Laboratory Procedure Manual

Analyte: **Hepatitis D Antibody**

Matrix: **Serum**

Method: **Anti-HDV Total International Immunodiagnostics (IID)**

First Published: **September 2025**

Revised:

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

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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	CLIA Approved
HEPBD_L	LBDHD	Hepatitis D antibody (anti-HDV)	Yes CDC-10626 (Previously listed as CDC-10328)

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, 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 people 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 outbreak, 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), but may 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 in combination with HBsAg is found in acute and chronic hepatitis B patients and, when detected alone, 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.

Hepatitis D, also known as “delta hepatitis”, only occurs in people who are also infected with the hepatitis B virus. This test is used in the diagnosis of hepatitis delta virus (HDV) infection in hepatitis B virus (HBV) infected individuals. This test will be used to diagnose serologic marker of HDV infection, anti-HDV. The test will be applied for individuals positive for hepatitis B surface antigen (HBsAg).

Certain geographic areas and populations have high prevalence of HDV infections. Global prevalence of HDV infection based on detection of anti-HDV is estimated to be 4.5%-15.6% of all chronic HBV-infected individuals. Prevalence within the Unites States is estimated to be between 1.2-1.6% and 5.0%-7.2% among HBsAg-positive individuals.

Examined participants aged 6 years and older in the NHANES 2021-2023 were eligible for aHDV testing.

Test principle:

The International Immunodiagnostics HDV Ab (IID HDV Ab) assay is a competitive enzyme immunoassay (EIA) for the determination of antibodies to Hepatitis D Virus in human serum with a “one-step” methodology. Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with Horseradish peroxidase (HRP), for a fixed amount of recombinant HDV protein coated on the microplate. The concentration of the bound enzyme on the solid phase is inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by adding the chromogen/substrate in the second incubation.

2. SAFETY PRECAUTIONS

Universal safety precautions should always be followed. Appropriate PPE will be worn during testing; this includes protective eyewear, lab coat, and gloves eQMS # PLAN-000001-DVHLB and USRMAN-000001-DVHLB.
Handle all specimens as if potentially infectious. Follow established safety and material handling procedures for a Biosafety Level 2 (BSL-2) in the Site-Specific Bloodborne Pathogen Program eQMS # PLAN-000001-DVHLB. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Keep these bags in in autoclave metallic bins according to CDC regulations and guidelines.
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.

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.

Handle with care ELISA kit components: recombinant HDV -specific antigen coated strips, controls, calibrators, wash buffer, enzyme conjugate, chromogen/Substrate and Stop solution. Do not expose the Chromogen/Substrate to strong light, oxidizing agents and metallic surfaces. Handle with precaution the reagents as they contain 0.1% Kalhon GC as preservatives. Avoid contact with skin and eyes. Wear suitable gloves.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

CDC Enterprise Laboratory Information Management System (ELIMS) has been used since January 1st, 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. All necessary information about ELIMS at CDC is maintained on an intranet site accessible by CDC laboratory personnel.

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

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum

Specimens Not Recommended

- Do not use turbid (visibly hyper lipemic-milky) and hemolyzed specimens. These specimens could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
- Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.

Specimen Collection and Preparation

- Collect specimens using standard procedures.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.
- The Anti-HDV Total test uses 100 µL of sample for each determination. If the test shows positive by initial screening, it is repeated in duplicate to confirm. Therefore, a minimum of 400ul sample is needed.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- Return to 2–8 °C (36–46 °F) as soon as possible after use
- Acceptable specimen conditions determined during the method validation: Serum stored for up to 5 days at 2–8 °C (36–46 °F) or stored below -20 °C (-4 °F) or stored at room temperature for not more than 8 hours
- Testing is acceptable in specimens subjected to up to 3 freeze/thaw cycles as determined by stability studies conducted as part of the method validation and approved by CDC CLIA office.

  Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29-A. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

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

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION

a. Instrumentation and Software

Equipment	Manufacturer	Temperature Range (Operating Condition)	Humidity (Operating Condition)
BioTek EPOCH Reader DXStem	Agilent	18°C (64°F) and 40°C (104°F)	10% to 85%
BioTek 50S 8 well Microplate Washer	Agilent	15° - 40°C (59° - 104°F)	Maximum relative humidity of 80%
37°C Incubator Model 100	ThermoScientific/ Precision	15°C to 40°C (59° - 104° F)	Maximum relative humidity of 80%

Software: Gen 5

Instrument Setup

- The ELISA microplate reader must be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes.
- When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol must be Installed in the operating system of the unit and validated as the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) must be validated and correctly set. Particular attention must be paid to avoid carry-over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results.

b. Required Materials Not Provided

- Laboratory eye protection equipment- Safety glasses
- Laboratory disposable coats
- Laboratory disposable gloves
- Single-channel micropipettes
- Multichannel pipette- 12 well or 8 well
- Adjustable Spacer Multichannel Pipette-8 well
- P20 and P200 air plugged pipette tips

- Caps- Nalgene™ General Long-Term Storage Cryogenic Tubes, 2mL Catalog Number: 5011-0020 (Thermofisher Scientific)
- 15 ml and 50 ml conical tube (Glassware Cat # 96135 and 95625)
- EIA grade or Deionized water
- Centrifuge (Eppendorf 5804)
- Reagent reservoirs
- Timer
- Biosafety cabinet
- EPOCH ABS Spec Reader DXStem or equivalent
- 50TS MICROPLATE WASHER 8 WELL
- Dry incubator at 37^0C
- Evaluated Internal control
- Bleach rite or equivalent
- Absorbent towelettes

Materials Provided in the kit
HDV AB EIA kit, Catalog. #288, International Immunodiagnostics (Foster City, CA)

- Antigen coated microwell strips
- Calibrator
- Positive and negative controls
- Conjugate
- Chromogen/Substrate
- Stop Solution
- 20X Wash Buffer
- Plate sealers

c. Reagent Preparation

Antigen coated microwells; Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green. indicating a defect in manufacturing in this case, call customer service.
Unused strips must be placed back into the aluminum pouch with the desiccant supplied, firmly zipped and stored at +2-8^{0}C. When opened the first lime, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control: Ready to use. Mix well on vortex before use.

Positive Control: Ready to use. Mix well on vortex before use

Calibrator: It is a low positive control. Add precisely the volume of EIA grade water, reported on Its label. to the lyophilized powder: let fully dissolve and then gently mix on vortex and make 250ul aliquots (one time use).
Note: The dissolved calibrator is not stable, therefore, each aliquot is for single use only and must be stored at - 20^0C. Discard the tube after one time use; do not freeze again.

Wash buffer concentrate: The whole content of the 20x concentrated solution must be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles. Note: Once diluted, the wash solution is stable for 1 week at +2-8^0C.

Enzyme conjugate: Ready to use, Mix well on vortex before use.
Avoid contamination of the liquid by oxidizing chemicals, dust or microbes. If this component must be transferred, use only plastic, and if possible, sterile disposable containers.

Chromogen/Substrate: Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid by oxidizing chemicals, air driven dust or microbes Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component must be transferred use only plastic, and if possible, sterile disposable container.

Sulfuric Acid: Ready to use, Mix wen on vortex before use.
Attention: Irritant (H315. H319; P280, P302+P352, P332+P313, P305+P351+P338. P337+P313, P362+P363).

7.CALIBRATION AND CALIBRATION VERIFICATION PROCEDURE

Calibration Verification Procedure

A calibrator sample (CAL) is a low positive control sample provided with every kit. The lyophilized powder should be hydrated with the specified amount of water as stated on the calibrator vial.
Note: The volume of water to dissolve varies from lot to lot, so it is important to always check the vial for water needed to rehydrate the calibrator.
Immediately make one-time aliquot vials (250ul) and store the rest aliquots at –20C.
It will be used in every run-in duplicate. If this calibrator tests negative, the run will be repeated.
If the second run fails, the testing personnel must contact the technical supervisor and open an NCE to investigate the failed controls.

Instrument Calibration Procedure

Instrument calibration is performed according to each manufacturer's requirements and DVH SOPs. Every run includes controls (low positive (in house), and high positive and negative (manufacturer provided) controls, and calibrator (mid positive control) and every 6 months an instrument comparison is run to include a negative, low positive (in house) and high positive (manufacturer provider) control, and calibrator (mid positive control) reviewed to ensure s/co ratios are within range. Every lot change includes a run of controls (negative, low positive (in house), high positive (manufacturer provided) and calibrator (mid positive control) plus 5 known samples (positive (n=2) and negative (n=3).
Tests will not be performed on instruments that fail to meet calibration requirements.

Quality Control Steps:

Quality Control of New Kit Lots

Quality control for every new lot of kits will include testing all the controls provided with the kit plus a panel of at least five known samples: positive (n=2) and negative (n=3) samples. The results will be analyzed. Once these data are reviewed, signed by the technical supervisor and documented in the CLIA suite (under Analytical Equipment MFC and Cal- go to ELISA- then to the folder ELISA HDV Ab IID Lot Verification testing of clinical specimens can proceed.

Quality Control with Every Run

Positive control, negative control and calibrator will be used in each run as provided by the kit manufacturer and described in the tables below.
Additionally, a single replicate of well-characterized in-house low-positive control (IHC) will be included in every test run, to ensure adequate performance.

Positive Controls
Positive control is provided in the kit and is ready to use.

Control	Frequency (every run, every extraction, etc.)	Expected Value
CONTROL +	Every run, single replicate	Reactive

Negative Controls
Negative control is provided in each kit and is ready to use.

Control	Frequency (every run, every extraction, etc.)	Expected Value
CONTROL -	Every run, in triplicate	Non-reactive

Additional Controls
Calibrator (Lyophilized) is provided in each kit. It is a low-level positive control.
A well characterized, In-house control (IHC) will be included in every test run, to ensure adequate performance, as stated in the validation report.

Control	Frequency (every run, every extraction, etc.)	Expected Value
CAL	Every run, in duplicate	Reactive
IHC	Every Run, single replicate	Reactive

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Procedure

- Dissolve calibrator with EIA grade water using the exact volume stated on the label and gently mix. Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20˚C. When it is thawed do not freeze again, discard it.
- Allow all other components to reach room temperature before opening the foil bag.
- Check that the desiccant has not turned dark green. If it does, do not use the product and call customer service.
- Make wash buffer: Prepare wash buffer in the wash buffer bottle connected to the plate washer by adding the entire contents of the 20X concentrated solution to 1200 ml EIA water. Mix well. Note: Once diluted the wash buffer is stable for 1 week at 2-8˚C.
- Place the required number of strips in the microplate holder. Leave A1 well empty for the blank. Store back the other strips into the bag in presence of desiccant at 2-8℃, sealed.
- Thaw the samples and spin at 5000 rpm for 5 minutes. Arrange the sample vials according to the plate layout by accurately cross checking the box map for sample IDs. A barcode scanner is connected to the plate reader computer. Open “Notepad” on this computer and scan the CUID from each tube into the “Notepad” using the attached scanner. Save the “Notepad” file (it will be used later to import CUIDs while reading the plate). Inside the biosafety cabinet, open the sample tubes and discard the caps.
- Starting from well A2, add 100 µl of each sample to be tested into the wells. Add 100 µl of negative control 100ul into wells B1, C1 and D1; add 100 µl of calibrator into wells E1 and F1; add 100ul positive control into well G1. Cover the plate with protective film before taking out from the biosafety cabinet. Incubate the plate at 37˚C +/-2 (this range was maintained in the validation study) for 60 min.
- While the plate is incubating, go back to biosafety cabinet to re-cap the sample tubes with new caps and store at 2-8°C until the end of the experiment.
- After incubation, remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Using automated plate washer program HDV (set for 4 washes, 350ul/well and soaking time 20 sec), adjust the strip number based on the number of strips being used.
- In all wells except for A1, add 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Then incubate at 37℃ for 60 min.
- Wash the plate as described above.

- Pipette 100 µL TMB/H_2O_2 mixture into each well, the blank wells included. Check that the reagent has been correctly added. Then incubate at room temperature for 20 minutes, sheltered from light.
- Pipette 100 uL 0.4M Sulphuric acid into all the wells to stop the enzymatic reaction using the same pipetting sequence as above. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.
- Measure the color intensity of the solution in each well, using a 450 nm filter (reading) and a 620-630nm filter (background subtraction), blanking the instrument on A1. Reading should be performed immediately after the addition of the stop solution, and no than 20 minutes after adding the stop solution.
- **Calculations**
  The results are calculated by means of a cut-off value determined with the following formula:
  Cut-off = (NC+PC)/5
  with NC being the averaged OD value of the negative controls and PC being the OD value for positive control.
- In the plate reader software, go to statistics and choose Blank 450-650nm and right click “select quick export.” This will generate an Excel spreadsheet. Save the file. This file is used for uploading the results into ELIMS.

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

Interpretation of Results
Since the IID HDV Ab assay is a competitive EIA, the concentration of the bound enzyme on the solid phase is inversely proportional to the amount of anti-HDV antibodies in the sample. OD values below the cut-off value are interpreted as ”Reactive”, while OD values observed above and equal to the cut-off value are interpreted as ”Non-Reactive”. After the initial screen, only the samples which come reactive will be tested in duplicate. If the result is 2/3 reactive then the final interpretation of the sample will be reported as “Reactive”. However, if the result is 1/3 reactive then the final interpretation of the sample will be reported as “Non-Reactive”.

Reporting Results

TS/GS will review all results and approve in ELIMS as described in the ELIMS Job-Aid for User Level 3 Post Analytic NHANES and Individual Specimen Reporting (GUI-000004-DVHLB).

Laboratory Interpretative Comments & Disclaimers
Resources for interpretation of viral hepatitis markers can be found on the Health Professionals Tools website: Viral Hepatitis | Viral Hepatitis | CDC
Disclaimer: *"This test has not been cleared or approved by the FDA. The anti-HDV test used is a commercially available test that has been validated for CLIA testing in the Diagnostic Reference Team at the CDC Division of Viral Hepatitis Laboratory Branch".*

9. REPORTABLE RANGE OF RESULTS

Results are expressed qualitatively as positive or negative for the presence of IgG anti- HDV antibody in the sample based on the cut off value on the run. No quantitative results are determined.

10. QUALITY CONTROL (QC) PROCEDURES

- Do not use expired or damaged reagents.
- Check that the desiccant has not turned dark green. If the desiccant is dark green, do not use the kit because there is a manufacturing error. Call customer service.
- If any of the controls fail, repeat the run.
- If the repeat run fails, the testing personnel must contact the technical supervisor and open an NCE to investigate the failed controls.

Replacement and Periodic Maintenance of Key Components

- Instruments are on service contract and, except for the routine daily, and monthly maintenance, are serviced by an Agilent technical representative.
- Laboratory personnel monitor and document refrigerator temperature, freezer temperature, and room temperature on a daily basis.
- All micro-pipettors used in testing clinical specimens are calibrated every 6 months. Pipettors that do not conform to specifications are removed from the workflow, disinfected and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

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

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

Interfering Substances: Interference on biological assays due to hemolysis, icterus, or lipemia (HIL) could represent a significant source of analytical errors leading to inaccurate interpretation of results. According to the manufacturer of the assay, samples containing sodium azide, or are hemolyzed or hyperlipemic (milky), should not be

tested. Additional instrumental measuring of the interfering substance such as bilirubin was conducted using VITROS Microsensor Technology to automatically measure the icteric index (ICT) reflecting bilirubin, while testing samples for HBsAg- a prerequisite testing before reflecting to anti-HDV. We assessed the presence of ICT in a subset of 231 NHANES samples tested between October 22, 2021, and January 17th, 2023, and in the panel of 80 specimens prepared for this validation that required testing for HBsAg using VITROS Immuno-analyzer, equipped with Microsensor Technology. ICT <5 determined by VITROS is VITROS Microsensor quality control.
Among specimens tested in the target population and those included in this validation, median ICT was 1.9 with range 1.9 to 18.0; normal value of <5 was in 307/311 (98.7%) and elevated ICT, but still within the threshold – in 4 (1.3%) of samples. None of the specimens were flagged with levels that can potentially cause interference of the HBsAg results. Therefore, specimens were reflexed for anti-HDV testing.

13. REFERENCE RANGES (NORMAL VALUES)

The reference value for anti-HDV in a healthy, unexposed population is non-reactive.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 15-25°C (59–77 °F) during preparation and testing only.

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

Other FDA-licensed tests for total anti-HDV 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.

Course of Action if a Test system becomes inoperable

- Sample Handling

Samples received frozen at -20^{0}C or below will remain frozen until test system becomes operable, or it is determined that samples should be returned to submitter or destroyed. If samples are already thawed and the test system becomes inoperable, testing personnel inform preanalytical CLIA personnel and technical supervisor that samples will be frozen immediately at -20°C or below and the freeze thaw cycles will be documented by preanalytical CLIA personnel. Samples can be tested by IID HDV Ab EIA after a total of three freeze/thaw cycles, according to the CLIA test validation.

- Testing delay communication

Submitters will be contacted by the CLIA Preanalytical TP within 24 hours of the test system becoming inoperable and give the option of returning the sample or destroying the specimen. DVH DRT will not forward sample to another laboratory.
Turn around time is stated in CDC Test Directory (Test Directory | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC). DVH DRT cannot provide test results earlier than stated in the test directory, if the test system is inoperable for longer than turn around time.

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 ELIMS, as described in Section 3, to track specimens.

For NHANES, residual specimens are stored frozen at -20^{o}C or below, 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 in each run. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

REFERENCES

U.S. Department of Health and Human Services. Healthy People. 2022. Available from: https://health.gov/our-work/national-health-initiatives/healthy-people

U.S. Department of Health and Human Services. 2020. Viral Hepatitis National Strategic Plan for the United States: A Roadmap to Elimination (2021–2025). Washington, DC. Available from: https://www.hhs.gov/hepatitis/viral-hepatitis-national-strategic-plan/index.html

National Academies of Sciences, Engineering, and Medicine. 2017. A national strategy for the elimination of hepatitis B and C. Washington, DC: The National Academies Press. Available from: http://www.nationalacademies.org/hmd/reports/2017/national-strategy-for-the-elimination-of-hepatitis-b-and-c.aspx

Chow S-K, Atienza EE, Cook L, Prince H, Slev P, Lapé-Nixon M, Jerome KR. Comparison of Enzyme Immunoassays for Detection of Antibodies to Hepatitis D Virus in Serum. Clinical and Vaccine Immunology. 2016. 23(8):732-734.

Hollinger FB, Dienstag J. Hepatitis B and D viruses. In: Murray T, Baron E, Phaller M, Tenover F, Yolken R (eds). *Manual of Clinical Microbiology*. Washington DC: American Society for Microbiology Press, 1995:1033–1049.

Ganem D. Hepadnaviridae and their replication. In: Fields B, Knipe D, Howley P, eds. *Fields Virology*. Philadelphia: Lippencott-Raven Publishers, 1996:2703–2737.

CDC-NIH. *Biosafety in Microbiological and Biomedical Laboratories – 3rd Edition*. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C., 1993.

CLSI. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline– Third Edition. CLSI document M29-A3 (ISBN 1-56238-567-4). CLSI. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898 USA, 2005.

NCCLS. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Fifth Edition. NCCLS document H4-A5 [ISBN 1-56238-538-0]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.

NCCLS. *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline – Second Edition*. NCCLS document H18-A2 (ISBN 1-56238-388-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1999.

CLSI. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline - Third Edition. CLSI document C24-A3 [ISBN 1-56238-613-1]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2006.

NCCLS. *Interference Testing in Clinical Chemistry; Proposed Guideline*. NCCLS document EP7-P (ISBN 1-56238-020-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1986.

National Committee for Clinical Laboratory Standards. Order Code EP5-T2. (1992) *Evaluation of Precision Performance of Clinical Chemistry Devices* - Second Edition.

NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Editio*n. NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.

Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. Lancet. 2011;378(9785):73-85. doi: 10.1016/S0140-6736(10)61931-9

Chen H-Y, Shen D-T, Ji D-Z, et al. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. Gut. 2019;68(3):512-521. doi: 10.1136/gutjnl-2018-316601

Stockdale AJ, Kreuels B, Henrion MYR, et al. The global prevalence of hepatitis D virus infection: systematic review and meta-analysis. J Hepatol. 2020;73(3):523-532. doi: 10.1016/j.jhep.2020.04.008

Wasuwanich P, Striley CW, Kamili S, Teshale EH, Seaberg EC, Karnsakul W, Hepatitis D-associated hospitalizations in the United States: 2010–2018 First published: 25 January 2022; https://doi.org/10.1111/jvh.13645

Wong RJ, Yang Z, Jou JH, John BV, Lim JK, Cheung R. Hepatitis Delta Virus Testing, Prevalence, and Liver-Related Outcomes Among US Veterans With Chronic Hepatitis B. Gastro Hep Adv. 2024 Oct 25;4(3):100575. doi: 10.1016/j.gastha.2024.10.015. eCollection 2025.PMID: 39906477

Gish RG, Cohen C, Holden LR, Raymond D, Razavi H, Stiehl L, Thomas DL, Wedemeyer H, Wong RJ, Glenn JS, Terrault N. A call to reclassify the delta hepatitis virus as an orphan disease. Hepatol Commun. 2025 Jun 9;9(7):e0746. doi: 10.1097/HC9.0000000000000746. PMID: 40489760; PMCID: PMC12151022.

Stark DL, Falekun S, Jorgensen S, Slev P. Prevalence of Hepatitis D in the United States.J Appl Lab Med. 2025 Jul 8:jfaf092. doi: 10.1093/jalm/jfaf092. Online ahead of print.PMID: 40626720