

HEPATITIS B VIRUS TESTING AND INTERPRETING TEST RESULTS 3

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Links to: Chapter 4: Natural history of chronic hepatitis B virus infection
Chapter 5: Primary prevention of hepatitis B virus infection
Chapter 6: Clinical assessment of patients with hepatitis B virus infection
Chapter 9: Hepatitis B virus-related hepatocellular carcinoma
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KEY POINTS

- The management of hepatitis B virus (HBV) infection requires a complex interpretation of multiple parameters.
- HBV DNA testing has an important role in the evaluation of chronic HBV and the assessment of the efficacy of antiviral therapy.
- HBV DNA level is predictive of the development of cirrhosis and hepatocellular carcinoma (HCC).
- Non-invasive methods of assessing hepatic fibrosis are being developed.
- Normal ALT level does not rule out significant hepatic disease.

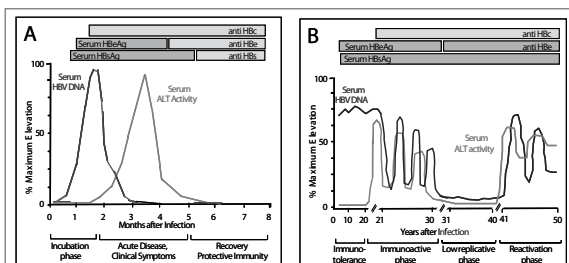
Hepatitis B is a complex disease, which can be defined using biochemical, serological, virological, and histological parameters. Management decisions are based on an accurate interpretation of these parameters. This chapter will detail the specific tests for hepatitis B virus (HBV) infection, discussing their interpretation and focusing on who should be tested.

1. Markers of HBV infection

The parameters used to define and characterise HBV infection include: HBV antigens and host antibodies; HBV DNA and genotype; biochemical markers, such as alanine aminotransferase (ALT); and the degree of hepatic fibrosis and inflammation (Figure 3.1).

Serological markers Hepatitis B surface antigen (HBsAg)

HBsAg is an antigen on the three proteins that make up the envelope of the HBV virion. It is secreted as lipoprotein particles in excess of virions by a ratio of greater than 1000:1. HBsAg



hepatitis B virus infection. *Hepatology* 2003;38(5):1075-86.

Figure 3.1: Temporal changes in serological, virological and biochemical parameters in HBV. (A) acute HBV and (B) chronic HBV infection¹

is usually detectable between week 4 and week 10 in acute infection. Chronic HBV infection is defined by the persistence of HBsAg for more than six months.

Antibody to surface antigen (anti-HBs)

This is a protective antibody that develops with the resolution of acute infection or following the successful vaccination against HBV. Very occasionally, anti-HBs and HBsAg can be found together, which has no known clinical significance.

Antibody to core antigen (anti-HBc)

The HBV core antigen is not found as a discrete protein in the serum. It is produced in the hepatocyte cytosol during HBV replication, surrounding the viral genome and the associated polymerase. It is then packaged within an envelope before secretion from the hepatocyte. The antibody to HBV core (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, complementing the corresponding increase in anti-HBc IgG over a three to six month period. Elevation of anti-HBc IgM usually signifies acute infection, but low elevations may also occur during the reactivation of chronic HBV. Anti-HBc IgG remains positive for life following exposure to HBV, however, unlike anti-HBs, anti-HBc is not a protective antibody. Most serological assays do not directly measure anti-HBc IgG, but test for total anti-HBc antibody.

Hepatitis B e antigen (HBeAg)

HBeAg is an accessory protein from the precore region of the HBV genome, which is not necessary for viral infection or replication.¹ It is, however, produced during active viral replication and may act as an immunogen or a tolerogen, leading to persistent infection.

Antibody to e antigen (anti-HBe)

While anti-HBe is not a protective antibody, its appearance usually coincides with a significant immune change associated with lower HBV

DNA replication ($<10^5$ copies/mL or 20,000 IU/ml). The loss of HBeAg and the development of anti-HBe is termed HBeAg seroconversion, and has been used as an end-point for treatment in HBeAg-positive people, as it has been shown that seroconversion is associated with a lower risk of disease progression.²

Virological markers

HBV DNA

With the advent of molecular amplification technology, such as the 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 treatment. PCR-based assays (target amplification assays) involve a process of 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.³ Initially, the unit of measurement was copies/mL, which is now being standardised to international units (IU)/mL, using a conversion factor of between 5–6 genome copies/mL = 1 IU/mL, depending on the assay. The introduction of real-time PCR has allowed for sensitivities ranging from 5–10 IU/mL up to 8–9 log₁₀ IU/mL.⁴ The level of 20,000 IU/mL (around 10^5 copies/ml) has been arbitrarily selected as the level below which there is a relatively low likelihood of hepatic damage, although this can still occur.⁵

The serum level of HBV DNA is a dynamic parameter in chronic HBV. The level of circulating HBV has recently been shown to be the strongest predictor of the development of cirrhosis and hepatocellular carcinoma (HCC).^{6,7} 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 (<300 copies/mL), to 36.2% and 14.9% respectively, in those with high HBV DNA (10^6 copies/mL). The incidence of both cirrhosis and HCC followed

dose response relationships with HBV DNA levels, which were independent of the HBeAg status and the ALT level. Importantly, the risk of HCC and cirrhosis started to increase significantly at 10^4 copies/mL, 1 log lower than the current level used to signify a low risk of progression (10^5 copies/mL). These studies also suggest that effective suppression of HBV replication with antiviral therapy should be expected to lower the incidence of significant fibrosis and HCC.

HBV DNA testing is now a vital part of the pre-treatment evaluation 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 clear that there is a population with HBV infection with active replication (high level HBV DNA) who are HBeAg negative and have 'precore mutant' HBV. This state occurs as a result of a mutation in this region of the HBV genome. A major problem with the use of current antiviral therapy is the development of resistance, characterised by a rise of ≥ 1 log IU/mL in the HBV DNA level while on therapy.⁸ The development of treatment resistance has important management implications. Based on increasing evidence of the importance of HBV DNA testing, the Medical Services Advisory Committee, within the Department of Health and Ageing, recently approved HBV DNA testing, recommending 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.⁹

HBV genotyping

Genotyping is determined by sequencing the HBV genome. It is defined as a $\geq 4\%$ divergence in the s antigen and $\geq 8\%$ divergence in the entire nucleotide sequence. There are eight currently recognised genotypes (A-H), which vary geographically, with the four most common genotypes being A-D. The most prominent genotypes in the Asia-Pacific region are B and C. Data now suggest that genotype may have an important influence on disease progression and treatment response.¹⁰ While the reasons are unclear, it appears that, in Asian populations, genotype B has increased rates

of HBeAg seroconversion, less aggressive liver disease and lower rates of HCC.¹¹ Furthermore, it has been observed that genotypes A and B have better response rates to interferon when compared to genotypes C and D.^{12,13} Currently, genotyping is only a research tool; patients are not routinely genotyped 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 aminotransferase (ALT)

The main biochemical marker used in viral hepatitis is the serum ALT level, used as a surrogate marker for necroinflammation in the liver. An elevated ALT is also associated with better serological response to antiviral treatment. However, some studies have suggested that significant liver fibrosis can occur in the context of a normal ALT level. Recent data show that between 12% and 43% of patients with chronic HBV and normal ALT levels have significant hepatic fibrosis (stage 2 fibrosis or greater).^{14,15} In part this may relate to what is currently considered a normal ALT. 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 the serum ALT was 30 IU/L for men and 19 IU/L for women, significantly lower than our current range.¹⁶

Histological markers

Liver biopsy

The two histological features on liver biopsy used in the assessment of HBV are fibrosis (stage of disease) and necroinflammation (grade of disease). Liver fibrosis is usually graded from 0-4 (1=limited portal fibrosis; 2=periportal fibrosis; 3=septal fibrosis linking portal tracts or central vein; and 4=cirrhosis with development of nodules and thick fibrous septa). Liver biopsy, either performed percutaneously or transjugularly in those with ascites or significant coagulopathy, has been the gold-standard investigation for determining the stage of HBV.

A number of different scoring systems have been developed to stage fibrosis and grade inflammation. Prominent among these are the Histological Activity Index (HAI), the Ishak modified HAI, and the METAVIR system.¹⁷⁻¹⁹ The development of significant fibrosis (stage 2 or greater) implies progressive disease and the need for treatment. Inflammation is graded on necroinflammatory score.

Liver biopsy has, however, a number of disadvantages. It is an invasive, uncomfortable, costly and time-consuming procedure that carries a small, but significant risk of complications. For this reason, some patients are unwilling to undergo the procedure. It also suffers from sampling bias, as scarring and necroinflammation may be heterogeneously distributed in the liver.

Non-invasive assessment of hepatic fibrosis

As a result of the problems associated with liver biopsy, non-invasive techniques to evaluate fibrosis, such as imaging-based and serum-based analyses, have been developed. A panel of blood tests that describe hepatic fibrosis has been proposed for chronic HBV.²⁰ However, rather than describe specific levels of fibrosis, the serum markers divide fibrosis into mild (METAVIR score: < F2) and severe (≥ F2). Currently, the most widely validated non-invasive serum-based tests are the Fibrotest® and Actitest®, which use a combination of biochemical markers: α2-macroglobulin, apolipoprotein A, haptoglobin, gamma-glutamyl-transpeptidase (GGT), bilirubin (Fibrotest®); B2-macroglobulin, apolipoprotein A, haptoglobin, GGT, bilirubin and ALT (Actitest®).

Table 3.1: Serological, virological and biochemical profiles of hepatitis B virus

| | HBsAg | Anti-HBs | Anti-HBc (total) | Anti-HBc IgM | HBeAg | Anti-HBe | HBV DNA (IU/mL) | ALT |
|---|-------|----------|------------------|--------------|-------|----------|-----------------------------|-----|
| Acute HBV | + | - | + | + | + | +/- | High | ↑ |
| Natural HBV immunity (resolved infection) | - | + | + | - | - | +/- | Absent | N |
| Vaccination | - | + | - | - | - | - | Absent | N |
| Chronic HBeAg positive | | | | | | | | |
| Immune tolerance phase | + | - | + | - | + | - | >20,000 IU/mL | N |
| Immune clearance phase | + | - | + | - | + | -/+ | >20,000 IU/mL (fluctuating) | ↑ |
| Chronic HBeAg negative | | | | | | | | |
| Immune control phase | + | - | + | - | - | + | <2,000 IU/mL* | N |
| Immune escape phase | + | - | + | - | - | + | >2,000 IU/mL* | ↑ |
| Occult HBV | - | - | + | - | - | +/- | Very low | N |
| Reactivation of HBV | + | - | + | +/- | + | +/- | >20,000 IU/mL | ↑ |
| +=positive, -=negative, N=normal, ↑=elevated. * HBV DNA cut-off levels may change in the future. | | | | | | | | |

Fibroscan® uses ultrasound elastography to measure liver stiffness. Increasing hepatic fibrosis leads to an increase in liver stiffness, measured in kiloPascals (kPa). In a French HBV-infected population (n=170), liver stiffness was correlated with METAVIR fibrosis score of > 2 and > 3 area under receiver operated curve (AUROC) of 0.81 and 0.92 respectively, equating to a likelihood of predicting an abnormal result.²¹ In another study of chronic HBV patients (n=183) comparing non-invasive liver elastography, Fibrotest® and liver biopsy, the best results were obtained using a combination of Fibroscan® and Fibrotest® with AUROC 0.88 ≥ stage 2 fibrosis, 0.95 ≥ stage 3 fibrosis and 0.95 = stage 4 fibrosis.²² These tests will become available in the future for the assessment and monitoring of HBV patients.

2. Clinical interpretation

The clinical states of HBV can be characterised using the serological, virological, biomedical and histological markers of infection (Table 3.1). The definition and characterisation of the phases of chronic hepatitis B infection are discussed in more detail in Chapter 4: Natural history of chronic hepatitis B virus infection and Chapter 6: Clinical assessment of patients with HBV infection.

Who should be tested?

In Australia, there are an estimated 90,000–160,000 people with HBV infection.²³ Individuals at high risk of HBV in developed countries are those who immigrated from high or intermediate prevalence countries or those engaging in risky behaviours.²⁴⁻²⁶ The majority of people in Australia with HBV infection were born in endemic regions: 33% in South-East Asia and 16% in North-East Asia. Other high-risk groups include Indigenous Australians, men who have sex with men (MSM) and injecting drug users, with rates of 16%, 8% and 5%, respectively.²³

Australia does not currently have a national HBV testing policy. Recent guidelines by the American Association for the Study of Liver Disease (AASLD) recommend screening

people born in high and intermediate prevalence countries, including immigrants and adopted children (Table 3.2).⁸ Other high-risk groups identified in the US guidelines include: household and sexual contacts of HBsAg-positive people; those with a history of injecting drug use; people with sexually sexual partners or any history of sexually transmitted infection; MSM; prison inmates; people with chronically elevated alanine and aspartate aminotransferase (ALT/AST) levels; people with HIV or HCV infection; patients undergoing haemodialysis; pregnant women.⁸ In Australia, these recommendations should also include Indigenous Australians. High-risk patients undergoing treatment with immunosuppressive agents should also be screened for HBV. Seronegative people (susceptible to infection) should be vaccinated.

Table 3.2: Countries of high to intermediate HBV prevalence; people born in these countries are at high risk for HBV infection and should be screened⁸

| |
|--|
| <ul style="list-style-type: none"> ▪ Asia (except Sri Lanka) |
| <ul style="list-style-type: none"> ▪ Africa |
| <ul style="list-style-type: none"> ▪ South Pacific Islands (except non-Indigenous populations of Australia and New Zealand) |
| <ul style="list-style-type: none"> ▪ Middle East (except Cyprus) |
| <ul style="list-style-type: none"> ▪ Europe: Greece, Italy, Malta, Portugal and Spain |
| <ul style="list-style-type: none"> ▪ Eastern Europe, all countries (except Hungary) |
| <ul style="list-style-type: none"> ▪ The Arctic (Indigenous populations) |
| <ul style="list-style-type: none"> ▪ South America: Argentina, Bolivia, Brazil, Ecuador, Guyana, Suriname, Venezuela, and the Amazon region of Colombia and Peru |
| <ul style="list-style-type: none"> ▪ Central America: Belize, Guatemala, Honduras, Panama |
| <ul style="list-style-type: none"> ▪ Caribbean: Antigua and Bermuda, Dominica, the Dominican Republic, Granada, Haiti, Jamaica, Puerto Rico, St Kitts and Nevis, St Lucia, St Vincent and Grenadines, Trinidad and Tobago, Turks and Caicos |

Testing usually involves evaluation of HBsAg and anti-HBs. Alternatively, anti-HBc can also be tested, but needs follow-up with HBsAg and anti-HBs testing. If HBsAg is positive, then further investigation is appropriate with anti-HBc, HBeAg, anti-HBe and ALT. HBV DNA assays are now available and should be used in the assessment and management of all patients with HBV infection.

Pre-test and post-test discussion

Providing support and information about the HBV testing procedure assists in minimising the personal impact on the patient of a positive diagnosis, changing health-related behaviour and reducing anxiety. Pre-test and post-test discussion forms an integral part of testing and should be relevant to the patient’s gender, cultural beliefs and practices, behaviour and language ²⁷. This includes considering local and cultural issues such as stigma, shame and concerns around confidentiality.

Pre-test discussion

The key points to be discussed during pre-test discussion for HBV include:

- Risk assessment and reasons for testing
- Information about prevention and risk reduction
- Confidentiality and privacy
- Testing process and window period
- Seeking informed consent
- Implications of a positive and negative result
- Medical consequences of infection
- Support mechanisms whilst waiting for test results and if result is positive

While not all these issues may be relevant to every patient, assumptions about the patient’s knowledge and risk practices should be avoided. The pre-test discussion ensures that prevention measures are in place, the patient is prepared for his/her test results, and the clinician’s ethical and legal responsibilities have been met.

Table 3.3 provides a summary of the key points to be addressed in the pre-test discussion.

| Table 3.3 Summary of HBV pre-test discussion |
|--|
| ▪ Reason for testing and risk assessment |
| ▪ Timing of risk and option of post-exposure prophylaxis (PEP) |
| ▪ Need for other STI and blood-borne virus testing |
| ▪ History of testing |
| ▪ Confidentiality and privacy issues around testing |
| ▪ Ensuring there is informed consent for the HBV test |
| ▪ Natural history of HBV and HBV transmission information (if appropriate) |
| ▪ Prevention of transmission and risk reduction through behaviour change |
| ▪ Implications of a positive or indeterminate test result, including availability of treatment |
| ▪ Implications of a negative test result |
| ▪ Explanation of the window period |
| ▪ General psychological assessment and assessment of social supports in the event of a positive result |
| ▪ Logistics of the HBV test: time taken for results to become available and the need to return for results |

Post-test discussion

All HBV test results must be given in person. As outlined for the pre-test discussion, giving a test result should also be conducted in a manner that is confidential, sensitive and appropriate to gender, cultural beliefs and practices, behavior, ongoing risk and language. The post-test discussion should be conducted where privacy is assured and where there will be no interruptions.

Giving a positive result

The key points to be discussed in relation to delivering a positive result and subsequent consultations have been summarised below (Table 3.4). In addition to the post-test discussion, patients newly diagnosed may benefit from the provision of written material

that reinforces key messages and provides details of local support services (Appendix 1 and 2). Clinicians inexperienced in managing patients with HBV should collaborate with more experienced general practitioners and/or specialists or specialist centres. See the ASHM Directory available at: www.ashm.org.au/ashm-directory

Table 3.4 Summary of post-test discussion: giving a positive result

| First post-test consultation |
|---|
| <ul style="list-style-type: none"> Establish rapport and assess readiness for the result |
| <ul style="list-style-type: none"> Give positive test result |
| <ul style="list-style-type: none"> Avoid information overload |
| <ul style="list-style-type: none"> Listen and respond to needs (the patient may be overwhelmed and hear little after being told the positive result) |
| <ul style="list-style-type: none"> Discuss immediate implications |
| <ul style="list-style-type: none"> Review immediate plans and support |
| <ul style="list-style-type: none"> Reassess support requirements and available services |
| <ul style="list-style-type: none"> Arrange other tests and the next appointment |
| <ul style="list-style-type: none"> Begin contact tracing process and discuss options available to facilitate this |
| Subsequent consultations |
| <ul style="list-style-type: none"> Treatment options, diet and exercise |
| <ul style="list-style-type: none"> Effect of diagnosis on relationships and information about prevention |
| <ul style="list-style-type: none"> Issues about disclosure |
| <ul style="list-style-type: none"> Assessment of contact tracing process and difficulties encountered |
| <ul style="list-style-type: none"> Access to life insurance may be affected |
| <ul style="list-style-type: none"> Workplace implications |
| <ul style="list-style-type: none"> Impact of other issues (eg. drug use, poverty, homelessness) on the ability to access health care and treatments |
| <ul style="list-style-type: none"> Referral for on-going counselling, social worker, or medical specialist, as appropriate |

Contact tracing

Contact tracing of individuals who may have been exposed during the infectious period of acute hepatitis should be undertaken to enable preventative measures to be implemented. Discussion with the patient regarding how to proceed with contact tracing may be appropriate. The clinician may ask the patient to consider recent blood-to-blood or sexual contacts as well as recent blood donations. It is recommended that primary care practitioners keep up to date with the relevant State or Territory guidelines.

Giving a negative result

Providing a negative test result provides an opportunity to reinforce harm reduction strategies and consider vaccination. If appropriate, the window period should be discussed and an appointment made for re-testing. Table 3.5 below provides a summary of the key points to be discussed in relation to giving a negative HBV test result.

Table 3.5 Summary of post-test discussion: giving a negative result

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| <ul style="list-style-type: none"> Explain the negative test result and the window period (if relevant) |
| <ul style="list-style-type: none"> Reinforce education regarding safe behaviours |
| <ul style="list-style-type: none"> Consider vaccination for hepatitis B, hepatitis A (if indicated), and, for women aged between 9 and 26, human papillomavirus (HPV) |
| <ul style="list-style-type: none"> Further discuss anxiety or risk behaviours |
| <ul style="list-style-type: none"> Discuss testing for other STIs |

Summary

While hepatitis B is a complex disease, an understanding of the parameters used to define HBV infection is crucial for the assessment and management of people with hepatitis B. With the emergence of new data, our accepted paradigms are changing, particularly relating to the importance and role of serum HBV DNA levels and the assessment of hepatic fibrosis. Ongoing research is needed to validate new data for routine clinical use.

References

1. Milich D, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 2003;38(5):1075-86.
2. Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, Haussinger D. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996;334(22):1422-7.
3. Lindh M, Hannoun C. Dynamic range and reproducibility of hepatitis B virus (HBV) DNA detection and quantification by Cobas Taqman HBV, a real-time semiautomated assay. *J Clin Microbiol* 2005;43(8):4251-4.
4. Weiss J, Wu H, Farrenkopf B, Schultz T, Song G, Shah S, Siegel J. Real time Taqman PCR detection and quantitation of HBV genotypes A-G with the use of an internal quantitation standard. *J Clin Virol* 2004;30(1):86-93.
5. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000–summary of a workshop. *Gastroenterology* 2001;120(7):1828-53.
6. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al for the REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *J Am Med Assoc* 2006;295(1):65-73.
7. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterol* 2006;130(3):678-86.
8. Lok AS, McMahon BJ. Chronic hepatitis B (AASLD guidelines). *Hepatology* 2007;45(2):507-39.
9. Medical Services Advisory Committee (MSAC). Application 1096: Hepatitis B DNA testing for chronic hepatitis B. Australian Government: Department of Health and Ageing, June 2007. Available at <http://www.health.gov.au/internet/msac/publishing.nsf/Content/app1096-1> (Accessed September 2007).
10. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology* 2004;40(4):790-2.
11. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterol* 2000;118(3):554-9.
12. Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000;33(6):998-1002.
13. Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002;36(6):1425-30.
14. Wang C, Shuhart M, Manansala J, Corey L, Kowdley K. High prevalence of significant fibrosis in patients with immunotolerance to chronic hepatitis B infection (abstr.). *Hepatology* 2005;42(4):573A.
15. Lai MD, Hyatt B, Afdal N. Role of liver biopsy in patients with normal ALT and high HBV DNA (abstr.). *Hepatology* 2005;42(4):720A.
16. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002;137(1):1-10.
17. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22(6):696-9.
18. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996;24(2):289-93.
19. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-32.
20. Myers RP, Tainturier MH, Ratziu V, Piton A, Thibault V, Imbert-Bismut F, et al. Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. *J Hepatol* 2003;39(2):222-30.
21. Marcellin P, DeLedinghen V, Dhumeaux D, et al. Non-invasive assessment of liver fibrosis in chronic hepatitis B using fibroscan® (abstr.). *Hepatology* 2005;42(4):715A.
22. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterol* 2005;128(2):343-50.

23. O'Sullivan B, Law M, Gidding H, Kaldor J, Gilbert G, Dore GJ. Hepatitis B in Australia: responding to a disease epidemic. Sydney: National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, 2000.
24. Mast EE, Margolis HS, Fiore AE, Brink EW, Goldstein ST, Wang SA, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP). Part I: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005;54(RR-16):1-31.
25. Mast EE, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP). Part II: immunization of adults. *MMWR Recomm Rep* 2006;55(RR-16):1-33; quiz CE31-34.
26. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepatol* 2004;11(2):97-107.
27. Bradford D, Hoy J, Matthews G, eds. HIV, viral hepatitis and STIs: a guide for primary care. Sydney: Australasian Society for HIV Medicine, 2008;92-4.