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Hepatitis B virus testing and interpreting test results

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KEY POINTS

- Opportunistic testing of people at risk of hepatitis B virus infection should be undertaken, particularly for people born in intermediate- and high-prevalence countries, and Aboriginal and Torres Strait Islander people (1).
- Testing for hepatitis B in a patient from a hepatitis B priority population aligns with the screening provisions of the Medicare Benefits Schedule (2) and presents an opportunity to diagnose, intervene and prevent illness and death.
- Informed consent should be obtained before testing, and test results should be conveyed in a safe and culturally appropriate manner.
- When testing for hepatitis B, the tests to be ordered are: hepatitis B surface antigen (HBsAg), antibody to surface antigen (anti-HBs) and antibody to core antigen (anti-HBc). Positive HBsAg indicates current infection, positive anti-HBs indicates immunity (through vaccination or past infection), and positive anti-HBc indicates past or current infection (this test may occasionally give a false-positive result).

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GESA Recommendation 2

GESA Recommendation 3

Introduction

Hepatitis B is a complex disease that can be defined using biochemical, serological, virological and histological parameters. Management decisions are based on an accurate interpretation of these parameters. This chapter discusses who should be tested for hepatitis B virus (HBV) infection, and details the specific tests for HBV infection and the interpretation of test results.

Who should be tested?

Guidelines from the GESA Australian consensus recommendation for the management of hepatitis B, National HBV testing policy, Asian Pacific Association for the Study of Liver (APASL), the American Association for the Study of Liver Diseases (AASLD), and the European Association for the Study of Liver (EASL) recommend screening people born in high- and intermediate-prevalence countries, including immigrants and adopted children (Table 3.1) (1-2, 4-6). Other high-risk groups identified include: household and sexual contacts of people positive for hepatitis B surface antigen (HBsAg), people with a history of injecting drug use, people with multiple sexual partners or any history of sexually transmitted infection, men who have sex with men, prison inmates, people with chronically elevated levels of alanine transaminase and aspartate aminotransferase (ALT/AST); people with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) infection, people undergoing haemodialysis and all pregnant women. In Australia, these recommendations should also include Aboriginal and Torres Strait Islander people, who account for about 7.2% of the Australian population with HBV infection (7). High-risk patients undergoing treatment with immunosuppressive agents, such as chemotherapy and antirejection therapy, should also be screened for HBV. Seronegative people (who are susceptible to infection) should be vaccinated.

Table 3.1 Priority populations for testing (adapted from Australian consensus recommendations for the management of hepatitis B 2022)

[See here for a full table in the guidelines](#)

Populations with higher prevalence of CHB

People who inject drugs
Men who have sex with men
Aboriginal and Torres Strait Islander peoples [†]
People living with chronic hepatitis C
People who have ever been incarcerated

People born overseas in regions with ≥2% CHB prevalence

People born in North-East Asia
People born in South-East Asia
People born in the Pacific Islands [‡]
People born in North Africa
People born in Central Asia
People born in Southern Europe
People born in Eastern Europe
People born in Sub-Saharan Africa

Populations with higher risk of onward transmission and/or adverse outcomes

	Reason
Pregnant women	Additional prevention measures for women with CHB further reduces transmission risk
People receiving immunosuppressive therapy	Risk of CHB exacerbation and death without prophylaxis
Health care workers ^{‡‡}	High risk of transmission (if performing exposure-prone procedures); treatment may be required to reduce viral load
People with other chronic liver disease (e.g. metabolic-associated fatty liver disease)	Risk of liver disease flare in people with comorbid disease
People undergoing renal dialysis [§]	Higher transmission risk and more severe disease progression
People living with HIV	Higher susceptibility to CHB and more severe disease progression

Household and sexual contact of people with CHB	Significant risk of transmission through household and sexual contact
Children born to mothers with CHB	Significant risk of transmission in infants born to mothers with high viral load, even with vaccination
People with multiple sexual partners	Risk of sexual transmission
[†] Māori and other indigenous peoples are also at higher risk of CHB and should be offered screening. [‡] All health care workers should be offered hepatitis B testing, while respecting their rights of privacy and legal protection in the workplace. [§] These people are also likely to have a higher prevalence of CHB.	

Gaining informed consent for testing

Hepatitis B is a life-long disease for many patients. It is important to provide support and information about the testing process, to minimise the impact of a positive diagnosis on patients and their families, change high-risk health-related behaviour and reduce anxiety. Informed consent for testing means that the person agrees to be tested on the basis of understanding the testing procedures, the reasons for testing and being able to assess the personal implications of a positive test result, including the need for further medical assessment.

The process of obtaining informed consent before testing needs to be conducted in a culturally appropriate and safe manner. It should acknowledge the patient's gender, cultural beliefs and practices, health literacy, behaviour and language; it should also consider local and cultural issues such as stigma, shame and concerns about confidentiality. This may be particularly relevant when dealing with patients from culturally and linguistically diverse (CALD) backgrounds, who may have low English proficiency, and Aboriginal and Torres Strait Islander people. The Good Medical Practice code of conduct recommends using qualified language or cultural interpreters to assist with communication (8), and providing information packs in the patient's first language (see [Health Professional Resources](#) for further resources). Finally, it is important to address the implications of a positive, negative or indeterminate test result, the medical consequences of infection and the support mechanisms available, both while awaiting test results and in the event that the result is positive. Consent should be discussed confidentially without other family members or a spouse present in the consultation room.

The Translating and Interpreting Service (TIS National) is available 24 hours a day, 7 days a week, and can be contacted via the Doctors Priority Line on 1300 131 450.

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GESA Consensus Recommendation 2

Diagnostic tests for hepatitis B

Serological testing for HBV infection relies on immunoassay techniques for the detection of antigens and antibodies in patient serum. Current serological tests for hepatitis B are highly sensitive and specific. Initial testing should include:

- hepatitis B surface antigen (HBsAg)
- antibody to surface antigen (anti-HBs)
- antibody to core antigen (anti-HBc).

Hepatitis serology tests are Medicare rebatable. However, to order all three diagnostic tests (HBsAg, anti-HBc and anti-HBs) simultaneously, and retain Medicare eligibility, the requesting doctor should write 'chronic hepatitis B' or a similar clinical justification for testing on the request slip. Table 3.2 outlines the test results and their interpretation.

Table 3.2 Hepatitis B test results and their interpretation (11)

Test	Result	Interpretation
HBsAg anti-HBc anti-HBs	Negative Negative Negative	Susceptible to infection (if at risk, vaccination should be recommended)
HBsAg anti-HBc anti-HBs	Negative Positive Positive	Immune due to resolved infection
HBsAg anti-HBc anti-HBs	Negative Negative Positive	Immune due to hepatitis B vaccination
HBsAg anti-HBc IgM anti-HBc* anti-HBs	Positive Positive Positive Negative	Acute HBV infection *(high titre)
HBsAg anti-HBc anti-HBs	Positive Positive Negative	Chronic HBV infection

HBsAg: hepatitis B surface antigen; anti-HBc: antibody to hepatitis B core antigen; anti-HBs: antibody to hepatitis B surface antigen; IgM anti-HBc: Immunoglobulin M antibody to the hepatitis B core antigen; HBV: hepatitis B virus

Refer to page 1 of the [Decision Making in hepatitis B Tool](#)

Serological markers

Hepatitis B surface antigen (HBsAg)

The presence of HBsAg signifies HBV infection. HBsAg is an antigen on the envelope of the HBV virion and is secreted as lipoprotein particles in excess of virions by a ratio greater than 1000:1. In acute infection, HBsAg usually becomes detectable during weeks 4–10. CHB virus infection is defined by the persistence of HBsAg for more than 6 months.

Recently, there is increasing interest in quantification of HBsAg, although this assay is not yet available for routine clinical practice and there is no Medicare rebate for the test. There have been studies that relate changes in level of HBsAg to treatment and long-term outcomes (9). HBsAg levels have also been related to outcomes in patients not treated with antiviral therapy (10). Previously, HBsAg has been related to degree of liver fibrosis and risk of relapse after stopping therapy (11). Based on these studies, the quantification of HBsAg may have a future clinical role in predicting HBV disease progression and outcomes, both in treated and untreated patients.

Antibody to surface antigen (anti-HBs)

Anti-HBs is a protective antibody that develops with the resolution of acute infection, or following successful vaccination against HBV. Occasionally, anti-HBs and HBsAg can be found together; this situation has no known clinical significance. Rarely, there may be a window where both HBsAg and anti-HBs can be negative during the seroconversion and clearance of HBV.

Antibody to core antigen (anti-HBc)

Anti-HBc immunoglobulin G (IgG) remains positive for life following exposure to HBV; however, unlike anti-HBs, anti-HBc is not a protective antibody. HBV core antigen is not found as a discrete protein in the serum. During HBV replication, it is produced in the cytosol of the hepatocyte, surrounding the viral genome and the associated polymerase. It is then packaged within an envelope before being secreted from the hepatocyte. Anti-HBc is an antibody to a peptide of this core protein, which has been processed within an antigen-presenting cell. In acute infection, anti-HBc immunoglobulin M (IgM) is found in high concentrations, which gradually decline over 3–6 months, while there is a corresponding increase in anti-HBc IgG. If acute hepatitis B is suspected through recent risk or presentation, or both, a test for anti-HBc IgM can be ordered to support the clinical suspicion. Elevation (to a high

Recent rise or persistence of HBsAg, a test for anti-HBc IgG can be ordered to support the chronic diagnosis. A rise (or a high titre) of anti-HBc IgM usually signifies acute infection, but low-grade elevations may also occur during reactivation or flares of CHB. Most serological assays do not directly measure anti-HBc IgG; rather, they test for the total anti-HBc antibody.

Conveying a test result

The result of a test should be conveyed in a culturally appropriate and safe manner, using a qualified language interpreter (who is gender and dialect appropriate) for patients with a low proficiency in English. Results need to be given promptly and in person, in a setting where privacy is assured. It is important to avoid information overload, and it is often useful to provide written material that is culturally and language appropriate (taking literacy levels into account), and details of support services (for further information see [ASHM Testing Portal - Conveying Hepatitis B Test Results](#)).

Conveying a hepatitis B test result: susceptible (non-immune)

A person is regarded as susceptible or non-immune when there is no documented history of completed vaccination, and the anti-HBs, anti-HBc and HBsAg results are all negative. This situation provides an opportunity to discuss hepatitis vaccination with the person, and to emphasise positive messages about safer behaviours (see: [Primary prevention of hepatitis B virus infection](#)).

Conveying a hepatitis B test result: immune

A person is regarded as immune when the anti-HBs titre is positive (> 10 IU/mL) in the setting of a previous completed vaccination, or the anti-HBc is positive (with or without anti-HBs being positive). Isolated anti-HBc positive results usually indicate distant resolved infection (with the anti-HBs titre having fallen below the threshold of the assay). However, the result is occasionally falsely positive and, rarely, isolated anti-HBc results can indicate a different hepatitis B status, as outlined in [Table 3.3](#).

For patients who have become immune through natural infection or vaccination, this result should be conveyed to the patient and clearly entered in their medical record, to avoid unnecessary repeat serologic testing or vaccination in the future. Patients who have become immune through natural infection should be advised that they may be at risk in settings of severe immunosuppression (see: [Complex situations: Co-infection and immunosuppression](#)).

Table 3.3 Isolated elevation of anti-HBc:

Test	Result
HBsAg	Negative
anti-HBc	Positive
anti-HBs	Negative

Interpretation possibilities include:

- distant resolved HBV infection – the most common interpretation, particularly in people born in HBV-endemic areas
- false positive result – more common in people with a low risk of past HBV infection
- resolving acute HBV infection – in the period between HBsAg loss and development of detectable anti-HBs
- passive transfer of maternal anti-HBc – in children up to 3 years of age
- occult HBV infection – where HBV DNA is detectable in the absence of HBsAg; this can be determined by detecting HBV DNA in serum, but this test is not Medicare rebatable in the absence of HBsAg (approximately 1% of people with this serological pattern)
- Confirmation via repeat serology should be considered.

HBsAg: hepatitis B surface antigen; anti-HBc: antibody to hepatitis B core antigen; anti-HBs: antibody to hepatitis B surface antigen; HBV: hepatitis B virus

Conveying a hepatitis B test result: confirmed infection

A positive HBsAg test result can have a significant impact on the patient, and those close or important to them. It is important to focus on the person's immediate needs, and to provide support and allow adequate time for questions. It is essential to use a language interpreter (who is gender and dialect appropriate) for patients with a low proficiency in English. The process of conveying a positive result should include:

- giving the test result in person, and in a confidential manner (without other family members or a spouse present) that is sensitive and appropriate to the gender, culture, behaviour, language and literacy level of the person who has been tested
- informing the patient about how hepatitis B is and is not transmitted, and how onward transmission may be prevented, including discussion about hepatitis B vaccination for partners, household contacts and other close contacts
- discussing strategies for disclosure to the patient's partner and family members, including discussion of the following (depending on whether the person has acute or chronic disease):
 - disclosure to children
 - whether current and future household and sexual contacts should be tested for hepatitis B, and subsequently vaccinated if they are found to be susceptible
- providing information about the legal considerations about disclosure of hepatitis B status (see: [Privacy, confidentiality and other legal responsibilities](#)).
- providing information about (and referral to) available support services (see [Patient Resources](#)).
- Arranging a follow-up appointment to check understanding and answer further questions.

Considerations

Considerations for acute hepatitis B

Information should be provided about the natural history of hepatitis B, and the importance of clinical monitoring to identify resolution of acute infection (which will occur in 95% of adults) or the possibility of going on to have CHB.

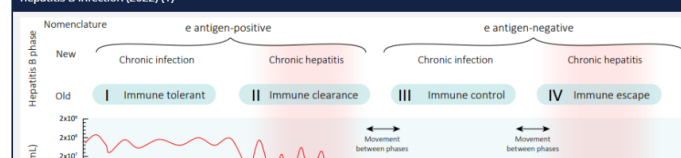
Considerations for chronic hepatitis B

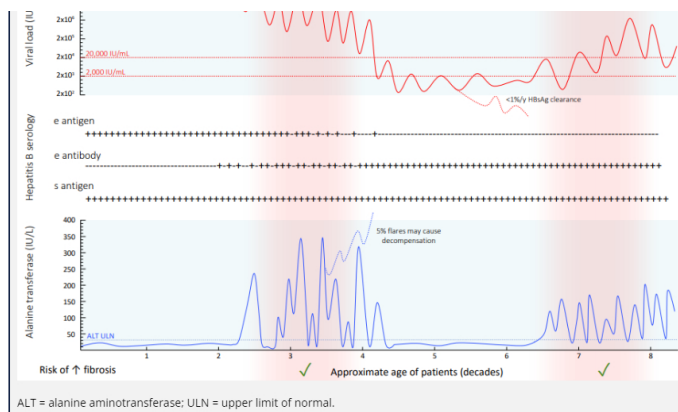
Information should be provided about the natural history of CHB, and the need for regular, ongoing clinical monitoring to detect progression of liver disease, determine the need for treatment and prevent liver cancer (see: [Clinical assessment of patients with hepatitis B virus infection](#)). Health maintenance strategies should be identified; such strategies could include alcohol minimisation, weight loss, smoking cessation and harm-reduction strategies, as appropriate. Discussion should also cover the availability, efficacy and timing of treatment options, including the fact that antiviral therapy may not be needed. It may be necessary to cover these issues over a period of time rather than in a single session; hence, a subsequent consultation should be arranged at the time of diagnosis.

Markers of hepatitis B virus infection

The parameters used to define and characterise CHB include HBV antigens and host antibodies; HBV DNA and genotype; biochemical markers such as ALT; and the degree of hepatic fibrosis and inflammation. The definition and characterisation of the phases of CHB infection ([Figure 3.1](#)) are discussed in more detail in [Natural history of hepatitis B virus infection](#). Information on which tests to order during initial assessment or ongoing management of CHB are discussed in more detail in [Clinical assessment of patients with hepatitis B virus infection](#).

Figure 3.1 Natural history of chronic HBV from the GESA Australian consensus recommendations for the management of hepatitis B infection (2022) (1)





Antigens

Hepatitis B e antigen

HBeAg is an accessory protein from the precore region of the HBV genome that is not necessary for viral infection or replication (13). It is, however, produced during active viral replication, and may act as an immunogen or a tolerogen, promoting persistent infection. The role of HBeAg quantification is unclear but it is an area of ongoing study.

Antibody to e antigen

Anti-HBe is not a protective antibody; however, its appearance usually coincides with a significant immunological change associated with better virological control and lower HBV DNA replication (< 2000 IU/mL). The loss of HBeAg and the development of anti-HBe is termed HBeAg seroconversion, and it has been used as an end-point for treatment in HBeAg-positive people, because seroconversion is associated with a lower risk of progression to liver disease (14).

Virological markers

Hepatitis B virus DNA

With the advent of molecular amplification technology such as polymerase chain reaction (PCR), it has become possible to directly quantify the level of HBV replication. This is now an integral part of HBV management, especially with the development of effective antiviral treatments. PCR-based assays (target amplification assays) involve lysing the virion and purifying the DNA, which is then amplified and quantified. Alternatively, signal amplification assays can quantify the level of HBV DNA from serum, and require no purification step. Currently, the PCR-based assays for HBV DNA detection have the best range of quantification, now being standardised to IU/mL (15). The introduction of real-time PCR has allowed for sensitivities ranging from about 5–10 IU/mL to about 8–9 \log_{10} IU/mL (16). Newer HBV DNA assays can detect HBV DNA at 4.5 IU/mL (17). The level of 20,000 IU/mL has been arbitrarily selected as the level below which there is a relatively low likelihood of hepatic damage, although damage can still occur (5, 6).

The serum level of HBV DNA is a dynamic parameter in CHB. The level of circulating HBV is the strongest predictor of the development of cirrhosis and hepatocellular carcinoma (HCC) (18, 19). In a large, prospective Taiwanese cohort ($n = 3653$) followed over 11 years, the incidence of cirrhosis and HCC ranged from 4.5% and 1.3%, respectively, in those with low HBV DNA (< 50 IU/mL), to 36.2% and 14.9%, respectively, in those with high HBV DNA ($> 100,000$ IU/mL). The incidence of both cirrhosis and HCC had dose-response relationships with HBV DNA levels, independent of HBeAg status and ALT level. Importantly, the risk of HCC and cirrhosis increased significantly at 10,000 IU/mL (18,19). Effective suppression of HBV replication with antiviral therapy may reduce the incidence of significant fibrosis and HCC.

HBV DNA testing is now a vital part of the initial evaluation, ongoing monitoring and assessment of the efficacy of antiviral treatment. Before the introduction of HBV DNA testing, HBeAg was used as the biomarker of HBV replication. However, it is now clear that there is a population, termed e antigen negative chronic HBV, with HBV infection with active replication (high-level HBV DNA) who are HBeAg negative. This state occurs as a result of a mutation in the precore or basal core promoter region of the HBV genome (see [Virology: viral replication and drug resistance](#)). A major problem with the use of some antiviral therapies is the development of drug resistance, defined as a rise of $\geq 1 \log_{10}$ IU/mL in the HBV DNA level while on therapy (6). The development of treatment resistance has important management implications. Many patients treated with nucleos(t)ide analogues are negative for HBV DNA, but remain positive for HBeAg, which probably represents the lack of effect of the drugs on the integrated HBV DNA in the hepatocytes. Based on increasing evidence of the importance of HBV DNA testing, the Medical Services Advisory Committee of the then Australian Government Department of Health and Ageing approved HBV DNA testing. The committee recommended one pre-treatment assay for monitoring of patients not on antiviral therapy, and up to four assays over 12 months for those on antiviral therapy (20).

Hepatitis B virus genotyping

Genotyping is determined by sequencing the HBV genome. It is defined as at least 4% divergence in the s antigen and at least 8% divergence in the entire nucleotide sequence. There are nine currently recognised genotypes (A–I), which vary geographically, with the four most common genotypes being A–D. The most prominent genotypes in the Asia and Pacific regions are B and C. Genotype may have an important influence on disease progression and treatment response (21). Although the reasons are unclear, it appears that, in Asian populations, genotype B has increased rates of HBeAg seroconversion, and is associated with less aggressive liver disease and lower rates of HCC than genotype C (22). Furthermore, genotypes A and B have better response rates to interferon when compared to genotypes C and D (23). Currently, genotyping is a research tool and is not routinely performed for HBV in Australia. However, it may become a relevant test in future clinical practice, to identify patients at greater risk for disease progression.

Biochemical markers

Alanine transaminase (ALT)

The main biochemical marker in viral hepatitis is the serum ALT level, which is used as a surrogate marker for necroinflammation in the liver. An elevated ALT is also associated with better serological response to treatment with pegylated interferon. However, some studies have suggested that significant liver fibrosis can occur in the context of a normal ALT level. Recent data show that 12–43% of patients with chronic HBV with normal ALT levels have significant hepatic fibrosis (stage 2 fibrosis or greater) (24, 25). Recent data has also suggested that the HBeAg positive Chronic Infection phase (previously referred to as ‘immune tolerant phase’) of HBV is associated with genetic damage associated with HBV related carcinogenesis (26). Importantly, ALT can be abnormal even in the current normal reported ranges. It is likely that the original data to determine normal reference ranges for ALT levels included people with subclinical liver disease, which led to an overestimation of what should be considered a normal ALT level. A large study of healthy blood donors revealed the upper limit of normal for serum ALT was 30 IU/L for men and 19 IU/L for women (Figure 3.2), significantly lower than the current range (27).

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Figure 3.2 Alanine transaminase (ALT) upper limit of normal

Beware of what the laboratory lists as ‘normal’ ALT.

Elevated ALT levels are:
 > 30 U/L in men
 > 19 U/L in women

Conclusion

Testing people at risk of HBV infection provides an opportunity to diagnose, intervene and prevent illness and death. It is essential that informed consent is gained before testing, and that test results are conveyed in a safe and culturally appropriate manner. Requesting all three serological tests – HBsAg, anti-HBc and anti-HBs – in a patient at risk of hepatitis B infection allows systematic interpretation of results to determine a patient's hepatitis B status, either as susceptible (to infection), immune through vaccination or resolved infection, or with chronic hepatitis B infection. Requesting these three tests avoids missed diagnoses, unnecessary vaccination and recalling patients or adding tests for diagnosis. The parameters used to define and characterise CHB infection include HBeAg and anti-HBe, HBV DNA, ALT and the degree of hepatic fibrosis and inflammation. Subsequent management and treatment decisions based on these results are discussed in detail in “[Natural history of hepatitis B virus infection](#)” and “[Clinical assessment of patients with hepatitis B virus infection](#)”.

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Introduction

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