HDV IgG ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAHDG1	HDV IgG ELISA	96 Tests

Intended Use:

The HDV IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG antibody to hepatitis D virus (HDV) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients with infection with hepatitis D virus.

Summary and Principle:

Hepatitis D is a liver diseases caused by Hepatitis D virus – a defective (36 nm – 43 nm) enveloped RNA virus, which requires co-infection with Hepatitis B virus (HBV) for its replication. Transmitted percutaneously or sexually through contact with infected blood or blood products, HDV is associated with the most severe forms of chronic and acute hepatitis in many Hepatitis B – HBsAg positive patients. Since the infection with HDV requires infection with HBV, the development of the disease depends on whether the two viruses infect simultaneously (coinfection) or whether the newly infected HDV patient is also a chronic HBV carrier (superinfection). The co-infection with HDV can lead to severe acute hepatitis disease with low risk of chronic stage development. Chronic HBV carrier patients superinfected with HDV are at risk of developing chronic HDV disease, which can lead to cirrhosis in 70%-80% of patients. The serological diagnosis of HDV is based on detection of specific HDV antibodies (anti-HDV) or antigens. Anti-HDV IgG antibodies are indicator of past or current delta infection. High titres of the antibody may be indicative of chronic or active infection. During HBV-HDV co-infection, detectable concentrations of anti-HDV IgG appear after the tenth week of exposure to the viruses and clearance during convalescence indicates recovery. During HDV superinfection, detectable levels of antibodies appear four to six weeks after exposure and failure to clear indicate possible progression to long, chronic carrier stage.
The HDV-IgG ELISA is based on a solid phase, two-step incubation method. The microwells are pre-coated with recombinant HDV antigens. Unknown samples are added to wells with a diluent and during the first incubation step, any anti-HDV antibodies present in the sample will bind to the coated HDV antigens. After washing, conjugate is added which is contains horseradish peroxidase conjugated to anti-human IgG antibody. During the second incubation, the HRP-conjugated anti-IgG will bind specifically to immobilised antigen-HDV-IgG complexes and label them. After washing to remove unbound Conjugate, Substrate solutions are added to the wells and the HRP on the immunocomplexes catalyses the hydrolysis of the substrate to a blue-coloured product. The reaction is stopped by the addition of acid Stop Solution which also turns the blue colour to yellow. The colour intensity can be measured spectrophotometrically and is proportional to the concentration of HDV-IgG in the sample. Wells containing samples negative for HDV IgG remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12 x 8 well plate)	Microwells containing HDV antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag provided together with the desiccant and return to 2 - 8°C. Once open, stable for one month at 2 - 8°C.
Negative Control Preserv.0.1% PoClin™300	1.0ml	Blue-coloured liquid. Protein-stabilized buffer tested non reactive for IgG antibody to HDV. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.
Positive Control Preserv.0.1% PoClin™300	1.0ml	Red-coloured liquid. Anti-HDV IgG antibody diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.
Conjugate Preserv.0.1% PoClin™300	12ml	Red-coloured liquid. HRP conjugated anti-human IgG antibody. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.
Sample Diluent	12ml	Green-coloured liquid. Serum base, casein, and sucrose solution. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.
Wash Buffer (20X) Detergent Tween-20	40ml	Colourless liquid. PH 7.4, 20 x PBS. 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.
Substrate Solution A	6ml	Colourless liquid. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.
Substrate Solution B	6ml	Colourless liquid. TMB (Tetramethyl benzidine) solution. Ready to use as supplied. Once open, stable for one month at 2 - 8°C
Stop Solution	6ml	Colourless liquid. 0.5M sulphuric acid solution. Ready to use as supplied. Once open, stable for one month at 2 - 8°C.

Sealable plastic bag, IFU and plate covers.

Materials required but not provided:

Distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, pipettes, disposable pipette tips, absorbent tissue, dry incubator or water bath, plate reader (single wavelength 450nm or dual wavelength 450/630nm), microplate wash system.

Sample Collection:

Collect serum and EDTA, sodium citrate or heparin plasma by separation after standard venipuncture technique. For serum, the blood must be allowed to clot fully but be separated as soon as possible as to avoid haemolysis of the RBC. Any visible particulate matter in the serum or plasma should be removed by centrifugation. Use only serum or plasma sample type.
Samples will be stable for analysis up to 7 days at 2 - 8°C or stored at -20°C.
Do not use samples with high content of haemoglobin, bilirubin or triglyceride as these substances can interfere in the ELISA.

Storage and Stability:

The components of the kit will remain stable until the expiry date indicated on the kit when stored at 2 -8°C. Do not freeze. To assure maximum performance of this kit protect the reagents from contamination with microorganism and chemicals.

Precautions and Safety:

1. Correct ELISA results are dependent on incubation time and temperature. To avoid false results, follow the test procedure exactly as specified.
2. Do not mix reagents from different lots or substitute reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance.
3. Make sure that all reagents are from the same lot and all within expiry. Do not use reagents beyond expiry.
4. IMPORTANT: Allow the reagents and samples to reach room temperature (15 - 25°C) before use. Shake reagents gently before use. Return all components, tightly sealed, to 2 - 8°C immediately after use.
5. Use different pipette tip for each sample and reagent in order to avoid cross-contamination. Only touch the pipette tip against the side of the well, never the bottom of the well.
6. Make sure timing of reagent additions are the same for all wells and for each procedure step.
7. Do not touch the underside of the wells; fingerprints or scratches may interfere with the Absorbance. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
8. Never allow the microplate wells to dry out between wash steps. Avoid the formation of air bubbles when adding the reagents.
9. Ensure that the temperature of the incubator is at 37°C.
10. The enzyme activity of the HRP-conjugate is affected by dust and strong chemicals including sodium hypochlorite, acids and alkalis. Do not let these substances contaminate the Conjugate reagent.
11. If using fully automated equipment do not cover the plate with a plate cover during incubation. The tapping out of well contents after washing, can also be omitted.
12. All samples from human origin should be considered as potentially infectious. Strict adherence to Good Laboratory Practice regulations will ensure personal safety.
13. WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These raw materials have been tested with approved kits and proved negative for and HBsAg and antibodies to HIV 1/2, HCV and TP. However, since no analytical method that can completely assure the absence of infectious agents handle reagents and samples as if potentially infectious. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are sourced from BSE/TSE free-geographical areas.
14. Pipette tips, vials, well strips and sample containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request. Reagents must be disposed of only in accordance with local or national regulations
15. Some reagent components may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Avoid contact of all reagents with skin and the mucosa but especially the Stop Solution, Substrate reagents and the Wash Buffer.
16. The Stop Solution contains sulphuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.
17. Proclin 300 is used as preservative and can cause a reaction on the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.
18. Indicators of deterioration of the reagents: The values of Positive and Negative controls fall out of the quality control range may indicate reagent instability or operator or equipment error. Where quality controls are out of range the results should be considered invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige Diagnostics representative.

Procedure:

Reagent preparation: Bring all reagents and samples up to room temperature (15-25°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 Wash buffer (20X) by adding the contents of the Wash Buffer bottle to 760 ml of distilled or deionized water. Alternatively add concentrate to distilled water in the ratio 1/20. All other reagents are supplied ready to use.

STEP 1

Preparation: Reserve 2 wells for Negative Control, 2 wells for Positive Control and one Blank (e.g. A1 – taking care that neither HRP conjugate nor any samples are added to the blank well). Note: If results are read using a plate reader having dual wavelength (450 / 600-650nm) then a Blank well need not be used. Assign samples to wells.

STEP 2

Addition of Diluent: Add 100 µl of Sample Diluent to each well except the Blank.

STEP 3

Addition of Sample: Add 10 µl of Positive control, Negative control, and samples to their assigned wells except the Blank. Mix by tapping the side of the plate gently.

STEP 4

Incubation: Cover the plate with the plate cover and incubate with shaking for 30 minutes at 37°C.

STEP 5

Wash Step: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

Addition of Conjugate: Add 100 µl of Conjugate to each well except the Blank.

STEP 7

Incubation: Cover the plate with the plate cover and incubate with shaking for 20 minutes at 37°C.

STEP 8

Wash Step: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 9

Addition of Substrate: Add 50 µl of Substrate Solution A and 50 µl of Substrate Solution B solutions to each well including the Blank. Incubate the plate at 37°C for 10 minutes in the dark.

STEP 10

Stopping the Reaction: Add 50 µl of Stop Solution to each well and mix gently until the blue colour changes completely to yellow.

STEP 11

Measurement: Calibrate the plate reader with the Blank well and read the absorbance of all wells within 10 minutes after stopping the reaction at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results.

Instruction for Washing:

To remove the potential for poor washing to cause false positive results and a high background, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. Assure that the microplate washer is adequately maintained. Ensure the liquid dispensing channels are not blocked or contaminated and that correct volume of wash buffer is dispensed each time into the wells.
If plates have to be washed manually, a process of 5 washing cycles, dispensing 350-400ul/well, allowing 60 second soak and aspirating the liquid 5 times is recommended. If poor results are observed with high background, increase the number of washing cycles and soak time per well. Capture all liquid waste aspirated and treat with 2.5% sodium hypochlorite for 24 hours before being disposed of in the appropriate way.

Quality Control and Calculation of the Results:

Each plate should be processed separately when calculating and interpreting the results of the assay, regardless of the number of plates assayed concurrently. The results are calculated by relating each sample absorbance (Abs) to the cut off value (CO) of the plate. If the reading is based on a single filter plate reader, the results are to be calculated by subtracting the Blank well Abs from the absorbances of the samples and the controls. If the results are taken from a dual filter plate reader, do not subtract the Blank well Abs value from the sample and control absorbances, just use the value obtained.

Calculation of the Cut-off value (CO) = Nc + 0.15
(Nc = the mean absorbance value for the Negative Control).

Assay Validation: The assay results are only valid if the following Quality Control criteria are met.
- The Abs value of the Blank well, which contains only Substrate and Stop solution, is < 0.080 at 450 nm.
- The Abs values of the Positive Control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The Abs values of the Negative Control must be < 0.100 at 450/630nm or at 450nm after blanking.

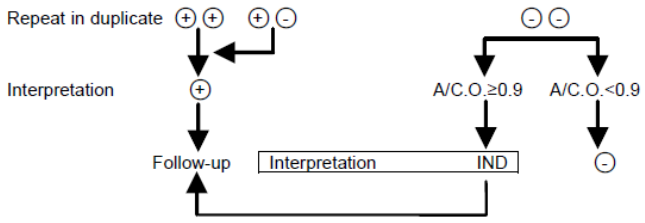
If one of the Negative Control Abs values does not meet the Quality Control criteria, it should be ignored, and the mean taken as the other Negative Control value. If both Negative Control Abs fail the Quality Control specifications, the whole run is invalid and must be repeated.

Example:
1. Assay Validation
Blank well Abs: A1= 0.025 at 450nm
Negative control Abs after blanking: 0.020 0.016
Positive control Abs after blanking: 2.125 2.235
All control values are within the stated quality control range
2. Calculation of Nc: = $\frac{(0.020 + 0.016)}{2}$ = 0.018
3. Calculation of the Cut-off: (CO) = 0.018 + 0.15 = 0.168

Interpretation of the Results:

Negative Results (Abs/CO < 1): Specimens giving Abs value less than the Cut-off value are negative for this assay, which indicates that no IgG antibodies have been detected with this HDV IgG ELISA kit, therefore there are no serological indications for infection with HDV.
Positive Results (Abs/CO ≥ 1): Specimens giving Abs value equal to or greater than the Cut-off value are considered initially reactive, which indicates that IgG antibodies to HDV have probably been detected with this HDV-IgG ELISA kit. Retesting in duplicate of any initially reactive sample is recommended. Repeatedly reactive samples should be considered positive for IgG antibodies to HDV and therefore the patient is probably infected with HDV.
Borderline (Abs/CO = 0.9 - 1.1): Specimens with Abs value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.
Follow up confirmation and supplementary testing of any positive sample with another analytical system such as PCR is required. Clinical diagnosis should not be established using this test as the sole source of data but integrate confirmatory test results and other clinical information.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP
ALL INITIALY REACTIVE OR BORDERLINE SAMPLES



IND = non interpretable

If, after re-testing of initially reactive samples, both wells show negative results (Abs/CO < 0.9), these samples should be considered negative and the original result must be classified as false positive. False positive results may occur due to several reasons, often associated with, but not limited to inadequate washing step.

- If after retesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive samples can be considered positive for anti-HDV IgG and therefore the patient is probably infected with HDV.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance Characteristics:

Clinical Sensitivity

The clinical sensitivity of this assay was determined using a set of samples obtained from 2500 hepatitis B acute and chronic patients of which 2400 samples were tested HBsAg positive. After testing these with an HDV RT-PCR kit, 150 samples were confirmed as co-infected with HDV. Using the Prestige Diagnostics HDV-IgG ELISA kit, 60 of the HDV positive samples tested positive for HDV-IgG and 60 samples were also found HDV-IgG positive when tested with a predicate HDV IgG ELISA kit. Clinical Sensitivity was therefore 100%.

Clinical Specificity

The clinical specificity of the HDV IgG ELISA kit was determined using a set of samples obtained from 500 healthy donors, confirmed negative for HDV. No false positive results were found which indicates 100% specificity of the ELISA.

Limitations:

1. Follow up confirmation and supplementary testing of any positive sample with another analytical system such as PCR is required. Clinical diagnosis should not be established using this test as the sole source of data but integrate confirmatory test results and other clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with this HDV-IgG ELISA are only indication that the sample does not contain detectable level of IgG antibodies to HDV and any negative result should not be considered as conclusive evidence that the individual is not infected with HDV.
3. Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
4. This kit is intended only for testing of serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
5. This kit is a qualitative assay and the results cannot be used to measure antigen concentration.

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. Centres for Disease Control and Prevention. Epidemiology and Prevention of Viral Hepatitis A to E: An Overview 2000.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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