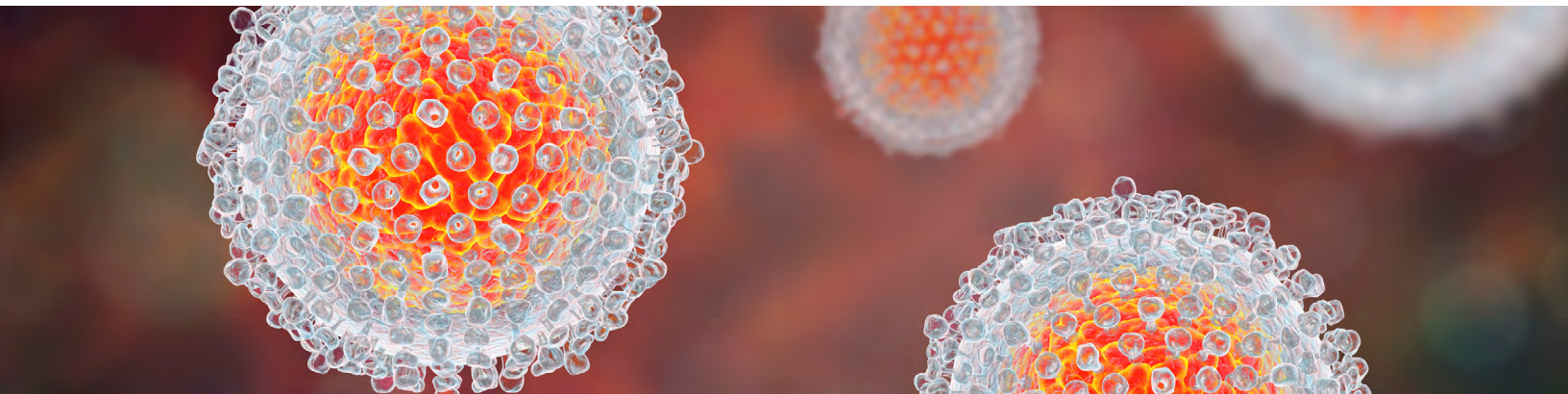
Hepatitis B and Hepatitis Delta Viruses: Best Practices for Testing, Reporting and Referral

Introduction

Hepatitis B virus (HBV) infection remains a significant global and domestic health concern. In the United States, an estimated 850,000 people are living with HBV, yet 67% remain unaware of their infection.[1] Although there is a highly effective HBV vaccine available, an estimated 70% of adults in the US were not vaccinated as of 2018.[2] In 2024, the US Centers for Disease Control and Prevention (CDC) released updated guidance recommending universal HBV vaccination in adults aged 19–59 years.[3]

HBV is transmitted through exposure to infectious blood or body fluids. Injection drug use was the most frequently reported risk factor among individuals with HBV infection, with multiple sexual partners being the next most common. Other reported exposures such as accidental needle sticks, surgical procedures, blood transfusions or household contact with someone infected with HBV were far less common. Healthcare-associated transmission of HBV in the US is now considered rare.[2] Hepatitis delta virus (HDV) is a small, circular RNA virus dependent on HBV for replication. Infection can occur as a coinfection or as a superinfection in someone already infected with HBV. HDV is transmitted through similar routes, with the highest risk observed in individuals with existing HBV infection who have bloodborne exposure.[3]

Following acute HBV infection, most healthy adults (approximately 90%) clear the virus spontaneously and develop protective immunity, whereas infants and young children have a much higher likelihood of progressing to chronic infection.[4] Chronic HBV infection is characterized by the persistence of HBV surface antigen (HBsAg) for more than six months.[5] HDV infection can occur as a co-infection with HBV or as a superinfection in someone already living with chronic HBV infection. HDV superinfection rarely resolves spontaneously and is associated with rapid progression to advanced liver disease. HDV accelerates progression to cirrhosis and increases the risk of hepatocellular carcinoma.[6] Despite this, HDV infection remains under-recognized, under-tested and inconsistently reported across the US.[7] HDV is not included in the notifiable disease list, contributing to the unknown prevalence of HDV infection in the US.



Who Should Be Tested or Screened

CDC updated its HBV screening and testing recommendations in 2023 to promote earlier diagnosis and intervention. The updated guidance recommends that all adults 18 years of age and older be screened at least once in their lifetime using a panel of HBV tests, which includes HBsAg, antibody to HBV surface antigen (anti-HBs) and total antibody to HBV core antigen (total anti-HBc). Pregnant women should be screened during every pregnancy, ideally in the first trimester, regardless of vaccination history.[2]

Beyond universal screening, additional screening is warranted for individuals with risk factors or ongoing exposures. These include persons born in regions with intermediate or high HBV prevalence; people who inject drugs; men who have sex with men; those living with HIV or hepatitis C virus; and persons with elevated serum levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) activity of unknown cause. Persons on hemodialysis or with household or sexual exposure to an HBV-infected person should also be tested. CDC additionally recommends that all healthcare personnel receive testing to confirm HBV vaccination and immunity through post-vaccination serologic testing, given the potential for occupational exposure to blood or body fluids.[8] Anti-HBs levels of >10 mIU/ml are considered seroprotective against HBV. Individuals with ongoing risk should undergo periodic retesting and anyone who requests HBV testing should receive it, regardless of risk disclosure.[2]

For HDV infection, there are varying recommendations on which HBsAg-positive individuals should be tested. Some professional organizations, such as the American Association for the Study of Liver Diseases, recommend targeted testing among individuals from HDV-endemic areas, persons who inject drugs, persons living with HIV or patients with unexpectedly severe liver disease or low HBV DNA levels.[9] Other recommendations suggest all patients with chronic HBV infection should be screened for HDV.[10,11,12] Screening for HDV infection starts with a HDV antibody test (anti-HDV) followed by HDV RNA testing for those patients testing positive for anti-HDV.[7]

Serologic Markers of HBV Infection

HBV infection is diagnosed and characterized using several key serologic markers. Their interpretation helps differentiate between acute, chronic, resolved or vaccine-induced immunity.

Table 1. Serological HBV Markers and Their Interpretations

Marker	Interpretation / Meaning
HBsAg[a] *(Hepatitis B surface antigen)*	Indicates current HBV infection, acute or chronic. Persistence for ≥ six months indicates chronic infection.
Anti-HBs *(antibody to HBsAg)*	Indicates immunity due to vaccination or recovery from natural infection in immunocompetent individuals. Levels ≥ 10 mIU/mL are considered seroprotective.
Total anti-HBc *(antibody to core antigen)*	Indicates previous or current infection (IgM + IgG).
IgM anti-HBc *(IgM antibody to core antigen)*	Indicates acute HBV infection.

a Used in HepB Vaccine. Individuals may be transiently positive for 30 days following inoculation of vaccine dose.

Mutant HBV Strains and Assay Limitations

Certain HBV variants, known as “escape mutants,” have mutations in the “a" determinant region of the HBV surface antigen that can reduce antibody binding. These mutations can produce false-negative HBsAg results in some commercial assays.[13] Most assays incorporate antibodies targeting additional epitopes to improve detection of a number of commonly occurring HBV mutants. Clinicians should verify whether the laboratory they send their samples for HBsAg testing use the appropriate assay able to detect mutant HBVs. **Table 2** provides an overview of FDA authorized assays and indicates if they claim the ability to detect various HBV mutants. In cases of suspected false negatives or discordant results, HBV DNA testing can confirm current infection.[4]

Table 2. Available Diagnostic HBsAg (Including Confirmatory) and Anti-HBs Assays

Manufacturer	Platform	HBsAg Assay	Mutant Detection	Positive HBsAg Confirmatory Testing Required	Quantitative Anti-HBs Assays (Assessment of Immunity)
Abbott	ARCHITECT	HBsAg Next Qualitative (HBsAgNx)	Yes	Required - HBsAg Next Confirmatory Assay	Architect Anti-HBs
		HBsAg Quantitative	Yes	Required - HBsAg Next Confirmatory V.1 Assay	
	Alinity i	HBsAg Next Qualitative (HBsAgNx)	Yes	Required - HBsAg Next Confirmatory Assay	Alinity Anti-HBs
		HBsAg Quantitative	Yes	Required - HBsAg Next Confirmatory V.1 Assay	
Bio-Rad	Manual	GS HBsAg EIA 3.0	Yes	Required - GS HBsAg Confirmatory Assay 3.0	MONOLISA Anti-HBs EIA
	Evolis				
Beckman Coulter	DxI 9000	Access HBsAg	Yes	Access HBsAg Confirmatory assay – Required for repeatedly reactive samples with S/CO values between 1–100	
DiaSorin	Liaison® XL/ LIAISON® XS	MUREX HBsAg	Yes	LIAISON Murex HBsAg Confirmatory Test - Required for HBsAg positive results with a S/CO of less than 140	MUREX Anti-HBs
QuidelOrtho	VITROS™	HBsAg	No	VITROS™ HBsAg Confirmatory Kit – Required for HBsAg positive results with a S/CO of less than 5.0. All positive results in pregnant women should be confirmed with supplemental testing.	Anti-HBs
Roche	Cobas e 411/601/602	Elecsys® HBsAg II	Yes	Required - Elecsys HBsAg Confirmatory Test	Elecsys® Anti-HBs II
Siemens	ADVIA Centaur	Centaur HBsAg II	Yes	Required - ADVIA Centaur HBsAg Confirmatory assay	Anti-HBs2 (AHBs2)

Typical Serologic Course

The classic serologic course of acute HBV infection involves a rise and fall of HBsAg and IgM anti-HBc. Total anti-HBc develops shortly after HBsAg and persists, while anti-HBs develops last and persists, slowly declining over time. In acute infection that resolves, HBsAg disappears while anti-HBs develops, signifying recovery and immunity (**Figure 1, page 5**). In chronic infection, HBsAg persists for more than six months and anti-HBs does not develop (**Figure 2, page 5**).[2]

While not covered in detail in this document, HBeAg is an indicator of active viral replication and increased infectivity. Testing for antibody to HBeAg (anti-HBe) can be used to track treatment response and the progression of chronic HBV infection. Testing for HBeAg and anti-HBe in HBV infected individuals is used to help assess the level of viral activity, infectivity and guide clinical management.[2]

Test Ordering and Available Assays

Initial HBV screening should use the triple panel (HBsAg, anti-HBs, and total anti-HBc). IgM anti-HBc testing should be ordered to differentiate acute from chronic HBV infection. See **Figure 1** and **Figure 2** depicting markers present in acute and chronic HBV infection. If HBsAg is positive, reflex testing for HBV DNA, HBeAg, anti-HBe and ALT should follow to assess infectivity and liver disease status.[2,4,9] See **Table 3 (page 6)** for interpretations of screening test results for HBV infection and how results differ in acute vs. chronic infection. The recommendation is also to consider testing for HDV infection.[14]

The American College of Obstetricians and Gynecologists (ACOG) recommends HBsAg screening early in every pregnancy, regardless of prior testing or vaccination status. Triple-panel screening (HBsAg, anti-HBs and total anti-HBc) is recommended for patients without a documented negative screen after age 18, those who have not completed the hepatitis B (HepB) vaccine series, or those with ongoing risk factors for HBV, regardless of vaccination history.[15]

HBV DNA tests are utilized to track how well a patient is responding to therapy, evaluate the risk of perinatal transmission, and identify occult HBV infection (that is, infection in individuals who test negative for HBsAg).[16]

Confirmatory (Neutralization) Test for Confirmation of Reactive HBsAg Screening Test

Confirmatory testing should be performed on reactive HBsAg samples as per the manufacturers' instructions. This neutralization assay helps to confirm if the sample is a true positive for HBsAg. Neutralization testing is done by retesting the original sample along with a portion of the same sample that has been "neutralized" using approved reagent containing anti-HBs. If the "neutralized" sample is non-reactive and the original sample remains reactive, this confirms the reactivity is due to HBsAg.[17]

Assays vary in sensitivity and mutant coverage. Selecting assays validated for mutant detection is recommended. Rapid or point-of-care HBsAg tests are increasingly available outside the US.

Quantitative Anti-HBs Assays (Assessment of Immunity)

Quantitative anti-HBs assays provide results to help determine whether an individual has achieved a seroprotective level of antibodies following vaccination or past infection. An anti-HBs level of ≥10 mIU/mL is considered as evidence

of immunity and confirms seroprotective serologic response. This information is especially helpful in individuals with ongoing exposure risk, such as healthcare personnel. These results guide decisions regarding revaccination or the need for additional vaccine doses in individuals who fail to mount an adequate antibody response.[16]

Figure 1. Acute HBV Infection

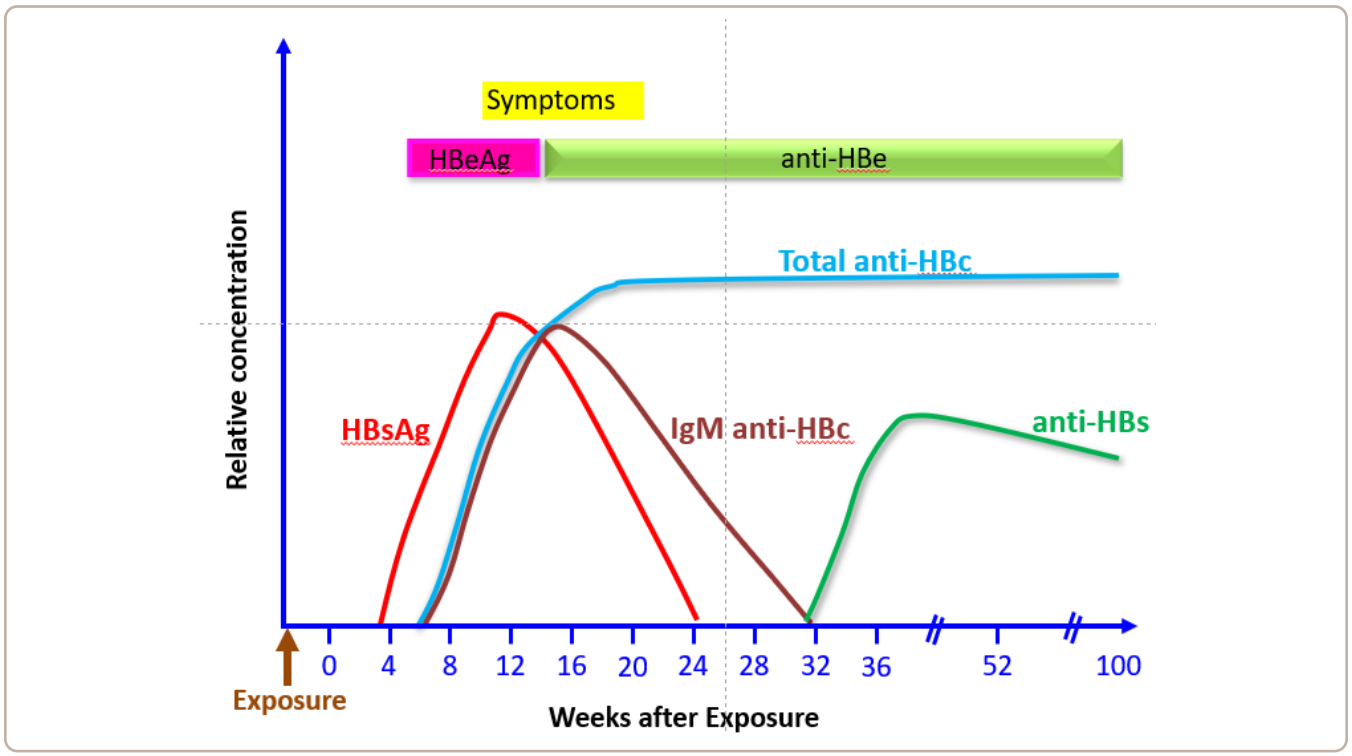
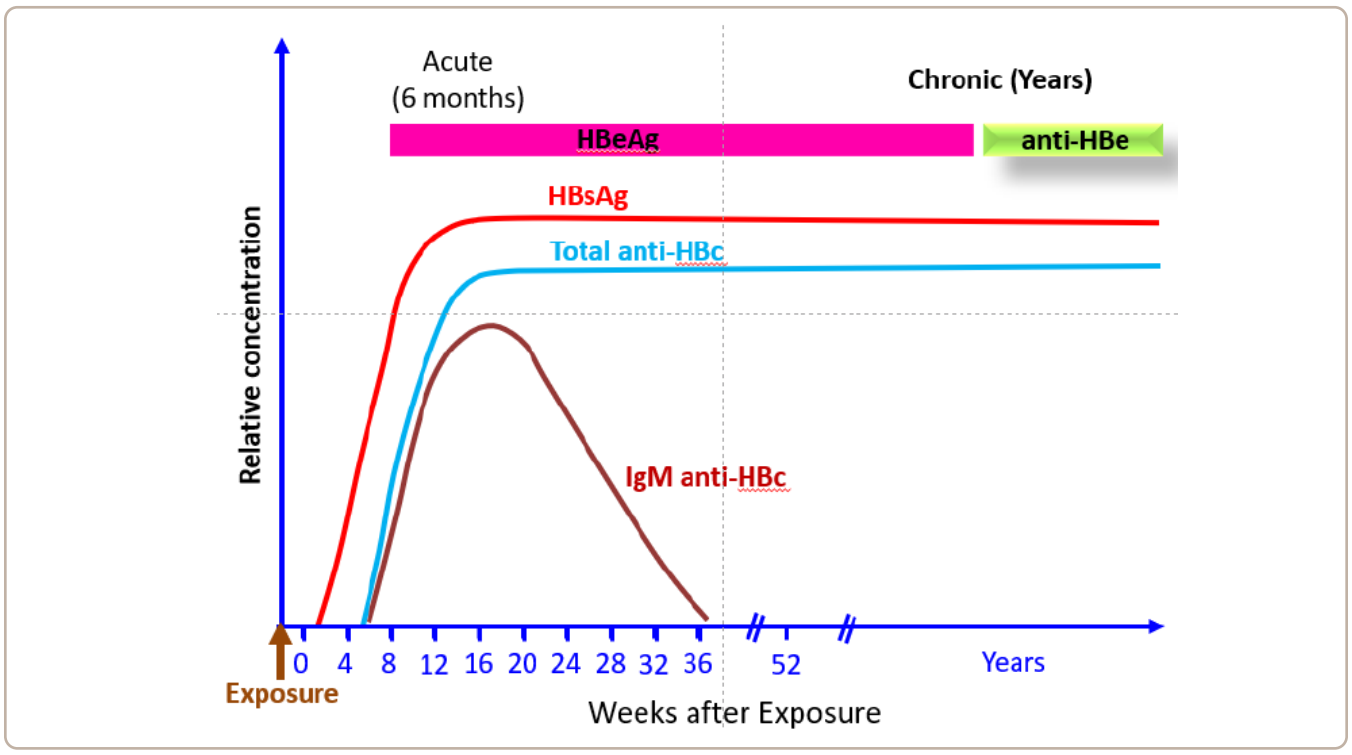


Figure 2. Chronic HBV Infection



Reporting Results

Laboratories should clearly report HBV test results according to manufacturer instructions for the assay(s) they are using, with interpretive comments. Acute, chronic and perinatal HBV infection are nationally notifiable conditions and should be reported to health departments as specified by jurisdiction regulations.[4,18]

Hepatitis D, however, is not a nationally notifiable disease, and only about half of states require reporting.[6] This inconsistency contributes to underestimation of HDV prevalence. Laboratories that detect HDV antibody or HDV RNA should consult local health departments for state-specific reporting guidance.

Interpretation of Hepatitis B Virus Test Results

Providers should be aware that mutant HBV strains, low-level infections or immunosuppression can complicate interpretation. When in doubt, follow CDC interpretive guidance or consult a specialist (**Table 3**).[2]

Table 3. Interpretation of Screening Test Results for HBV Infection and Recommended Actions[a]

Clinical state	HBsAg	Anti-HBs	Total anti-HBc[b]	IgM anti-HBc	Action[c]
Acute infection	Positive	Negative	Positive	Positive	Link to HBV infection care
Chronic infection	Positive	Negative	Positive	Negative[d]	Link to HBV infection care
Resolved infection	Negative	Positive	Positive	Negative	Counsel about HBV infection reactivation risk
Immune (immunity inferred from receipt of previous vaccination)	Negative	Positive[e]	Negative	Negative	Reassure if history of HepB vaccine series completion; if partially vaccinated, complete vaccine series per ACIP recommendations
Susceptible, never infected	Negative	Negative[f]	Negative	Negative	Offer HepB vaccine per ACIP recommendations
Isolated core antibody positive[g]	Negative	Negative	Positive	Negative	Depends on cause of positive result

a Table adapted from: Screening and Testing for Hepatitis B Virus Infection: CDC Recommendations — United States, 2023 | MMWR

b Total anti-HBc is a measure of both IgM and IgG antibodies to HBcAg.

c Source: Abara WE, Qaseem A, Schillie S, et al. Hepatitis B vaccination, screening, and linkage to care: best practice advice from the American College of Physicians and the Centers for Disease Control and Prevention. Ann Intern Med 2017;167:794–804.

d IgM anti-HBc also might be positive in persons with chronic infection during severe HBV infection flares or reactivation.

e Immune if anti-HBs concentration is >10 mIU/mL after vaccine series completion.

f Anti-HBs concentrations might wane over time among vaccine responders (Source: Schillie S, Vellozzi C, Reingold A, et al. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2018;67[No. RR-1]:1–31).

g Can be the result of a past infection when anti-HBs levels have waned, occult infection, passive transfer of anti-HBc to an infant born to an HBsAg-positive gestational parent, a false positive, or mutant HBsAg strain that is not detectable by laboratory assay.

Hepatitis Delta Virus Testing

Testing for HDV infection should be performed in all HBsAg-positive individuals or, at a minimum, in those with risk factors such as origin from endemic areas, injection drug use, living with HIV or severe liver disease and low or undetectable HBV DNA levels.[7,9]

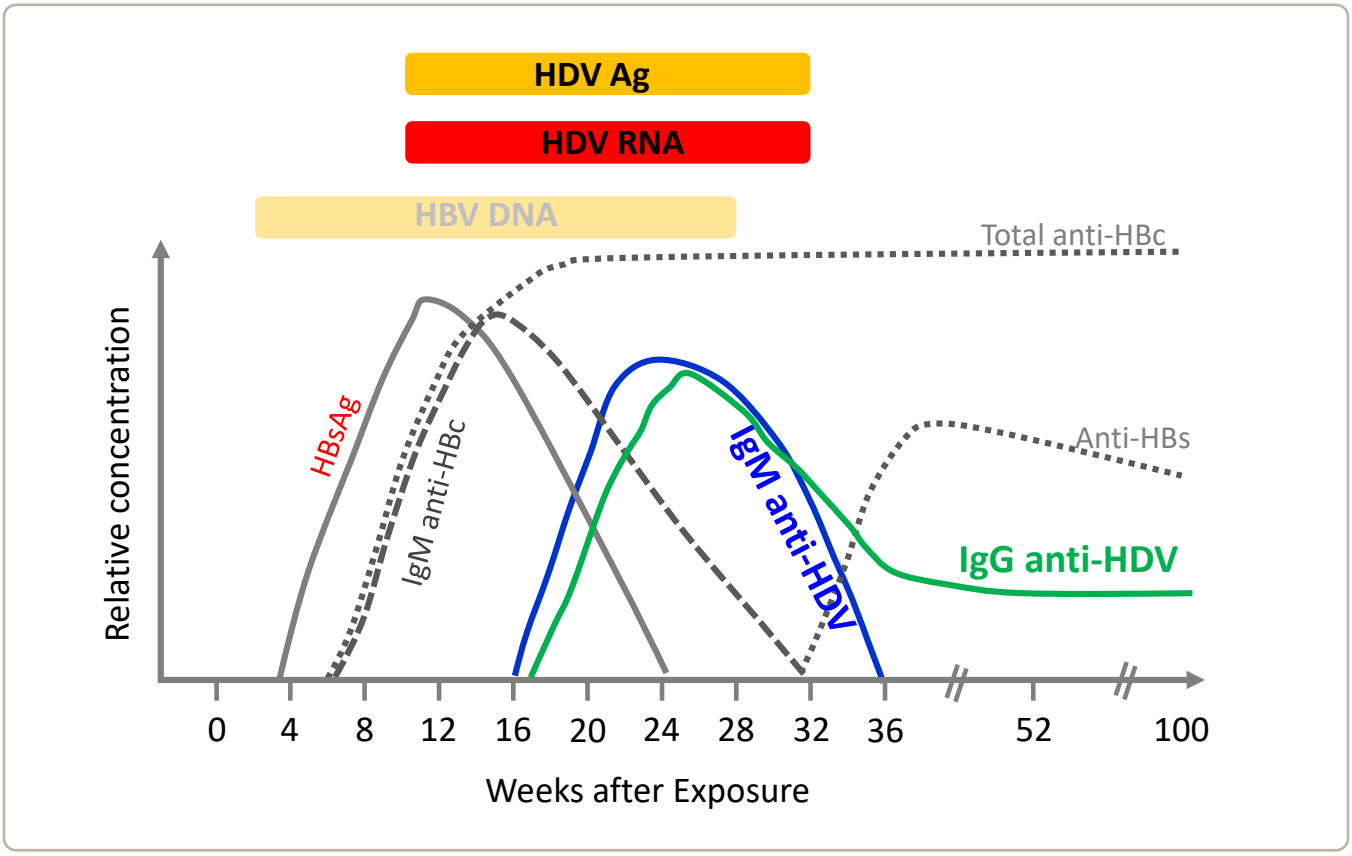
Screening begins with an anti-HDV antibody test, followed by reflex to nucleic acid test (NAT) for detection of HDV RNA for diagnosis of current HDV infection and viral load determination. Current infection is defined by detectable HDV RNA. Antibody positivity with undetectable HDV RNA indicates past infection.[6] **Figure 3** shows the serologic course of HDV superinfection.

Availability of HDV testing in the US remains limited. Only a small number of commercial laboratories currently offer anti-HDV or HDV RNA testing. These assays are typically validated as laboratory-developed tests, rather than FDA-cleared or FDA-approved assays.

Major CLIA-certified commercial reference laboratories offering HDV RNA testing include:

- **Quest Diagnostics:** Quantitative HDV RNA testing using a real-time PCR assay (test code 37889)
- **LabCorp:** HDV RNA quantitative PCR assay (test number 550653)
- **ARUP Laboratories:** HDV RNA detection and quantification by quantitative PCR (test code 2013881)
- **Mayo Clinic Laboratories:** HDV RNA detection and quantification using real-time RT-PCR (Test Id HDVQU)

Figure 3. Serological Course of HDV Superinfection



References

1. US Department of Health and Human Services. Viral Hepatitis in the United States: Data and Trends [Internet]. Available from: https://www.hhs.gov/hepatitis/learn-about-viral-hepatitis/data-and-trends/index.html
2. Conners EE. Screening and Testing for Hepatitis B Virus Infection: CDC Recommendations — United States, 2023. MMWR Recomm Rep. 2023;72. doi:10.15585/mmwr.rr7201a1
3. CDC. Hepatitis D [Internet]. 2024 [cited 2026 Mar 31]. Hepatitis D Basics. Available from: https://www.cdc.gov/hepatitis-d/about/index.html
4. CDC. Viral Hepatitis [Internet]. 2026 [cited 2026 Mar 31]. Hepatitis B Surveillance Guidance. Available from: https://www.cdc.gov/hepatitis/php/surveillance-guidance/hepatitis-b.html
5. Hepatitis B, acute and chronic 2024 Case Definition | CDC [Internet]. 2024 May 30 [cited 2026 Mar 31]. Available from: https://ndc.services.cdc.gov/case-definitions/hepatitis-b-acute-and-chronic-2024/
6. Asselah T, Rizzetto M. Hepatitis D Virus Infection. Hardin CC, editor. N Engl J Med. 2023 Jul 6;389(1):58–70. doi:10.1056/NEJMra2212151
7. Pan C, Gish R, Jacobson IM, Hu KQ, Wedemeyer H, Martin P. Diagnosis and Management of Hepatitis Delta Virus Infection. Dig Dis Sci. 2023 Aug 1;68(8):3237–48. doi:10.1007/s10620-023-07960-y
8. Sarah Schillie MD, Claudia Vellozzi MD, Arthur Reingold MD, Aaron Harris MD, Penina Haber MPH, John W. Ward MD, et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep. 2018;67. doi:10.15585/mmwr.rr6701a1
9. Terrault NA, Lok ASF, McMahon BJ, Chang K, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018 Apr;67(4):1560–99. doi:10.1002/hep.29800
10. Cornberg M, Zoulim F, Gish R, Jacobson IM, Kushner T, Lampertico P, et al. Best practices for screening, testing, diagnosing, and treating patients with hepatitis D (delta) virus based on global expert review and recent guidelines. Antiviral Therapy. 2025 Aug;30(4):13596535251349380. doi:10.1177/13596535251349380
11. Brunetto MR, Ricco G, Negro F, Wedemeyer H, Yurdaydin C, Asselah T, et al. EASL Clinical Practice Guidelines on hepatitis delta virus. Journal of Hepatology. 2023 Aug;79(2):433–60. doi:10.1016/j.jhep.2023.05.001
12. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection [Internet]. [cited 2026 Apr 1]. Available from: https://www.who.int/publications/i/item/9789240090903
13. Lazarevic I. Clinical implications of hepatitis B virus mutations: Recent advances. World J Gastroenterol. 2014 Jun 28;20(24):7653–64. doi:10.3748/wjg.v20.i24.7653 PubMed PMID: 24976703; PubMed Central PMCID: PMC4069294.
14. Kushner T, Cohen SM, Ahn J, Wong RJ. AGA Clinical Practice Update on Management of Hepatitis Delta: Commentary. Gastroenterology. 2025 Oct;169(5):1063–9. doi:10.1053/j.gastro.2025.07.037
15. Viral Hepatitis in Pregnancy: ACOG Clinical Practice Guideline No. 6. Obstet Gynecol. 2023 Sep 1;142(3):745-759. doi: 10.1097/AOG.0000000000005300. PMID: 37590986. https://pubmed.ncbi.nlm.nih.gov/37590986/
16. CDC. Epidemiology and Prevention of Vaccine-Preventable Diseases [Internet]. 2025 [cited 2026 Mar 31]. Chapter 10: Hepatitis B. Available from: https://www.cdc.gov/pinkbook/hcp/table-of-contents/chapter-10-hepatitis-b.html
17. Background – Diagnostics for Testing for Hepatitis B and C Infection. In: WHO Guidelines on Hepatitis B and C Testing [Internet]. World Health Organization; 2017 [cited 2026 Mar 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK442281/ PubMed PMID: 30418726.
18. Osiowy C, Giles E, Tanaka Y, Mizokami M, Minuk GY. Molecular Evolution of Hepatitis B Virus over 25 Years. J Virol. 2006 Nov;80(21):10307–14. doi:10.1128/JVI.00996-06 PubMed PMID: 17041211; PubMed Central PMCID: PMC1641782.

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