Laboratory Procedure Manual

Analyte: Hepatitis D Antibody
Matrix: Serum
Method: ETI-AB-DELTAK-2
DiaSorin (REF p2808)
Method No.:

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As performed by: *Assay Development and Diagnostic Reference Laboratory*
Laboratory Branch
Division of Viral Hepatitis
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

Contact: *Saleem Kamili, PhD (+1-404-639-4431); sek6@cdc.gov*

Important Information for Users

The National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP) periodically refines this laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, has been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HEPBD_H	LBDHD	Hepatitis D antibody

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The method for qualitative anti-HD determination is a simultaneous competitive assay. Anti-HD present in the sample and labeled anti-HD antibodies complete for a fixed quantity of HDAg bound to the solid phase. The quantity of enzyme tracer bound to the solid phase and consequently the enzyme activity are inversely proportional to the anti-HD concentration present in samples or controls.

Enzyme activity is measured by adding a colorless chromogen/substrate solution. The enzyme action on chromogen/substrate produces a color which is measured with a photometer.

The Delta antigen/antibody system (HDAg/Anti-HD) is related to HBV infection but immunologically distinct from its known reactivities; it is the expression of the Delta virus (HDV.Hepatitis D Virus), cause of severe liver disease in HBsAg carriers.

HDV is a 35-37nm particle containing low molecular weight RNA and HDAg, with an outer coat of HBsAg obtained from HBV. HDV is a defective virus and its replication requires helper functions provided by HBV.

HDAg has been detected in liver and in serum and induces a specific antibody response (anti-HD antibodies) both IgG and IgM class.

2. SAFETY PRECAUTIONS:

Handle with care chromogen, substrate and blocking reagent. Avoid chromogen, substrate and blocking reagent coming into contact with oxidizing agents of metallic surface

Do not eat, drink smoke or apply cosmetics in the assay laboratory

Do not pipette solutions by mouth.

Avoid direct contact with all potentially infectious materials by using articles such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of assay.

Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.

All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each Country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Liquid waste containing acid must be

neutralized before treatment. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 200, p. 2143). A minimum of one hour at 121°C is usually considered adequate though the user must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators. Blocking reagent (Council Directive 99/45/EC): Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Consider all serum specimens for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of infectious contamination hazards. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished. Biosafety Level 2 containment and practice as described in CDC/NIH publication #88-8395 are recommended for handling test specimens and kit reagents.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Raw data are transcribed manually from instrument readout sheets into a computerized database. This database was custom-designed for the management of CDC Assay Development and Diagnostic Reference Laboratory (ADDRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. Test values are compared with a cutoff value calculated from controls. Results are expressed as "positive" or "negative" for anti-HDV. Other information in the database may typically include the ADDRL identification number, the specimen number, the date collected, the date tested, and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer.

b. Finished data are reviewed by the laboratory supervisor and transmitted to the NCHS along with the other NHANES IV data.

c. Files stored on the CDC LAN are automatically backed up nightly by CDC Data Center staff.

d. Documentation for data system maintenance is contained in hard copies of data records for 2 years.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

Either human serum or plasma may be used. The anticoagulants citrate, EDTA and heparin have been tested and may be used with the assay. Borderline or low-positive results obtained from EDTA-plasma specimens should be evaluated with care. Blood should be collected aseptically by venipuncture, and allowed to clot, and the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity,

lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing.

Grossly hemolyzed or lipemia samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. If the assay is performed within 48 hours of sample collection, the samples should be kept at 2-8ºC; otherwise they should be aliquoted and stored deep-frozen (-20ºC or below). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles.

Samples containing sodium azide should not be assayed.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Required Materials not Provided
--Vertical reading photometer with the following instrument specifications wavelength: dual wavelength, 450nm and 620-630nm bandwidth: < 10nm. Absorbance range: 0 absorbance units to > 2.5 absorbance units repeatability: better than or equal to 0.005 absorbance units, or 1 % whichever is greater. Drift less than 0.005 absorbance units per hour.
-- Thermostatically-controlled humid chamber with the following specifications: temperature: 37ºC+/- 1ºC
-- Manual or automatic equipment for rinsing wells with the following instrument specifications. Volume dispensed: 300-370ul number of wash cycles: 5 . soak time: 30 seconds. Aspirate the last aliquot of dispensed liquid: yes.
-- Micropipettes with disposable tips (50, 100ul) (50ul: trueness +/-3%, precision 2% 100ul: trueness +/- 2%, precision 1%)
--Glassware
-- Distilled water.

b. Materials Provided
--- 2 precut cardboard sealers suitable for 1 to 12 strips
---2 cardboard sealers suitable for 12 strips (one plate)
--- pouch sealer
---Coated strips 12-8 well strips
---Enzyme tracer 1 vial. 0.5 ml
---Negative Control 1 vial. 2ml
---Positive Control 1 vial. 2ml
---Tracer diluent 1 vial. 17.5 ml
---Wash Buffer 1 vial. 40 ml
---Chromogen 1 vial. 9 ml
---Substrate 1 vial. 9ml

---Blocking reagent 1 vial. 30ml
---Number of tests 96

c. Reagent Preparation

Reagents provided in the kit

1) Coated strips: Wells are coated with biotinylated anti-HD IgG (human) and recombinant HDAg. Ready to use, the strips should be kept at 2-8ºC. Bring the coated strips to room temperature before opening the pouch to avoid development of condensed water in the wells. Place unused strips in the pouch, securely reseal and store at 2-8ºC.

2) Enzyme Tracer (conjugate): The vial contains 0.5 ml human anti-HD Fab fragments conjugated to horseradish peroxidase (HRP), phosphate buffer, BSA stabilizers and preservatives. The solution should be diluted 1:50 with tracer diluents (c.5) (e.g., 100ul tracer + 4.9 ml diluents). Prepare only amount of working enzyme tracer needed for the run and keep the concentrated enzyme tracer at 2-8ºC. The working enzyme tracer can be stored for one week at 2-8ºC after preparation.

3) Negative Control: The vial contains 2ml human serum/plasma non-reactive for anti-HD and preservatives. Ready to use, the reagent should be stored at 2-8ºC up to expiration date.

4) Positive Control: The vial contains 2ml human anti-HD antibodies, PBS buffer and preservatives. Ready to use, the reagents should be stored at 2-8ºC.

5) Tracer diluents: The vial contains 17.5ml human serum/plasma, PBS buffer, newborn calf serum, and preservatives. Ready to use, the reagent should be stored at 2-8ºC up to the expiration date. The solution is used to dilute the enzyme tracer (c.2).

6) Wash buffer (25x): The vial contains 40ml PBS buffer, Tween 20 and preservatives. Dilute the vial contents to one liter with distilled water and store for one week at 2-8ºC. The reagent is used to rinse wells. If crystallization occurs at 2-8ºC, warm the wash buffer to 37ºC and mix well before diluting.

7) Chromogen: The vial contains 9ml tetramethylbenzidine derivative in buffer solution. Mix the solution 1:1 with substrate (c.8) (e.g., 1 ml chromogen + 1 ml substrate). After dilution chromogen/substrate can be stored for 8 hours at room temperature, away from the light.

8) Substrate: The vial contains 9ml buffer solution containing H_2O_2. The solution should be mixed 1:1 with chromogen (c.7).

9) Blocking reagent: The vial contains 30ml 1N sulfuric acid (R 36/38, S 26). Ready to use, the reagent should be stored at 2-8ºC up to expiration date.

Storage of reagents:
Upon receipt, store all reagents at 2-8ºC, away from intense light. Do not freeze. Once opened the reagents of this kit are stable for eight weeks when properly stored, unless otherwise stated. The kit is stable for one week when used throughout the day for eight hours at room temperature and stored overnight at 2-8ºC.

Reagents should not be used past the expiration date. The expiration date of the kit is reported on the external label. The expiration date of each component is reported on the respective vial label.

d. Standards Preparation

This method does not involve the use of conventional calibrators or standards.

e. Preparation of Quality Control Material

(1) Kit positive and negative controls are prepared and quality controlled by the manufacturer.

(2) In-house controls are prepared according to ADDRL specifications.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

This method does not involve the use of conventional calibrators or standards. Calibration is based on the results of defined "positive" and "negative" controls.

Positive and negative control reagents are supplied with each test kit. The assay cutoff value is automatically calculated from values obtained from these controls and entered in the DMS by the analyst.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

Bring reagents to room temperature (20-25°C) before assaying. Perform all assay steps without stopping. Number sufficient strips to run 3 negative controls, 2 positive controls and unknowns in singlicate. Samples and controls should be subjected to the same process and incubation time. Prepare one blank well containing chromogen/ substrate only. A disposable tip should be used for dispensing each sample and control.

b. Sample Preparation

(1) Bring serum specimens to 20-25°C. Serum samples may stratify when frozen or stored at 4-8°C for extended periods. Mix them gently before testing.

(2) Identify the reaction tray wells for each specimen or control.

c. Instrument Procedure

Adjust the thermostatically-controlled humid chamber to 37º+/- 1ºC/

1. Dispense 50ul negative control and positive control I into their respective wells.
2. Dispense 100ul diluted enzyme tracer into all wells except for the blank well
3. Apply a cardboard sealer in order to prevent evaporation.
4. Incubate for three hours +/- 15min at 37ºC) +/1ºC.
5. Prepare chromogen/substrate just before the end of incubation.
6. When incubation has been completed, discard the cardboard sealer and rinse the strips. Use a suitable automatic or semiautomatic washer for washing strips. Aspirate the liquid and rinse each well five times with a volume of wash buffer ranging from 0.30 to 0.37 ml. Avoid overflow from the reaction wells. When either an automatic or semiautomatic washer is used, the soak time between each rinse should be 30 seconds.
7. Dispense 100ul chromogen/substrate solution into wells.
8. Incubate for 30+/- 2 min at room temperature, away from intense light.
9. Dispense 100ul blocking reagent into all wells in the same order and at the same rate as for chromogen/substrate.
10. Measure the absorbance of specimens with a photometer at 450/630nm within one hour of adding the block reagent. When dedicated equipment is used, absorbance values are provided automatically after selecting the suitable protocol. When another vertical reading photometer is used, blank the instrument with the blank, record the absorbance at 450/630nm for each specimen and subtract the 630nm absorbance value from 450nm absorbance value.

d. Recording of Data

Raw optical density values for each specimen are manually entered by the analyst into the Data Management System (DMS), where the data are processed and interpreted according to the cutoff value calculated automatically by the DMS based on the formula provided in the Instructions for Use.

e. Calculations of results

The cut-off value is determined by adding the mean absorbance for negative control values (NCx) multiplied by 0.5 to the mean absorbance for the positive controls values (PCx) multiplied by 0.5.

Cut-off value = 0.5 NCx + 0.5 PCx

f. Interpretation of results

The presence or absence of anti-HD is determined by comparing the absorbance of the unknown samples to that of the cut-off value.
The unknown samples with the absorbance values less than or equal to the cut-off value should be considered reactive for anti-HD. The unknown samples with absorbance values greater than the cut-off value should be considered non-reactive.

Samples with absorbance values within +/- 10% of cut-off value must be retested in order to confirm the initial result. Samples which are repeatedly reactive should be considered positive. Samples which are non-reactive at the second test should be considered negative.

g. Replacement and Periodic Maintenance of Key Components

(1) Instruments are on service contract and except for the most basic daily maintenance are serviced by a technical representative.

Laboratory personnel monitor and document refrigerator temperature, freezer temperature, and room temperature on a daily basis

(2) All micropipettors used in testing clinical specimens are calibrated every 6 months. Pipettors that do not conform to specifications are autoclaved and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

9. REPORTABLE RANGE OF RESULTS

Final results are expressed qualitatively as positive or negative for the presence of anti-Hepatitis D antibody in the sample. No quantitative results are determined.

10. QUALITY CONTROL (QC) PROCEDURES

Always validate quality control with the following steps when evaluating results.

The mean negative control absorbance value must be greater than or equal to 0.600 NCx > 0.600.

The mean positive control absorbance value must be less than or equal to 0.080 PCx < 0.080.

The difference between the mean negative control absorbance value and the mean positive control absorbance value (NCx – PCx) must be greater than or equal to 0.500 NCx – PCx > 0.500.

If not, the run is invalid and must be repeated.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

a. If controls do not conform to specifications, reject the results and reanalyze all samples. Do not use data from non-qualifying test runs.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Bacterial contamination, repeated freezes-thaw cycles or heat inactivation of the specimens may affect the absorbance values of the samples with consequence alteration of IgM anti-HD levels.

13. REFERENCE RANGES (NORMAL VALUES)

A normal human serum should be negative for hepatitis D antibodies.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25°C during preparation and testing for 4 hours.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other tests for total anti-Hepatitis D antibody may be substituted but must be accompanied by validation data to show substantial equivalence with these assays. Test methods may not be substituted without approval from NCHS.

Alternative methods of storage are not recommended. In case of system failure, samples should be refrigerated at 4-8°C for no more than 5 days. For longer periods, the specimens should be stored at -20°C until the system is functioning properly.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database (Section 3) to track specimens.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data. For NHANES, residual specimens are stored frozen and returned to the NCHS specimen bank after testing for each cycle has been completed.

19. Summary Statistics and QC graphs

Qualitative assays are assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

REFERENCES

None