u chain) are pre-coated on the polystyrene microwell strips. During the first incubation stage, and after the patient's serum or plasma is diluted, any IgM antibodies present will be captured in the wells. Next, all other components of the sample are washed out, especially any IgG antibodies. What becomes visible after adding purified HDV antigens conjugated to horseradish peroxidase (HRP), is the distinct HDV IgM captured on the solid phase. In the course of the second incubation stage, conjugated antigens will individually react solely with the specific HDV IgM antibodies. Washing the wells to remove unbound conjugates is important at this juncture so that the conjugated antigens can individually react only with the specific HDV IgM antibodies. At this point, when (anti-u)-(HDV-IgM)-(HDV antigen-HRP) immunocomplex is present, 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 antibody in the sample. Colorless wells appear when samples are negative for HDV-IgM.

Assay principle scheme: Antibody Capture ELISA

Ab(p)+IgM(s)→[Ab(p)–IgM(s)+(Ag)ENZ]→[Ab(p)–IgM(s)–(Ag)ENZ]→blue→yellow (+)

Ab(p) →[Ab(p) + (Ag)ENZ] → [Ab(p)] → no color (-)

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

Ab(p)–precoated anti-IgM antibodies (anti-μ chain);
IgM(s) – HDV IgM antibodies in sample;
(Ag)ENZ– HRP conjugated HDV antigens,

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 anti-IgM antibodies (anti-μ chain).
  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℃.
- **NEGATIVE CONTROL** 1vial
  Yellowish liquid filled in vial with green screw cap 0.5 ml per vial
  Protein- stabilized buffer tested
  non-reactive for HDV IgM.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied. Once open, stable for one month at 2-8℃.
- **POSITIVE CONTROL** 1vial
  Red liquid filled in a vial with red screw cap. 0.5 ml per vial
  Purified anti-HDV IgM antibodies 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 filled in a white vial with red screw cap. 12 ml per vial.
  Horseradish peroxidase-conjugated HDV antigens.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
- **SPECIMEN DILUENT** 1vial
  Blue liquid filled in a white vial with blue crew cap. 12ml per vial.
  Protein buffer solution.
  Preservatives: 0.1% ProClin 300
- **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℃.
- **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 black vial with black screw cap. 7 ml per vial.
  TMB solution. Tetramethylbenzidine dissolved in citric acid.
  Ready to use as supplied. Once open, stable for one month at 2-8℃.
- **STOP SOLUTION** 1vial
  Colorless liquid filled in a white vial with white screw cap. 7 ml per vial
  Diluted sulfuric acid solution (2.0M H_2SO_4). Ready to use as supplied.

- **PLASTIC 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**

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℃.
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.
11. Normal saline solution for dilution of the samples.

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 deterioration.

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.

3. **Sample preparation:** Each sample must be diluted 1:10 with normal saline.

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°C, do not freeze. To assure maximum performance of this HDV-IgM 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 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 from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that the reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry data stated on labels or boxes.
3. Allow the reagents and samples to reach room temperature(18-30℃) before use. Shake reagent gentl y 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 wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. 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, do not touch 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 not less than 2 hours at 121℃ or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal.
15. The Stop Solution contains 2M H2SO4. 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 and 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°C). 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 stock Wash buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer. Mark three wells as Negative control **(e.g. B1, C1, D1)**, two wells as 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

Step2 Specimen Dilution: Before adding, dilute each specimen 10 times with normal saline.

Step3 Adding Samples: Dispense **100 µl** of **Sample diluent** provided with the kit into each well except ' in the Positive, Negative controls and Blank wells. Add **10 µl** of saline diluted samples into each well and **100 µl** controls into their respective wells. Mix by tapping the plate gently. **(Note: to avoid cross-contamination use a separate disposable pipette tip for each specimen, Negative Control or Positive Control.)**

Step4 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C. 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 3 times with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the last washing cycle, turn down the strips plate onto blotting paper or clean towel, and tap the plate to remove any remainders.

Step6 Adding HRP-Conjugate: Add 100µl of HRP-Conjugate Reagent into each well except for the blank.

Step7 Incubating: Cover the plate with the plate cover and incubate for 30 min at 37°C (as Step 4).

Step8 Washing: Aspirate the liquid and rinse each well 5 times with wash buffer (as step 5).

Step9 Coloring: Add 50µl (or one drop) of Chromogen A and 50µl (or one drop) Chromogen B solution into each well including the Blank and mix gently. Incubate the plate at 37°C for 10 minutes avoiding light. The enzymatic reaction between Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HDV-IgM 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 IgM 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 CONTROL

Each microplate should be considered separately when calculating and interpreting the results, 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 <u>(C.O.) = *Nc × 2.1</u>

***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 controls OD value	0.02	0.012	0.016

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

2. Calculation of Cut-off (C.O.)= 0.05 x 2.1=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 ran

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 IgM class antibodies to HDV have been detected with this HDV IgM ELISA kit. Therefore, there are no indications for possible current infection with hepatitis D virus.

Positive Results (S/C.O.≥1)： Samples giving an absorbance greater than or equal to Cut-off value are considered initially reactive, which indicates that IgM class antibodies to HDV have probably been detected with this HDV IgM ELISA kit. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples can be considered positive for IgM antibodies to HDV. 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 IgM antibodies to HDV. Follow up and supplementary testing of and positive sample 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 hepatitis B acute and chronic 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-IgM ELISA kit, 107 of the HDV RT-PCR confirmed positive samples were found positive for HDV-IgM and 107 samples were confirmed HDV-IgM positive when tested with another commercially available HDV-IgM ELISA kit. Sensitivity 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 test.

Analytical specificity:

1. No interferences have been observed when testing patients with other HDV-unrelated clinical conditions like HIV, HCV, HAV, TP.
2. No interference was observed from rheumatoid factors up to 2000U/ml.
3. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

Reproducibility		Within Run		Between Run	
Specimen Type	**Runs**	**Mean OD**	**CV%**	**Mean OD**	**CV%**
Weak positive	10	0.257	8.1%	0.221	8.3%
Moderate positive	10	0.978	7.3%	0.904	7.5%
Strong positive	10	1.856	4.6%	1.782	4.7%
Positive control	10	1.948	4.2%	1.901	4.3%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of the ELISA assays. The test is design to achieve performance characteristics of high sensitivity and specificity. However, in very rare cases some HDV mutants or subtypes can remain undetectable. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive result should 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 label.

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 Centers for Disease Control and Prevention. Epidemiology and Prevention of Viral Hepatitis A to E: An Overview 2000.Hepatitis Delta: WHO/CDS/CSR/NCS 2001.1

SUMMARY OF THE ASSAY PROCEDURE:	
Dilute sample with normal saline	1:10
Add sample diluent	100µl
Add sample	10µl
Add controls	100µl
Incubate	30 minutes
Wash	3 times
Add HRP-Conjugate	100µl
Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	10 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/630nm

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:	
Microwell plate	One/ 96 well
Negative/Positive control	One each/ 0.5ml
HRP-Conjugate	One/ 12ml
Sample diluent	One/ 12ml
Wash Buffer	One/ 50ml
Chromogen A/B/Stop Solution	One each/7ml
Note: the components of individual kits are not interchangeable	

Date Adopted	Reference No.
2005-02-18	DA- AccuDiag™ HDV IgM ELISA-2012

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