Laboratory Procedure Manual

Analyte: **Hepatitis D Antibody**

Matrix: **Serum**

Method: Anti-HDV IgG WES

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As performed by: **Diagnostic Reference Team**
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Important Information for Users

The National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention periodically refines these 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, have 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_K	LBDHD	Hepatitis D antibody

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical relevance:

Hepatitis is inflammation of the liver most often caused by a virus. Viral hepatitis is a major public health problem of global importance because of the ongoing transmission of viruses that cause the disease and increased morbidity and mortality associated with the acute and chronic consequences of these infections. Global and US goals have been established for elimination of viral hepatitis as a public health threat by 2030.

In the US, the most common types of viral hepatitis are hepatitis A, B, and C. Effective vaccines are available to help prevent hepatitis A and hepatitis B. No vaccine is available for hepatitis C; however, highly effective, well-tolerated treatment can cure hepatitis C virus infection. Hepatitis D virus infection is less common in the US and can occur only among persons with hepatitis B virus infection. Hepatitis E infection also is less common in the US. These five hepatitis viruses, also called hepatitides, are well-characterized for detection with laboratory assays and are monitored in U.S. public health surveillance systems.

NHANES viral hepatitis data are used to monitor progress toward goals in *Healthy People* and the HHS *Viral Hepatitis National Strategic Plan*, which in turn support US and global viral hepatitis elimination goals. The viral hepatitis laboratory and interview components of NHANES complement data from outbreaks, case-based surveillance, vital statistics, health care systems, and cohort studies that can provide timely, detailed, or longitudinal information for subnational geographic areas and disproportionately affected populations, such as persons experiencing homelessness or living in correctional facilities; however, these sources lack information available from NHANES, such as race, ethnicity, education, income, and health status and behavior.

Viral hepatitis data from NHANES are available beginning with the Second NHANES conducted during 1976-1980 for hepatitis A and hepatitis B, and with the Third NHANES conducted during 1988-1994 for hepatitis C, hepatitis D and hepatitis E.

An estimated 300 million people worldwide are persistent carriers of hepatitis B virus (HBV). Infection with HBV results in a wide spectrum of acute and chronic liver diseases that may lead to cirrhosis and hepatocellular carcinoma. Co-infection with hepatitis D virus (HDV) in persons with acute or chronic hepatitis B virus (HBV) infection can lead to fulminant hepatitis.

Transmission of HBV occurs by percutaneous exposure to blood products and contaminated instruments, sexual contact and perinatally from HBV-infected mothers to their unborn child.

HBV infection produces an array of unique antigens and antibody responses that, in general, follow distinct serological patterns.

Hepatitis B surface antigen (HBsAg), derived from the viral envelope, is the first antigen to appear following infection and can be detected serologically as an aid in the laboratory diagnosis of acute HBV infection.

Anti-HBc is detectable shortly after the appearance of hepatitis B surface antigen (HBsAg). As the appearance of anti-HBsAg may be delayed after HBsAg clearance, anti-HBc is sometimes the only serological marker for HBV infection and potentially infectious blood. Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection.

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), a 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) in both the IgG and IgM classes.
Test principle:

The NHANES viral hepatitis laboratory component tests for anti-HBc, HBsAg among anti-HBc positive specimens, and anti-HDV among HBsAg positive specimens.

Examined participants aged 6 years and older in the NHANES 2019-March 2020 sample were eligible for the anti-HBc, HBsAg, and anti-HDV tests.

Test principle:

Hepatitis D antibody is measured using the Anti-HDV IgG WES Assay. The method for qualitative anti-HDV determination is a solid phase direct immunoassay.

HDV antibodies in the serum/plasma are captured to the cross-linked HDV antigen bound to the capillary, and a signal is produced from the HRP-labeled secondary antibody. The signal is measured and digitally recorded after an 8-second exposure.

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 surfaces.

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.

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

The Data Management System (DMS) was used through December 31, 2019.

The run information can be uploaded into the computerized database (DMS) after the run information is exported by the software. This database was custom-designed for the management of CDC Division of Viral Hepatitis (DVH) Laboratory Branch (LB) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. In August 2019, laboratory data management was transferred to the CDC Enterprise Laboratory Information System (ELIMS), where NHANES functionality was reproduced and improved over time to include more process automation. DMS was maintained in parallel through December 31, 2019, when it was discontinued. Finished DMS data were reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. Files stored on the CDC Local Area Network (LAN) were automatically backed up nightly by CDC Data Center staff. Documentation for data system maintenance was maintained with printed copies of data records for 2 years.

CDC Enterprise Laboratory Information System (ELIMS) is has been used since January 1, 2020, for accessioning, test results processing, reporting and storage. Finished ELIMS data are reviewed by the laboratory supervisor and transmitted to the NCHS along with other NHANES data. All information about the accessioned specimens, traceability of the diagnostic process, test runs and reported results are stored in the ELIMS database, are archived after 12 months and can be retrieved any time upon request.

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.

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

- Glassware
- Molecular Grade Water
- WES/JESS Detection Module
- WES/JESS 12-230 kDa Separation Module
- HDV Antigen
- IgG Secondary Antibodies
- 70% Ethanol
- 96-well plate
- Pipettes
- Sterile barrier tips

b. Materials Provided
  a. Antibody Diluent 2 (AD2)
  b. DTT
  c. Fluorescent 5X Master Mix
  d. 10X Sample Buffer
  e. Biotinylated Ladder

Storage of reagents:

Upon receipt, store all reagents at 2-8ºC (36–46°F), away from intense light. Do not freeze.

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

Provided with the kit.

e. Preparation of Quality Control Material

(1) Negative human serum

(2) Hepatitis D positive human serum

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

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

The negative control needs to be negative, with no band detected at 57-59 kDa. The positive control sample needs to be positive, with a 57-59 kDa band present.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

Samples and controls should be subjected to the same process and incubation time.

b. Sample Preparation

(1) Bring serum specimens to 20-25°C (68–77°F). Serum samples may stratify when frozen or stored at 4-8°C (39–46 °F) for extended periods. Mix them gently before testing.

c. Instrument Procedure

Before testing, thaw up to 22 samples plus anti-HDV positive and negative controls.

Start boiling 500 m of water in a 2 L beaker on a hot plate.

Dilute the samples 1:50 in Antibody Diluent 2 (AD2).

Vortex each sample.

Dispense 49 µl of AD2 into 24 wells of the 96 well plate.

Add 1 µl of the first serum sample to the first well. Using a new pipette tip, add 1µl of the next serum sample to the next well, etc. If there are less than 24 samples run, use AD2 in empty wells.

Once all samples have been diluted in AD2, gently shake the plate and place it on the bench while all other reagents are prepared.

Prepare the reagents as described below.

Remove three tubes from the EZ Standard Pack.

- DTT (Clear Tube)- Gently pierce the foil seal with a pipette tip and add 40 µl of molecular grade water to make a 400 mM solution. Gently pipette to mix.
- Fluorescent 5X Master Mix (Pink Tube)- Gently pierce the foil seal with a pipette tip, add 20 µl of 10x Sample Buffer and 20 µl of prepared 400 mM DTT solution. Gently pipette to mix.
- Biotinylated Ladder (Green Tube with Pink Pellet)- Gently pierce the foil seal with a pipette tip and add 20 µl of molecular grade water. Gently pipette to mix.

Prepare a 0.1X dilution of 10X sample buffer. Add 99 µl of molecular grade water and 1 µl of 10x Sample Buffer to a centrifuge tube. Gently vortex to mix.

Prepare Antigen: HDV-234-A. In a centrifuge tube, mix 79 µl of 0.1x Sample Buffer, 1 µl of HDV antigen and 20 µl of 5x Fluorescent Master Mix. Close centrifuge tube and gently vortex to mix.

Denature by heating at 95°C (203°F) for 5 minutes, open lid to let steam escape, close centrifuge tube, briefly centrifuge and then store on ice.

Preparation of goat anti-human secondary antibody:

In a centrifuge tube, mix 499 µl of AD2 with 1 µl of goat anti-human secondary antibody. Gently vortex to mix and store on ice.

Preparation of Luminol Peroxide: In a centrifuge tube, mix 200 µl of Luminol-S and 200 µl of Peroxide; gently pipette to mix and store on ice.

Preparation of ProteinSimple WES Microplate: Carefully peel back top half of foil, holding top l eft corner of bottom half of foil to prevent from peeling.

Add 10 µl of AD2 to each well of Row B, and the first well of Row C.

Add 10 µl of the first serum sample dilution to the second well of Row C, discard pipette tip and use a new one to add 10 µl of the next serum sample to the next well; repeat for all prepared serum samples and the anti-HDV positive and negative control dilutions.

Place 10 µl of Streptavidin-HRP in the first well of Row D.

Place 10 µl of prepared goat anti-human secondary antibody dilution in each of the remaining wells of Row D.

Place 15 µl of prepared Luminol Peroxide into each well of Row E.

Place 3 µl of prepared HDV antigen in each of the remaining wells of Row A.

In the first well of Row A, place 5 µl of the prepared Biotinylated Ladder.

Leave all wells of Row F empty.

Place 500 µl of Wash Buffer in the first 15 large wells below Row F.

Place reusable plate cover on the microplate, and centrifuge at 2500 RPM for 5 minutes. Balance with a new unused WES microplate with cover.

After centrifuging the plate, carefully peel back the foil at the bottom of the microplate. Pop any bubbles in the solution using a clean pipette tip.

Start WES run:

Turn on WES Instrument.

On the connected computer, open Compass Software.

Select File → Start New Run.

Ensure parameters are set for the size 25 plate, at 2-230 kD.

Lightly tap metallic sensor at the top of the machine to open the door.

Insert capillary cartridge into the cartridge holder; once it is properly inserted, the light will change colors from orange to blue.

Place microplate in the plate holder.

Gently close door and press “Start” in the Compass Program.

Once the assay is complete, change image exposure to 8 seconds.

Navigate to the “Scan” tab and identify samples that fall within the acceptable range for positivity (57-59 kDa) by panning over each lane using the computer mouse.

Right click in empty space towards the right of the scan and select “Copy.”

Select “.jpg” and click “Save.” Select desired folder and save the scanned image to the computer.

d. Recording of Data

The Data Management System (DMS) was used through December 31, 2019.

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.

CDC Enterprise Laboratory Information System (ELIMS) has been used since January 1, 2020.

Raw optical density values for each specimen are automatically transferred from the EISA reader to the ELIMS using a Data Collection Unit (DCU), where the data are processed and interpreted according to the cutoff value calculated automatically by the ELIMS based on the formula provided in the Instructions for Use.

e. Calculations of results

No calculations are needed.

f. Interpretation of results

The presence or absence of anti-HD is determined by detection of a band in the range of 57-59 kDa.

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 daily.

(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.

Test Limit of Detection

The lowest amount of anti-HDV that can be detected with the VITROS Anti-HDV Qualitative test was determined by serial dilution of a strong positive specimen. 1:256 dilution of a positive sample achieved 100% hit rate.

10. QUALITY CONTROL (QC) PROCEDURES

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

Negative control should have no bands in the 57-59 kDa range.

Positive control should have a single band in the range of 57-59 kDa.

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

There are no known interfering substances, and none were tested.

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 (68–77 °F) 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 (39–46 °F) for no more than 5 days. For longer periods, the specimens should be stored at -20°C (-4 °F) 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.

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