Hepatitis B virus DNA testing

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Printed copies of the report can be obtained from:

The Secretary
Medical Services Advisory Committee
Department of Health and Ageing
Mail Drop 106
GPO Box 9848
Canberra ACT 2601

Enquiries about the content of the report should be directed to the above address.

The Medical Services Advisory Committee (MSAC) is an independent committee which has been established to provide advice to the Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC recommendations do not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

This report was prepared by the Medical Services Advisory Committee with the assistance of Dr John Gillespie, Ms Antje Smala, Dr Nathan Walters, and Dr Liesl Birinyi-Strachan from M-TAG Pty Ltd, a unit of IMS Health. The report was endorsed by the Minister for Health and Ageing on 4 June 2007.

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Contents

Tables

Figures

Executive summary

The procedure

This report considers deoxyribonucleic acid (DNA) tests used as part of the clinical management of patients with chronic hepatitis B infection. The main techniques applied currently to assess hepatitis B virus (HBV) DNA are signal amplification by hybridisation, or branched DNA assays; and target sequence amplification by polymerase chain reaction assays.

Optimal clinical management requires a precise, sensitive and accurate assay to monitor response to antiviral therapy over time. The dynamic quantification ranges of available HBV DNA assays vary considerably but none covers the full range of HBV DNA values observed in treated and untreated chronic hepatitis B patients. Recently developed polymerase chain reaction assays are highly sensitive. These new generation assays enable quantitation of serum HBV DNA in samples with high viral loads without dilution and associated loss of accuracy.

Medical Services Advisory Committee—role and approach

The Medical Services Advisory Committee (MSAC) was established by the Australian Government to strengthen the role of evidence in health financing decisions in Australia. MSAC advises the Minister for Health and Ageing on the evidence relating to the safety, effectiveness and cost-effectiveness of new and existing medical technologies and procedures, and under what circumstances public funding should be supported.

A rigorous assessment of evidence is thus the basis of decision making when funding is sought under Medicare. A team from the Medical Technology Assessment Group (M-TAG) Pty Ltd, a unit of IMS Health, was engaged to conduct a systematic review of literature on hepatitis B DNA testing for chronic hepatitis B. An advisory panel with expertise in this area then evaluated the evidence and provided advice to MSAC.

MSAC's assessment of DNA testing for chronic hepatitis B

Clinical need

Hepatitis B is one of the world's most common infectious diseases. It is estimated that in Australia between 91,500 and 163,000 (0.49% and 0.87% of the population, respectively) people are chronically infected with hepatitis B. About half of the infected population are immigrants from southern or north eastern Asia (O'Sullivan et al 2004). Chronic HBV infection is associated with a 30 per cent risk of hepatic cirrhosis and carries a 5 to 10 per cent risk of people with the virus developing hepatocellular carcinoma. Estimates indicate that there are about 1200 deaths in Australia annually that are associated with chronic HBV and its complications.

Chronic hepatitis B infection is manifested by persistence of the virus and HBV surface antigen (HBsAg) in serum, production of hepatitis B e (HBeAg) viral antigens and HBV

DNA in the liver. The presence of HBeAg and HBV DNA is associated with an increased risk for developing hepatocellular carcinoma.

The primary goal of HBV treatment is to prevent clinical complications of chronic hepatitis B, including cirrhosis and hepatocellular carcinoma. Recent studies indicate that this can be achieved by durable suppression of viral replication.

The drug therapies currently reimbursed in Australia for chronic hepatitis B are lamivudine, entecavir, interferon-α, and adefovir dipivoxil. Lamivudine, entecavir and interferon-α are available as first line treatments for chronic hepatitis B as Section 100 items on the Pharmaceutical Benefits Scheme (PBS). Adefovir dipivoxil (PBS Section 100, monotherapy or combined with lamivudine) is available only for patients with lamivudine-resistant HBV. Lamivudine resistance occurs as a consequence of long term antiviral treatment for most chronic hepatitis B patients. The current Section 100 access guidelines mandate HBV DNA testing to verify presence of active hepatitis B replication.

Safety

Specimens for HBV DNA testing can be collected simultaneously along with samples for use in other tests, such as biochemical markers. Because specimens are collected using standard blood collection methods there are unlikely to be major safety issues relating to HBV DNA testing.

Effectiveness

Initial assessment of patients prior to antiviral therapy

The research question was:

'To what extent is hepatitis B virus (HBV) DNA testing safe, and effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and cost-effective in the initial assessment of patients with chronic hepatitis B prior to receiving antiviral therapy relative to current clinical practice?'

Monitoring of patients not receiving antiviral therapy

The research question was:

'To what extent is hepatitis B virus (HBV) DNA testing safe, and effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and cost-effective in monitoring patients with hepatitis B who do not require treatment relative to current clinical practice?'

Monitoring of patients receiving antiviral therapies

The research question was:

'To what extent is hepatitis B virus (HBV) DNA testing safe, and effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and cost-effective in monitoring of patients with chronic hepatitis B receiving antiviral therapy relative to current clinical practice?

Direct evidence

No studies were identified that had specific objectives of assessing additional benefits derived from HBV DNA testing on health outcomes compared with current clinical practice.

Studies by Chan et al (2002) and Lampertico et al (2005) informed assessment of the value of HBV DNA testing on patient outcomes. These studies compared outcomes between groups of patients before and after two different antiviral therapies were available.

HBV DNA testing and HBsAg positive renal transplant patients

Chan et al (2002) reported evidence to support that HBV DNA testing improves outcomes by reducing mortality risk among HBsAg positive renal transplant patients. This study considered HBV DNA testing to identify transplant patients whose DNA levels were likely to escalate. Patients were pre-emptively administered lamivudine, with or without elevation of alanine aminotransferase (ALT) levels.

When the pre-emptive strategy of HBV DNA testing with lamivudine therapy was readily available throughout the post-transplant period, patient survival was similar to HBsAg negative patients. When unavailable, patient survival declined and the relative risks of death and liver-related death increased. The study's design did not facilitate making associated additional comparative value assessments about benefits provided by HBV DNA and ALT testing.

HBV DNA testing and monitoring among patients undergoing treatment using lamivudine

Lampertico et al (2005) assessed outcomes of adefovir dipivoxil therapy among chronic hepatitis B e-antigen (HBeAg)-negative patients who were lamivudine resistant. Adefovir dipivoxil was administered either at the time point of genotypic resistance (rising serum HBV DNA levels) or phenotypic resistance (elevated ALT levels). Genotypic resistance can onlt be detected by measuring serum HBV DNA levels. Comparing adefovir dipivoxil treatment outcomes between genotypic and phenotypic resistance cohorts enable assessment of the impact of HBV DNA testing on patient outcomes.

Patients in the genotypic resistance cohort had a significantly greater response when adefovir dipivoxil therapy was co-administered with lamivudine, compared with the phenotypic resistance cohort patients. Greater response was measured as the proportion of patients who had a significantly greater response compared with the phenotypic resistance cohort, as measured by the proportion of patients whose HBV DNA levels were undetectable after three months of adefovir dipivoxil therapy. Normalisation of ALT levels was time dependent in the phenotypic resistance cohort. In contrast, ALT levels were normal throughout the study period in the genotypic patient cohort. Patients in the genotypic cohort were administered adefovir dipivoxil at first signs of lamivudine resistance.

Lampertico et al (2005) found that alanine aminotransferase (ALT) testing alone may not be the most effective means of detecting the initial development of lamivudine resistance. Including regular HBV DNA testing can improve monitoring of patients undergoing lamivudine monotherapy. This study provides evidence that HBV DNA

testing has the potential to alter management of patients undergoing lamivudine therapy and can result in improvements in short term outcomes.

Linked evidence

Because direct evidence relating to HBV DNA testing on patient outcomes was not considered applicable to all research questions in this assessment, linked evidence was required.

Accuracy studies

Initial assessment and monitoring of patients not receiving antiviral therapies

Studies by Chan et al (2003), Lindh et al (2000), Manesis et al (2003), Peng et al (2003), and Seo et al (2005) enabled accuracy assessment of serum HBV DNA testing to be made for initial assessment or monitoring of patients not undergoing antiviral therapy.

Manesis et al (2003) and Seo et al (2005) assessed the value of HBV DNA testing to differentiate inactive HBeAg negative carriers from HBeAg negative active chronic hepatitis B patients. Manesis et al (2003) reported that serum HBV DNA with a cut point of 30,000 copies/mL had the best diagnostic performance to differentiate active from inactive carriers. Analysis of the study design found potential for patient misclassification. Evidence was also found to suggest that HBV DNA testing contributes additional diagnostic performance to differentiate these patient groups by immunoglobulin anti-hepatitis B core (Hbc) complex measurement. Seo et al (2005) reported that a suitable serum HBV DNA cut point could not be established without misclassifying patients. Both studies were considered to provide poor quality evidence because the index test (HBV DNA test) was interpreted with knowledge of the reference standard.

Lindh et al (2000) and Peng et al (2003) reported HBV DNA test assessments to predict liver histology and damage. Results reported by Lindh et al (2000) indicated that increased serum HBV DNA levels were associated with extensive liver damage. This was true among HBeAg negative patients, but there was no association between serum HBV DNA levels and liver damage among HBeAg positive patients. Results reported by Peng et al (2003) provided modest evidence that increased serum HBV DNA level is associated with increased liver damage among HBeAg negative patients. Both studies were considered poor quality because the index test was interpreted with knowledge of the reference standard.

Chan et al (2003) reported that serum HBV DNA levels greater than 10^5 copies/mL at the time of, or after seroconversion, are predictive of HBeAg reversion. This study lacked sufficient reference standard reporting and was considered to offer poor quality evidence.

The accuracy studies considered did not include sufficient data to assess the value of HBV DNA testing in addition to other tests, such as alanine aminotransferase, serology, or liver histology. The additional value of HBV DNA testing was reported by Manesis et al (2003).

Monitoring of patients receiving antiviral therapies

Lamivudine

The value of HBV DNA testing among patients treated with lamivudine was assessed by Buti et al (2001) and Zollner et al (2001). Buti et al (2001) reported using real-time polymerase chain reaction. Negative HBV DNA test results at month three of therapy was found to be a sensitive indicator of sustained response. When HBV DNA testing was positive at month 3, this was a moderately specific indicator of lack of sustained response. This study was considered poor quality because the index test was also the reference standard

Zollner et al (2001) reported sequential HBV DNA measurement by real time polymerase chain reaction during lamivudine therapy. HBV DNA was assessed to determine its value to predict HBeAg seroconversion and emergence of drug resistance. Results from this study indicated that HBV DNA tests reporting a value of <log 2 copies/mL at month 12 of therapy was the optimal time to predict seroconversion or resistance to lamivudine. Patients with HBV DNA levels above the detection limit at 12 and 15 months did not have seroconversion (negative predictive value = 100%), indicating that these patients did not respond to therapy. Evidence reported by this study was also considered to be poor quality because interpretation of the index test was not independent from the reference standard.

The additional value of HBV DNA testing was not reported by these studies.

Interferon

Lindh et al (2001) and van der Eijk et al (2006) assessed the value of HBV DNA testing to predict treatment response among patients undergoing interferon treatment.
Lindh et al (2001) sought to determine whether pre-treatment HBV DNA levels could predict sustained virological response among patients treated with interferon who had undergone earlier priming doses of prednisolone. There was limited value in differentiating non-sustained responders from sustained responders when a serum HBV DNA threshold value of <log 8.7 copies/mL was used.

Van der Eijk et al (2006) evaluated HBV DNA testing by real-time polymerase chain reaction assay as a response predictor among HBeAg positive chronic hepatitis B patients who were treated with interferon-α. Log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 12 was better able to predict the proportion of patients who would not respond to treatment.

The additional value of HBV DNA testing was not reported by these studies.
Both studies were considered poor quality evidence because the index test was part of the reference standard.

Serum HBV DNA and clinical outcomes

The absence of studies that assessed the accuracy or predictive ability of HBV DNA testing to predict long term clinical outcomes, such as cirrhosis and hepatocellular carcinoma, meant that it was considered necessary to provide supporting evidence indicating the link between HBV DNA levels and clinical outcomes. These studies did not assess whether the test changed outcomes, but offer supportive evidence that knowledge of serum HBV DNA levels can assist to determine patients' prognoses.

Systematic review

A systematic review by Mommeja-Marin et al (2003) investigated the relationship between serum HBV DNA levels and clinical outcomes. Although this systematic review did not show that the (additional) using the test affects outcomes, evidence was provided that HBV DNA levels can be predictive of outcome and may illustrate treatment efficacy.

Hepatocellular carcinoma risk

The relationship between serum HBV DNA levels and hepatocellular carcinoma was investigated by Chen et al (2006), Harris et al (2003) and Okhubo et al (2001). The long term, population based prospective study by Chen et al (2006) found that increased HBV DNA levels at baseline indicated an independent risk factor for hepatocellular carcinoma among both HbeAg negative and positive patients. Patients with elevated serum HBV DNA levels, who did not have liver cirrhosis, carried increased risk of developing hepatocellular carcinoma. These analyses involved sampling HBV DNA at a single point. This approach did not capture potential variations in viral replication over the follow-up period or relationship to hepatocellular carcinoma. Additional analysis indicated that persistently elevated serum HBV DNA levels lead to an increased risk of developing hepatocellular carcinoma.

Okhubo et al (2001) found that serum HBV DNA level measured when hepatocellular carcinoma was diagnosed was an independent prognostic factor for survival. Lower HBV DNA levels were associated with increased patient survival.

Stable or unstable serum HBV DNA seroconversion was found to be a prognostic factor for hepatocellular carcinoma death by Harris et al (2003). Patients who became HBV DNA negative spontaneously, regardless of whether levels were stable were found to have an increased risk of hepatocellular carcinoma death. Evidence indicating that low serum HBV DNA levels at baseline are associated with a reduced risk of hepatocellular carcinoma death was also presented

Cirrhosis risk

The relationship between serum HBV DNA levels and risk of cirrhosis was explored by the REVEAL-HBV study. Iloeje et al (2005) reported that increased baseline serum HBV DNA levels were found to be associated with a greater cumulative incidence of cirrhosis. Serum HBV DNA levels were found to be an independent risk factor for cirrhosis. The study's methodology required HBV DNA to be sampled at a single point which did not capture potential variations in viral replication during the follow-up period.

Risk of hepatocellular carcinoma among patients with HBV-related cirrhosis

Ishikawa et al (2001) and Mahmoud et al (2005) investigated the association between serum HBV DNA levels and development of hepatocellular carcinoma among patients with HBV-related cirrhosis. Both studies reported that elevated serum HBV DNA was the strongest prognostic factor for hepatocellular carcinoma among patients with HBV-related cirrhosis.

Risk of hepatocellular carcinoma recurrence after resection

Kubo et al (2000) and Kubo et al (2003) assessed the value of HBV DNA testing as a prognostic factor for hepatocellular carcinoma recurrence after resection. These studies suggest that evidence of high serum HBV DNA levels before resection is a significant prognostic indicator of recurrent hepatocellular carcinoma.

Summary of evidence for effectiveness of serum HBV DNA testing

A review was conducted to identify evidence relating to the effectiveness of HBV DNA testing for chronic hepatitis B patients.

Direct evidence

There was limited evidence that:

- HBV DNA testing in addition to ALT testing alters patient management and improves short term health outcomes when used to identify drug resistance among patients undergoing lamivudine therapy
- HBV DNA test monitoring improved health outcomes among HbsAg positive renal transplant patients. The additional value provided by HBV DNA testing for these patients could not be measured.

Linked evidence: Accuracy studies

HBV DNA testing, initial assessment and monitoring of patients not undergoing antiviral therapy

There was limited evidence that HBV DNA testing used in initial assessment or monitoring of patients not undergoing antiviral therapy:

- HBV DNA testing enabled differentiation between inactive HBeAg negative carriers and HBeAg negative active chronic hepatitis B patients
- increased serum HBV DNA levels were associated with increased liver damage among HBeAg negative patients
- elevated serum HBV DNA levels were predictive of HbeAg reversion.

There was insufficient evidence for the additional value of HBV DNA testing in these patient groups.

HBV DNA testing and monitoring among patients undergoing antiviral therapy

There was limited evidence to support that HBV DNA testing used to monitor patients undergoing antiviral therapies:

- HBV DNA testing can predict sustained response to lamivudine therapy
- HBV DNA testing can predict HbeAg seroconversion or resistance to lamivudine
- HBV DNA testing can predict patients who would not respond to lamivudine therapy
- pre-treatment HBV DNA levels can differentiate between sustained and non-sustained responders among patients treated with interferon-α
- HBV DNA testing at baseline and during treatment can predict patients who would not respond to interferon treatment.

There was insufficient evidence for the additional value of HBV DNA testing in these patient groups.

Linked evidence: Serum HBV DNA testing and clinical outcomes

Evidence concerning the relationship between HBV DNA levels and long term clinical outcomes is also summarised:

- a systematic review provided evidence that HBV DNA testing can predict outcomes and illustrate treatment efficacy
- serum HBV DNA levels were shown to be indicative of survival following hepatocellular carcinoma diagnosis and risk of death from hepatocellular carcinoma
- elevated serum HBV DNA levels were associated with increased risk and cumulative incidence of hepatocellular carcinoma
- elevated serum HBV DNA levels were associated with increased risk and cumulative incidence of cirrhosis
- elevated serum HBV DNA levels were a prognostic factor for hepatocellular carcinoma among patients with HBV-related cirrhosis
- elevated serum HBV DNA levels were prognostic of recurrence following surgical resection to treat hepatocellular carcinoma.

Cost-effectiveness

Based on price information for the four different test systems currently available in Australia (Digene Hybrid Capture II assay, Bayer Versant HBV 3.0 test, Roche COBAS TaqMan HBV test, and Qiagen [Artus] RealArt HBV PCR), each HBV DNA test would cost Medicare Australia about $130, if listed for reimbursement.

Research questions were formulated to assess economic considerations associated with use of HBV DNA testing:

1. to conduct initial assessments of patients with chronic hepatitis B infection before initiating drug treatment
2. to monitor patients not undergoing antiviral treatment, and
3. to monitor patients undergoing antiviral therapy.

Testing of all newly reported HBV infections, assuming patients were tested once, would create demand for around 6500 tests per year. Based on historic incidence, future demand is forecast to remain stable at this rate.

The number of chronic hepatitis B patients to be monitored while not undergoing antiviral drug treatment is expected to be low and stable at about 8200 patients annually. Each patient would be tested annually to monitor disease course.

Increased HBV DNA testing could be expected if used to monitor patients undergoing antiviral treatment, including interferon. Numbers of patients treated with lamivudine

with or without co-administration of adefovir, or those treated with interferon, is expected to increase over time, generating higher demand for HBV DNA testing. Patients receiving antiviral treatments are expected to be tested quarterly; people treated with interferon would be tested three times during a defined 12-month period. The total number of monitoring tests was calculated to be around 4700 to 5900 tests per year.

Total demand is forecast to be about 20,000 tests annually. The expected cost to Medicare Australia would be between $2.5 and $2.7 million per year.

Other healthcare funders are also likely to benefit from listing HBV DNA testing on the Medicare Benefits Schedule because the test can identify patients who would not benefit from particular drug treatments. Patients who test negative for hepatitis B virus could discontinue drug treatment, and appropriate early interventions applied when drug resistance occurs. A detailed assessment of these benefits is not included because current evidence does not support changing long term outcomes following HBV DNA testing.

Recommendation

MSAC has considered the safety, effectiveness and cost effectiveness of the use of hepatitis B assays in the pre-treatment assessment and in the monitoring of patients with chronic hepatitis B.

MSAC finds there is sufficient evidence of the safety, effectiveness and cost effectiveness of hepatitis B assay in the pre-treatment and in the monitoring of patients with chronic hepatitis B.

MSAC recommends that public funding be provided for the use of hepatitis B assay in patients with chronic hepatitis B.

MSAC further recommends that the number of hepatitis B assays for pre-treatment assessment or for the monitoring of patients with chronic hepatitis B who are not on antiviral therapy be restricted to one assay in a twelve month period and for patients on antiviral therapy the number of assays be restricted to four assays in a twelve month period.

–The Minister for Health and Ageing accepted this recommendation on 4 June 2007–

Introduction

The Medical Services Advisory Committee (MSAC) has reviewed the use of hepatitis B DNA diagnostic testing for chronic hepatitis B. MSAC evaluates new and existing health technologies and procedures for which public funding is sought in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

MSAC's terms of reference and membership are at **Appendix A**. MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, oncology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

This report summarises the assessment of current evidence for hepatitis B DNA testing for the chronic phase of the disease.

Background

DNA testing for chronic hepatitis B

The procedure

Deoxyribuonucleic acid (DNA) tests used in the management of hepatitis B viral infection were assessed for this report. Measurement of hepatitis B virus (HBV) DNA occurs during routine clinical assessment and monitoring of people with chronic hepatitis B infections. Serum samples are obtained during clinical assessment which are analysed for viral serology, alanine aminotransferase (ALT) and HBV DNA levels.

The serum sample is processed for DNA testing. The resulting sample is then assessed using one of the available HBV DNA diagnostic assay kits. This involves further sample preparation, DNA amplification and detecting HBV DNA specific to the assay kit.

There are two main techniques to assess HBV DNA—either through signal amplification (sometimes referred to as hybridisation or branched DNA assays) or target sequence amplification (polymerase chain reaction [PCR] assays). Target sequence amplification techniques use primers to target specific HBV DNA sequences. Target sequences then undergo PCR amplification and direct detection to quanitify HBV DNA. Signal amplification techniques use target probes (ribonucleic acid [RNA] or oligonucleotide sequences) which combine with target DNA to form hybrid sequences. These hybrid sequences are then isolated, amplified and quantitatively assessed, typically by measuring a chemiluminescent reaction (Chen et al 2006).

Diagnostic assay kits quantify HBV DNA levels present in samples. Assay kits have different minimum detection levels for HBV DNA (see Table 1). Diagnostic capabilities may differ between kits, an aspect which was considered in this assessment.

Table 1 Minimum detection levels of HBV DNA diagnostic assay kits

Assay	Minimum detection level
Signal amplification assays	
Genostix	4 to 5 x 10^5 c/mL[a]
Digene HBV test hybrid capture II (standard)	1.4 x 10^5 c/mL
Digene HBV test hybrid capture II (ultra sensitive)	4.7 x 10^3 c/mL
Quantiplex	7 x 10^5 c/mL
Target sequence amplification assays	
Microwell plate Amplicor HBV monitor	10^3 c/mL
Cross-linking naxcor polymerase chain reaction	5 x 10^5 c/mL
Real time polymerase chain reaction	300 c/mL
Cobas Amplicor HBV monitor	4 x 10^2 c/mL
Polymerase chain reaction beacon molecular detection	100 c/mL
TMA-HPA	5 x 10^3 c/mL

Abbreviations: c/mL, copies per millilitre; HBV, hepatitis B virus; TMA-HPA, transcription-mediated amplification and hybridisation protection assay

[a] Corrected value 4 to 5 x 10^8 c/mL

Source: Mommeja et al (2003)

Intended purpose

The viral load, defined as the amount of HBV DNA contained in a serum sample, is considered to be a measure of viral replication level. These levels can be used to identify optimal timing for initiation, continuation, altering or termination of antiviral therapy (Keeffe et al 2004).

The purpose of HBV DNA testing is:

- for initial and ongoing assessment of viral load among patients with chronic hepatitis B who are not undergoing antiviral drug treatment, and
- for subsequent viral load monitoring among patients with chronic hepatitis B undergoing antiviral treatment.

Reference standard

Diagnoses made with new tests must be compared with the true status of the relevant disease to ascertain the test's accuracy. In practical and ethical terms it is often unfeasible to determine unequivocal disease status. Proxy measures—such as another diagnostic test or clinical judgement—may be applied to stand for disease states. The best available measure of disease is called the reference standard.

In this review, the reference standard to assess and monitor chronic hepatitis B infection is histologically examined hepatic tissue obtained by biopsy. Other reference standards regarded as approporiate included clinical outcomes, such as disease progression (cirrhosis, cancer, death); ALT normalisation; viral load (determined by HBV DNA testing); durable seroconversion; and quality of life. Histological sampling combined with clinical outcomes is also regarded as an appropriate reference standard.

Clinical need and burden of disease

The World Health Organization estimates that more than 350 million people globally are chronically infected with the hepatitis B virus (Kao et al 2002; McMahon 2004). In Australia, chronic hepatitis B prevalence is between 91,500 and 163,000 (0.49% and 0.87% of the Australian population, respectively). About half of all infected people migrated from southern or north eastern Asia (O'Sullivan et al 2004).

Chronic hepatitis B infection is associated with a 30 per cent risk of cirrhosis and 5–10 per cent risk of developing hepatocellular carcinoma. About 1200 people in Australia (Gust 1996), and about a million people worldwide (Kao et al 2002; McMahon 2004), die from chronic hepatitis B infection and its complications annually. Australia implemented universal hepatitis B vaccination for all newborns in 2000, but impact on hepatitis B incidence is unlikely to be evident for at least another 15 years.

Natural history

About 3–5 per cent of adults exposed to the hepatitis B virus will develop chronic infection—circulating hepatitis B surface (HBsAg) and hepatitis B e (HBeAg) antigens will be evident in these people. HBeAg seroconversion, where there is clearance of HBeAg and development of HBeAg (anti-HBe) antibodies, can occur spontaneously or result from treatment. Although these people may have circulating HBsAg particles, levels of HBV DNA are low or undetectable. These people have a reduced risk of hepatic decompensation and a twofold lower risk of death (McMahon 2004). Up to 30 per cent of infected people may have repeated ALT flares which can lead to progressive fibrosis (McMahon 2004). Hepatitis B viral infection does not persist among people with acute disease. People with acute hepatitis B virus initially test positive for HBsAg, HBeAg, and HBV DNA. These markers disappear over time.

Although HBeAg can be regarded as a hepatitis B virus replication marker, the type of HBV genotype (A–H) determines the percentage of people (1–25%) who develop HBeAg (pre-core) escape mutants during HBeAg seroconversion. The genotype variants prevent formation of HBeAg but allow for moderate-to-high levels of viral replication. Genotype variants are indicated by persistently elevated serum ALT levels, absence of HBeAg, presence of anti-HBe, and moderate to high HBV DNA levels (typically $>10^5$copies/mL). This form of infection is termed HBeAg-negative chronic hepatitis B. Chronic hepatitis B can be classified as either of two major forms: HBeAg positive and HBeAg negative.

Chronic hepatitis B has four phases: replicative/immune tolerant, HBeAg clearance, low or non-replicative and replicative or re-activating.

The replicative/immune tolerant phase is characterised by high levels of HBV DNA, HBeAg positivity, minimal liver inflammation, normal liver enzyme levels (generally), and low risk of progression to liver disease.

HBeAg clearance occurs in the second phase. HBV DNA levels fluctuate and decline during this stage, HBeAg is lost, and seroconversion (development of anti-HBe antibodies) occurs. There is a moderate-to-high degree of liver inflammation and liver enzymes, and liver disease progression is often rapid. This phase can be protracted and many people remain HBeAg positive for years, if not indefinitely. During seroconversion some patients develop the pre-core or basic core promoter variant of the virus that results in HBeAg negative chronic hepatitis B. People whose HBeAg clearance phase is short often progress to the third phase of low or non-replicative chronic hepatitis B.

The third phase is associated with low to undetectable levels of serum HBV DNA, persistently normal liver enzymes and negligible liver inflammation, minimal liver damage and low risk of liver disease.

People in phase three can progress to the fourth phase of chronic hepatitis B—the replicative or re-activating phase. Features of this phase are that HBV DNA levels increase, HBeAg is usually negative, serum enzyme levels are elevated and there is potential for further liver disease progression. Immune suppression, resulting from steroid therapy or chemotherapy, can contribute to development of replicative disease.

Spontaneous flares of disease activity during the natural course of chronic hepatitis B may lead to progressive hepatic fibrosis, cirrhosis and carcinogenesis (Perrillo 2001). Mortality rates at five years are 16 per cent for those with compensated cirrhosis, where the liver is damaged but can still function (Realdi et al 1994; Perrillo 2004), and 65–86 per cent (in the absence of liver transplantation) for people with decompensated cirrhosis, and whose liver function is severely impaired (Perrillo 2004).

The primary goal of treating hepatitis B is to prevent clinical complications of chronic disease. Recent data indicate that this goal can be achieved by durable suppression of viral replication. The optimal target level for HBV DNA suppression, associated with favourable alteration of natural history (if any), has not yet been conclusively identified. Cohort studies of chronic hepatitis B patients have shown that the risk of progression to advanced liver disease complications is considerably higher among patients with replicative disease compared with non-replicative disease (Realdi et al 1994; Villeneuve 1994). Control of serum HBV DNA levels has important clinical implications.

Serum HBV DNA and treatment for chronic hepatitis B

A panel of USA-based hepatologists have developed a treatment algorithm for chronic hepatitis B that uses serum HBV DNA levels to inform clinical decisions about treatment initiation (Keeffe et al 2004). Specifically, a serum HBV DNA threshold of $\geq 10^4$–10^5 IU/mL was recommended as a candidacy baseline for patients with HBeAg positive chronic hepatitis B. A lower threshold ($\geq 10^3$–10^4 IU/mL) was recommended for patients with HBeAg negative chronic hepatitis B. A further recommendation addresses treatment for patients with decompensated cirrhosis.

The treatment algorithm was subsequently revised to include a serum HBV DNA threshold level for treatment candidacy of 20 000 IU/mL or more for patients with HBeAg-positive chronic hepatitis B (Keeffe et al 2006). Other modifications to the earlier treatment algorithm include a serum HBV DNA threshold of 2000 IU/mL for patients with HBeAg-negative chronic hepatitis B and 200 IU/mL or more for patients with decompensated cirrhosis (Keeffe et al 2006).

The American Association for the Study of Liver Disease (AASLD) practice guidelines (Lok 2004) recommend that patients with decompensated cirrhosis patients whose HBV DNA $<10^4$–10^5 IU/mL should be referred to a liver transplant unit for assessment and treatment. These data also recommend that treatment criteria be based on elevated serum ALT, and/or liver biopsy findings.

Therapies available to treat chronic hepatitis B in Australia are:

1. lamivudine
2. interferon-α (including α-2a and α-2b, which are also available as investigational agents for HBV in their pegylated forms)
3. adefovir dipivoxil
4. entecavir.

Lamivudine, interferon-α and entecavir are available as first line treatments for chronic hepatitis B as Section 100 items on the PBS. Adefovir dipivoxil (monotherapy or combined with lamivudine) (also Section 100) is available only for patients with

lamivudine-resistant HBV. Lamivudine resistance occurs as a consequence of the need for long term antiviral treatment for most chronic hepatitis B patients (Locarni et al 2004).

HBV DNA testing is an essential criterion to establish patient eligibility for treatment with lamivudine, interferon-α, adefovir dipivoxil, or entecavir.

Existing procedures

Viral serology

Hepatitis B virus surface antigen (HBsAg and HBeAg) measurement is used in diagnosis and during routine clinical assessment and monitoring of chronic infection. Confirmation of serological marker HBsAg in serum taken over six months or more indicates chronic hepatitis B infection. Active HBV replication is indicated by the presence of the serological marker HBeAg in serum samples. Absence of this marker does not necessarily indicate that viral replication is not occurring, even among patients who were previously HBeAg positive, because some hepatitis B virus variants have mutations that prevent serological detection of this antigen (Keeffe et al 2004).

Alanine aminotransferase (ALT) levels

Alanine aminotransferase (ALT) enzyme level measurement is used to monitor liver inflammation and inform routine clinical assessment. ALT levels within the normal range are generally regarded as predictive of quiescence of inflammatory activity, and above normal levels indicate active inflammation (Keeffe et al 2004). Elevated ALT levels can indicate hepatitis B infection, but a number of other conditions can also cause active liver inflammation.

Liver biopsy

Liver biopsy is used in the clinical assessment of chronic hepatitis B infection. Biopsied liver tissue is histologically examined to determine the extent of liver disease. It is a minimally invasive surgical technique using a biopsy needle that involves percutaneous sampling, with or without CT or ultrasound imaging, of a small portion of the liver (Zaman et al 2006). Liver biopsy complications may include pain and intrahepatic and/or subcapsular bleeding. Arteriovenous fistula, haemorrhage, bile peritonitis, bacteraemia, sepsis, pneumothorax and haemothorax are less common complications. There is a small risk of mortality associated with liver biopsy (Zaman et al 2006).

Biopsied liver tissue is typically assessed by a pathologist using a histological grading scale to determine disease extent. The Knodell histology activity index, Scheuer system, Ishak system, Ishak modified histology activity index and the METAVIR system are common grading scales. The Knodell histology activity index is shown in Table 2.

Table 2 Knodell histology activity index

Periportal +/– bridging necrosis		Intralobular degeneration and focal necrosis		Portal inflammation		Fibrosis	
None	0	None	0	None	0	None	0
Mild piecemeal necrosis	1	Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in less than a third of lobules or nodules)	1	Mild (few inflammatory cells in less than a third of portal tracts)	1	Fibrous portal expansion	1
Moderate piecemeal necrosis (involves <50% of the circumference of most portal tracts)	3	Moderate (involvement of one to two-thirds of lobules or nodules)	3	Moderate (increased incidence of inflammatory cells in one to two-thirds of portal tracts)	3	Bridging fibrosis (portal-portal or portal-central linkage)	3
Marked piecemeal necrosis (involves >50% of the circumference of most portal tracts)	4	Marked (involvement of more than two-thirds of lobules or nodules)	4	Marked (high density of inflammatory cells in more than two-thirds of portal tracts)	4	Cirrhosis	4
Moderate piecemeal necrosis plus bridging necrosis [a]	5						
Marked piecemeal necrosis plus bridging necrosis [a]	6						
Multilobular necrosis	10						

[a] Hepatitis activity index (HAI) score is the combined scores for necrosis, inflammation, and fibrosis
Source: Brunt EM (2000). 'Grading and staging the histopathological lesions of chronic hepatitis: The Knodell histology activity index and beyond'. *Hepatology* 31: 241–246. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

HBV DNA testing would be used as an additional test in routine clinical assessment and monitoring of people with chronic hepatitis B infection. The relative value of HBV DNA testing to viral serology, ALT enzyme level measurement, and liver biopsy were considered in this assessment.

Marketing status of the technology

Commercial HBV DNA diagnostic assay kits are available in Australia. They are exempted from the current regulatory requirements of the Therapeutic Goods Act, 1989.

Current reimbursement arrangement

There is currently no reimbursement arrangement with the Medicare Benefits Scheme regarding HBV DNA testing.

Approach to assessment

Research questions and clinical pathways

Initial assessment of patients with chronic hepatitis B before undergoing antiviral therapy

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) developed *a priori* for evaluation of HBV DNA testing in initial assessment of patients with chronic hepatitis B infection before antiviral therapy is given in Table 3.

Table 3 PPICO criteria for the use of HBV DNA testing in the initial assessment of patients with chronic hepatitis B before undergoing antiviral therapy

Population	Prior tests[a]	Index test	Comparator	Outcomes
Chronic hepatitis B patients before undergoing antiviral therapy	History and physical examination Laboratory test to assess liver disease Tests to rule out other causes of liver disease Tests for co-infection with other viruses Consider screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology	HBV DNA testing	Current clinical practice, including: HBsAg and HBeAg serology, ALT levels and/or liver biopsy	Change in clinical management Change in clinical outcomes Diagnostic accuracy

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBsAG, surface antigen of the hepatitis B virus; HBeAG, hepatitis B e antigen

[a] These tests are not required before HBV DNA testing (advisory panel advice)

The research question for this indication, based on these criteria, was as follows.

To what extent is hepatitis B virus (HBV) DNA testing:

- safe, and
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in the initial assessment of patients with chronic hepatitis B prior to receiving antiviral therapy relative to current clinical practice?

The clinical pathways[1] for the initial assessment of patients with chronic hepatitis B infection before antiviral therapy are shown in Figure 1 and Figure 2. Figure 1 illustrates

[1] Clinical pathways also illustrate HBV DNA test use for monitoring chronic hepatitis B patients not receiving antiviral therapy.

the clinical pathway without availability of HBV DNA testing. Figure 2 shows the clinical pathway including availability of HBV DNA testing.

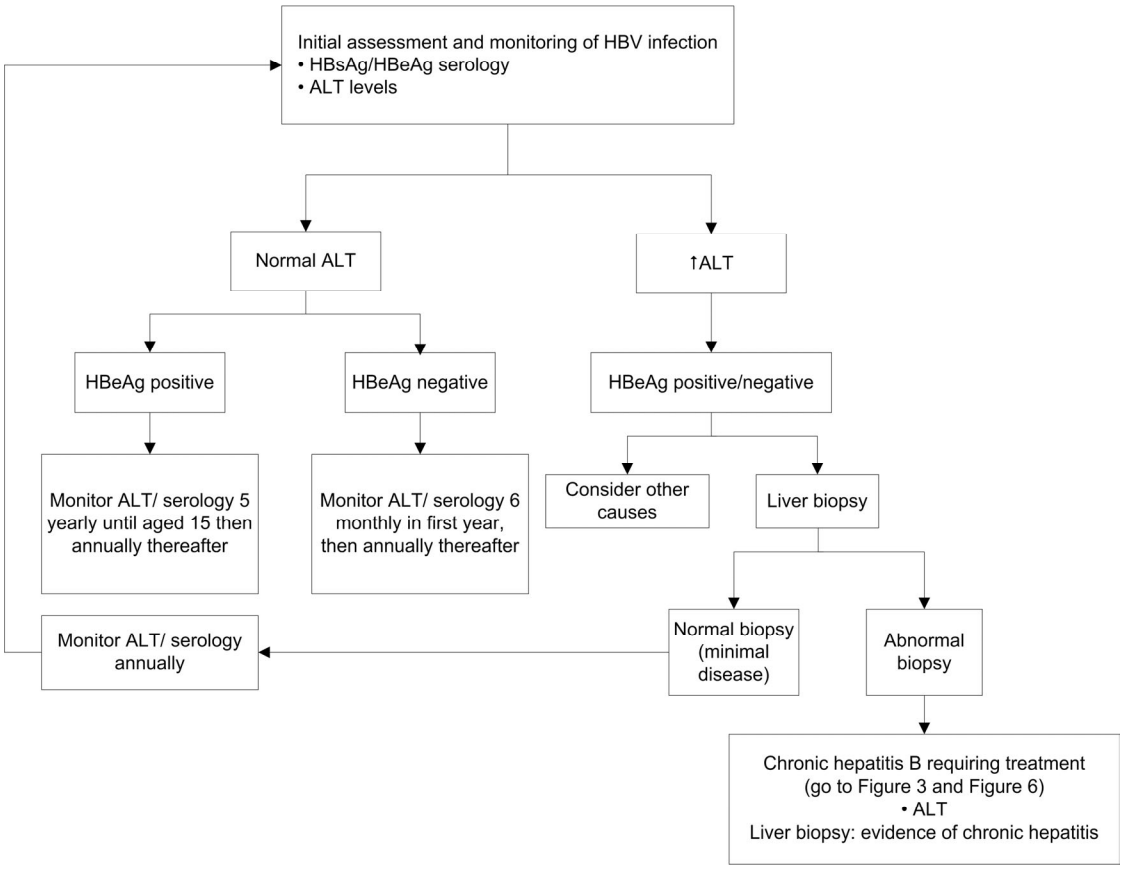

Figure 1 Clinical pathway: Initial assessment of patients with chronic hepatitis B prior to receiving antiviral therapy and monitoring of chronic hepatitis B patients not undergoing therapy, HBV DNA testing not available

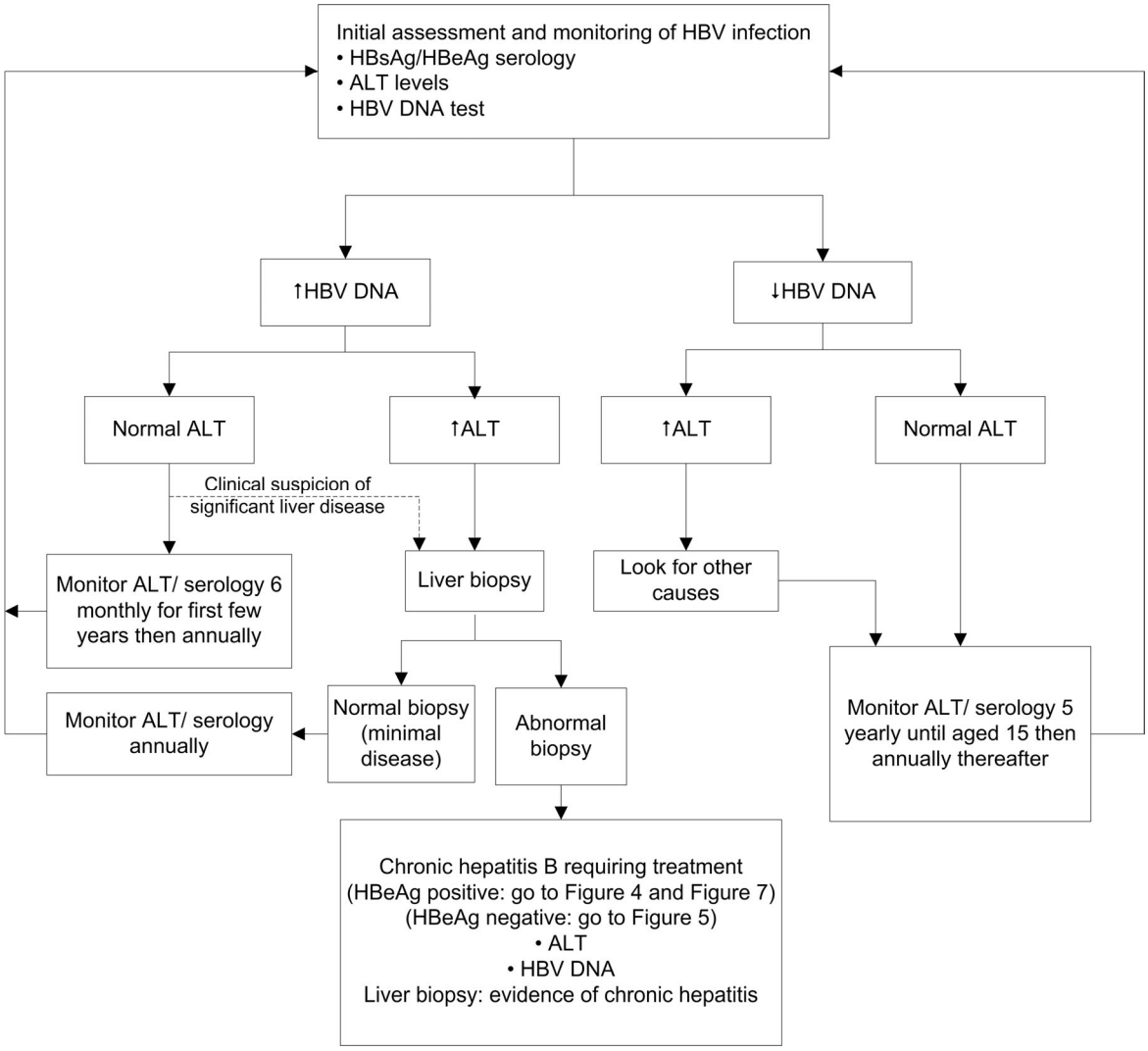

Figure 2 Clinical pathway: Initial assessment of patients with chronic hepatitis B before undergoing antiviral therapy and monitoring of chronic hepatitis B patients not undergoing therapy, with HBV DNA testing available

[a] Current recommendations for treatment thresholds differ between HBeAg positive patients (10^5), HBeAg negative patients (10^4) and inpatients with cirrhosis or liver decompensation (10^3)

Monitoring of patients with chronic hepatitis B not receiving antiviral therapy

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) developed *a priori* for evaluation of HBV DNA testing to monitor chronic hepatitis B patients who were not undergoing antiviral therapy are presented in Table 4.

Table 4 PPICO criteria for the use of HBV DNA testing in monitoring patients with chronic hepatitis B not receiving antiviral therapy

Population	Prior tests[a]	Index test	Comparator	Outcomes
Chronic hepatitis B patients not undergoing treatment	History and physical examination Laboratory test to assess liver disease Tests to rule out other causes of liver disease Tests for co-infection with other viruses Consider screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology, HBV DNA testing	HBV DNA testing	Current clinical practice, including: HBsAg and HBeAg serology and/or ALT levels	Change in clinical management Change in clinical outcomes Diagnostic accuracy

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBsAG, surface antigen of the hepatitis B virus; HBeAG, hepatitis B e antigen

[a] These tests are not required before HBV DNA testing (advisory panel advice)

The research question for this indication, based on these criteria, was as follows.

To what extent is hepatitis B virus (HBV) DNA testing:

- safe, and
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in the monitoring of patients with hepatitis B not requiring treatment relative to current clinical practice?

The clinical pathways for monitoring patients with chronic hepatitis B not receiving therapy are shown in Figure 1 and Figure 2. Figure 1 indicates the clinical pathway without HBV DNA testing available**;** Figure 2 displays the clinical pathway with HBV DNA testing available.

Monitoring of patients with chronic hepatitis B receiving antiviral therapy

The PPICO criteria (target population, prior tests, index test, comparator, outcomes) developed *a priori* to evaluate HBV DNA testing to monitor chronic hepatitis B patients undergoing antiviral therapy are presented in Table 5.

Table 5 PPICO criteria for the use of HBV DNA testing in the monitoring of patients with chronic hepatitis B receiving antiviral therapy

Population	Prior tests [a]	Index test	Comparator	Outcomes
Chronic hepatitis B patients undergoing antiviral treatment	History and physical examination Laboratory test to assess liver disease Tests to rule out other causes of liver disease Tests for co-infection with other viruses Consider screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology HBV DNA testing	HBV DNA testing	Current clinical practice, including: HBsAg and HBeAg serology, ALT levels, and/or liver biopsy	Change in clinical management Change in clinical outcomes Diagnostic accuracy

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBsAG, surface antigen of the hepatitis B virus; HBeAG, hepatitis B e antigen

[a] These tests are not required before HBV DNA testing (advisory panel advice)

The research question for this indication, based on these criteria, was as follows.

To what extent is hepatitis B virus (HBV) DNA testing:

- safe, and
- effective (including diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes), and
- cost-effective

in monitoring chronic hepatitis B patients who are undergoing antiviral therapy, relative to current clinical practice?

Clinical pathways for monitoring chronic hepatitis B patients undergoing antiviral therapy are represented in Figures 3–7. Figure 3 illustrates the clinical pathway omitting availability of HBV DNA testing. Clinical pathways that include availability of HBV DNA testing are presented in Figure 4 (HBeAg positive patients), and Figure 5 (HBeAg negative patients). Clinical pathways for patients undergoing interferon therapy are represented in Figure 6 (without HBV testing) and Figure 7 (with HBV testing).

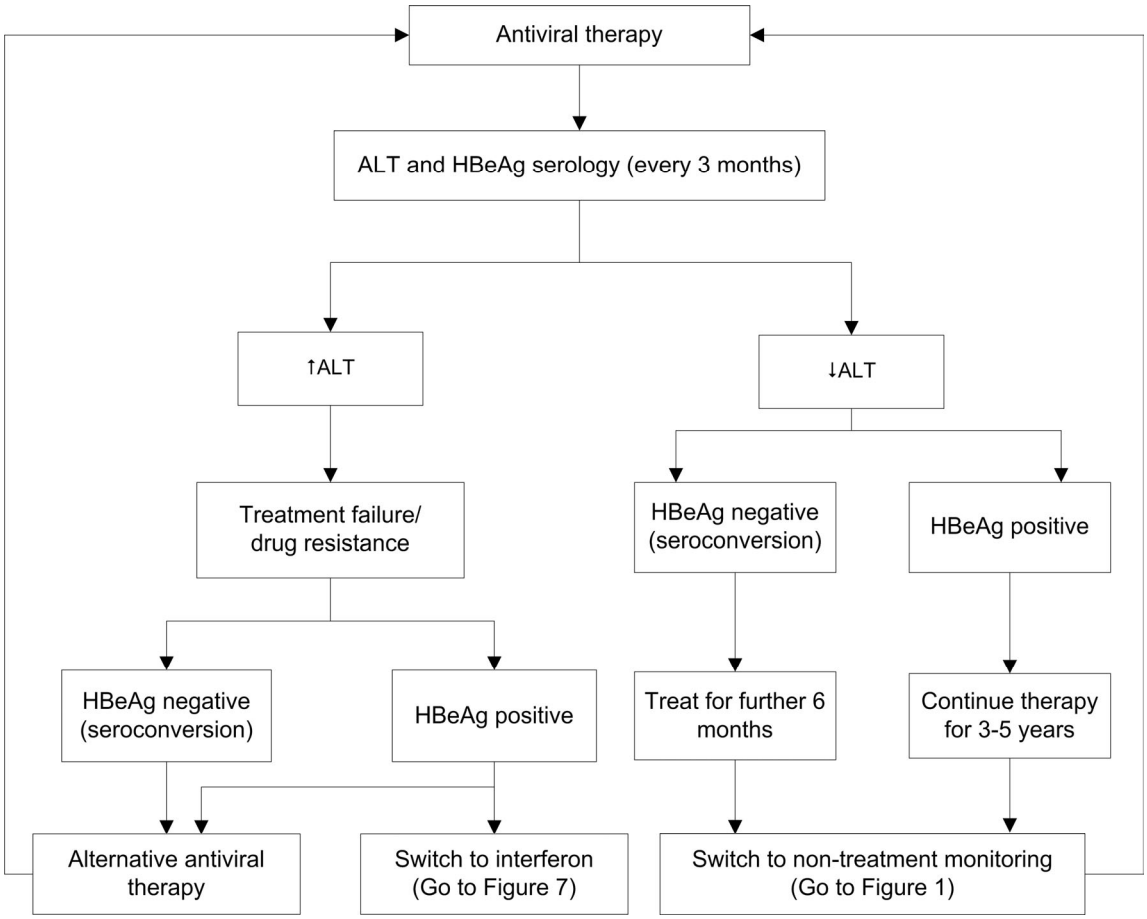

Figure 3 Clinical pathway: Monitoring chronic hepatitis B patients undergoing antiviral therapy without HBV DNA testing available

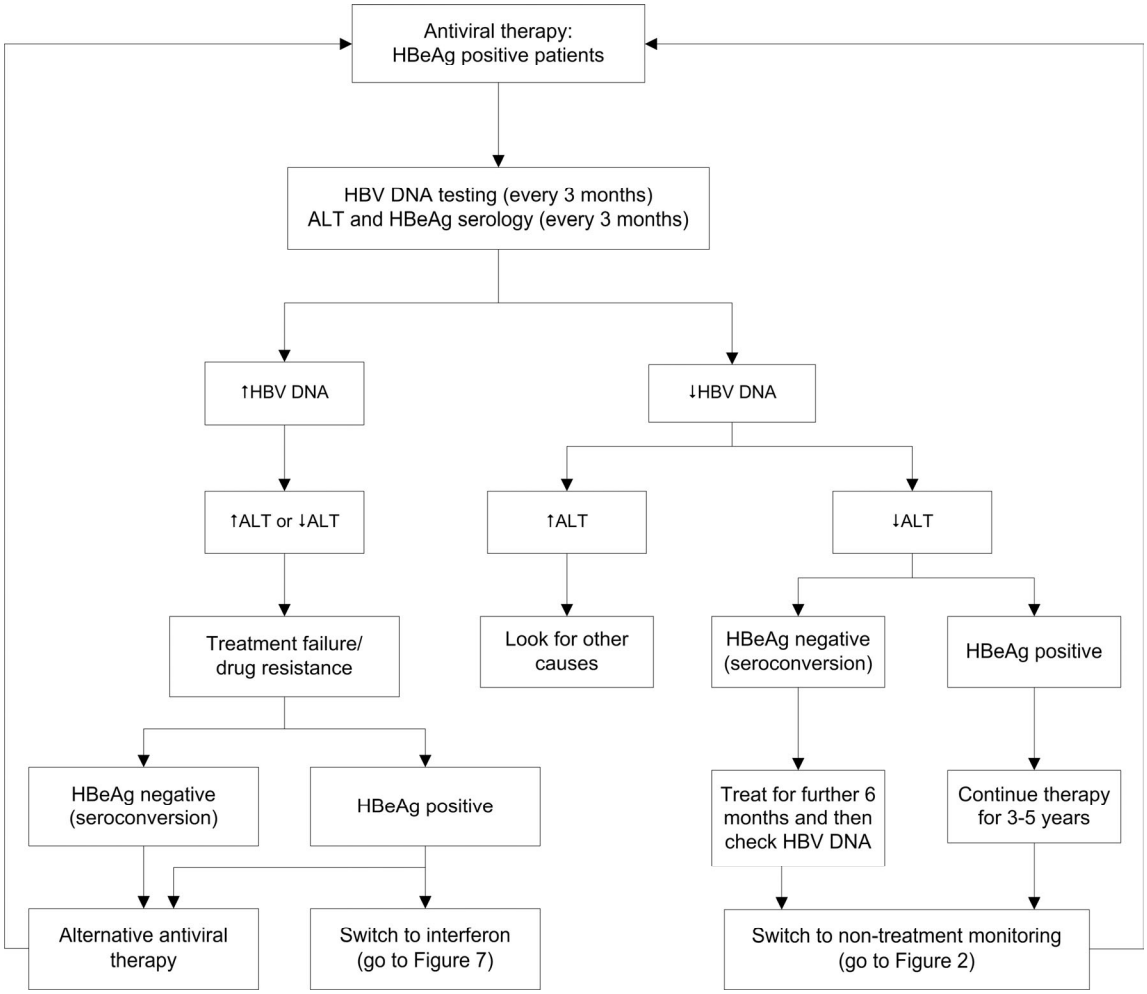

Figure 4 Clinical pathway: Monitoring HBeAg positive chronic hepatitis B patients undergoing antiviral therapy with HBV DNA testing available

[a] HBV DNA testing every three months in the first year after commencing therapy is necessary in HBeAg positive patients with mild liver disease if ALT returns to normal

Note: In the case of drug resistance, abnormal DNA refers to a minimum increase of 1 log above nadir

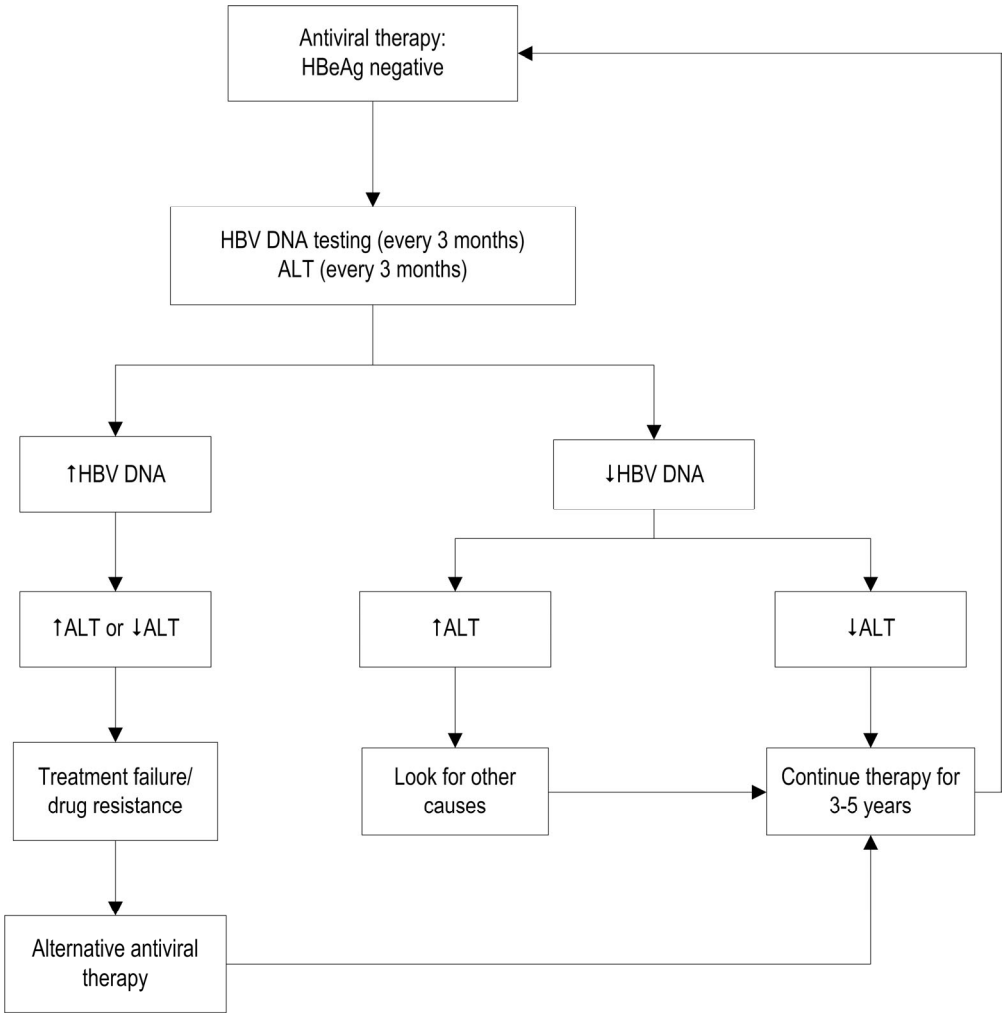

Figure 5 Clinical pathway: Monitoring HBeAg negative chronic hepatitis B patients undergoing antiviral therapy with HBV DNA testing available

[a] Current recommendations for treatment thresholds differ HBeAg negative patients (10^4) and in patients with cirrhosis or liver decompensation (10^3)

Notes: The advisory panel indicated that HBeAg negative patients undergo life-long oral antiviral therapy
In the case of drug resistance, abnormal DNA refers to a minimum increase of 1 log above nadir

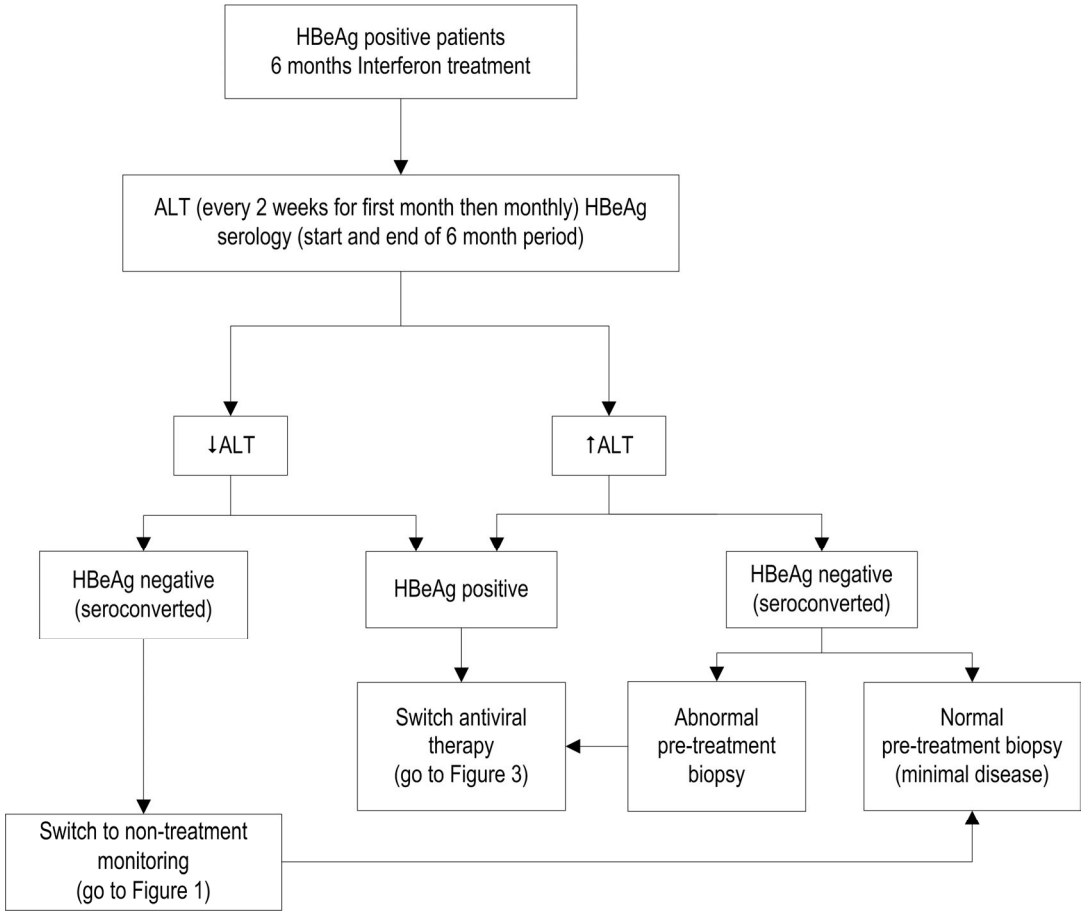

Figure 6 Clinical pathway: Monitoring HBeAg positive chronic hepatitis B patients undergoing interferon without HBV DNA testing available

Note: Assessment of liver disease was based on first pre-treatment biopsy

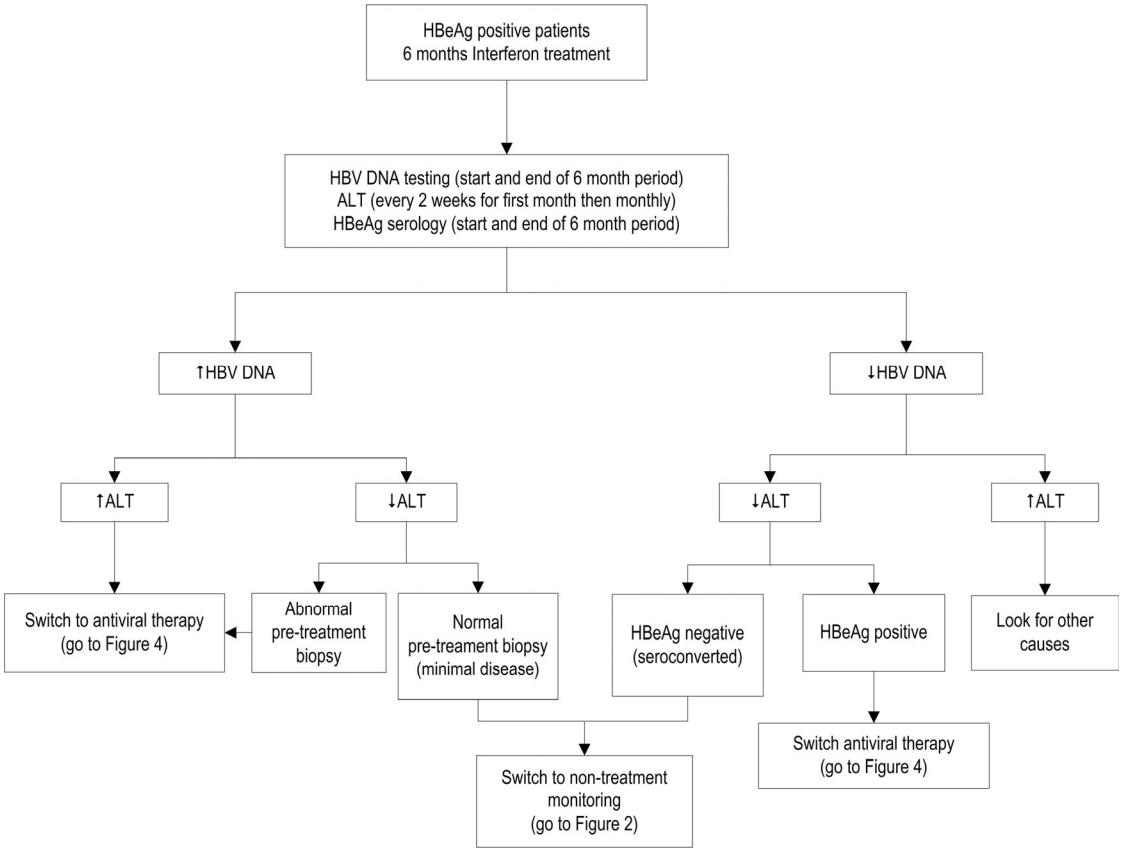

Figure 7 Clinical pathway: Monitoring HBeAg positive chronic hepatitis B patients undergoing interferon therapy with HBV DNA testing available

[a] Current recommendations for treatment thresholds differ between HBeAg positive patients (10^5) and in patients with cirrhosis or liver decompensation (10^3)

Note: Assessment of liver disease was based on first pre-treatment biopsy

Assessment framework

Types of evidence

A systematic review of the medical literature was undertaken to identify relevant studies that examined the value of HBV DNA testing to assess and monitor patients with chronic hepatitis B in relation to commencing or continuing antiviral therapy. Direct evidence regarding the impact of HBV DNA testing on health outcomes was sought. The literature search was not limited by outcomes or comparators. Indirect evidence concerning the impact of HBV DNA testing on clinical management and diagnostic accuracy was assessed where studies offering direct evidence were absent or limited.

Literature review

A search of the medical literature was conducted to identify all relevant studies and reviews published before April 2006. Primary database searches were conducted as indicated in Table 6.

Search strategy

Primary databases

Table 6 Electronic databases searched to review HBV DNA testing for chronic hepatitis B literature

Database	Period covered/date searched
PreMedline and Medline	1966 to April week 1, 2006
EMBASE	1980 to 2006, week 15
Cochrane Library	Issue 2, 2006 (5 May 2006)

The search terms included the following (as determined from the PPICO criteria):

- hepatitis B
- DNA, deoxyribonucleic acid
- hepatitis B virus, HBV, hepatitis B surface antigens
- viral DNA, HBV DNA, hepatitis B virus DNA
- test, assay, nucleic acid amplification techniques, gene amplification, nucleic acid hybridisation, polymerase chain reaction, versant, amplification, hybrid capture, Digene, Cobas, Amplicor, Roche PCR
- viral load, viral burden, viral dynamics, viral decline, virology, viral quantification, virus examination, DNA level, DNA value, DNA quantitation, DNA concentration, DNA determination.

Complete details of the literature searches performed using primary and secondary databases are presented in **Appendix E**. Additional searches were conducted as required to locate quality of life, epidemiological and economic information.

Selection criteria

Initial assessment of patients with chronic hepatitis B prior to antiviral therapy

Table 7 Selection criteria: HBV DNA test studies considering initial assessment of chronic hepatitis B patients before antiviral therapy

Research question: To what extent is HBV DNA testing safe, effective and cost-effective in initial assessment of chronic hepatitis B patients before undergoing antiviral therapy relative to current clinical practice?

Selection criteria	Inclusion	Exclusion
Study design	Studies with ≥10 patients	Non-systematic reviews, letters, opinion pieces, surveys, non-human or *in vitro* studies
Population	Chronic hepatitis B patient not treated with antiviral therapy	Wrong indication Patients co-infected with HCV, HDV, HIV and other viruses
Prior tests[a]	History and physical examination Laboratory test to assess liver disease Tests to rule out other causes of liver disease Tests for co-infection with other viruses Screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology	Prior HBV DNA testing
Index test	HBV DNA test use for assessment and monitoring of chronic hepatitis B patients as currently approved by the TGA	Wrong usage Wrong test
Comparator	Current clinical practice	Wrong comparator
Reference standard	Liver biopsy / clinical outcomes	Inadequate reference standard No reference standard
Outcomes		
Direct evidence studies	Effect on health outcomes	Wrong outcomes Inadequate data reporting Case-control studies Case-referent studies
Accuracy studies	Diagnostic performance	
Management studies	Effect on clinical management	

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus, HIV, human immunodeficiency virus; TGA, Therapeutic Goods Administration

[a] These tests are not required before HBV DNA testing (advisory panel advice)

Monitoring patients with chronic hepatitis B not receiving antiviral therapy

Table 8 Selection criteria: HBV DNA test studies monitoring chronic hepatitis B patients who were not undergoing antiviral therapy

Research question: To what extent is HBV DNA testing safe, effective and cost-effective in monitoring patients with active hepatitis B who are not undergoing treatment relative to current clinical practice?

Selection criteria	Inclusion	Exclusion
Study design	Studies with ≥10 patients	Non-systematic reviews, letters, opinion pieces, surveys, non-human or *in vitro* studies
Population	Chronic hepatitis B patients not treated with antiviral therapy	Wrong indication Patients co-infected with HCV, HDV, HIV and other viruses
Prior tests[a]	History and physical examination	No specific exclusion criteria
	Laboratory test to assess liver disease	
	Tests to rule out other causes of liver disease	
	Tests for co-infection with other viruses	
	Screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology	
	HBV DNA testing	
Index test	HBV DNA test use for assessment and monitoring of chronic hepatitis B patients as currently approved by the TGA	Wrong usage Wrong test
Comparator	Current clinical practice	Wrong comparator
Reference standard	Liver biopsy / clinical outcomes	Inadequate reference standard No reference standard
Outcomes		
Direct evidence studies	Effect on health outcomes	Wrong outcomes Inadequate data reporting Case-control studies Case-referent studies
Accuracy studies	Diagnostic performance	
Management studies	Effect on clinical management	

Abbreviations: HBV, hepatitis B virus; TGA, Therapeutic Goods Administration

[a] These tests are not required before HBV DNA testing (advisory panel advicel)

Monitoring patients with chronic hepatitis B receiving antiviral therapy

Table 9 Selection criteria: HBV DNA test studies monitoring chronic hepatitis B patients undergoing antiviral therapy

Research question: To what extent is HBV DNA testing safe, effective and cost-effective in monitoring chronic hepatitis B patients undergoing antiviral therapy relative to current clinical practice?

Selection criteria	Inclusion	Exclusion
Study design	Studies with ≥10 patients	Non-systematic reviews, letters, opinion pieces, surveys, non-human or *in vitro* studies
Population	Chronic hepatitis B patients undergoing antiviral therapy	Wrong indication Patients co-infected with HCV, HDV, HIV and other viruses
Prior tests[a]	History and physical examination Laboratory test to assess liver disease Tests to rule out other causes of liver disease Tests for co-infection with other viruses Screening for hepatocellular carcinoma, HBsAg / HBeAg / anti-core serology HBV DNA testing	No specific exclusion criteria
Index test	HBV DNA test use for assessment and monitoring of chronic hepatitis B patients as currently approved by the Therapeutic Goods Administration	Wrong usage Wrong test
Comparator	Current clinical practice	Wrong comparator
Reference standard	Liver biopsy / clinical outcomes	Inadequate reference standard No reference standard
Outcomes		
Direct evidence studies	Effect on health outcomes	Wrong outcomes Inadequate data reporting Case-control studies Case-referent studies
Accuracy studies	Diagnostic performance	
Management studies	Effect on clinical management	

Abbreviation: HBV, hepatitis B virus

[a] These tests are not required before HBV DNA testing (advisory panel advice)

Search results

Results from safety and effectiveness searches and management and health outcomes searches were pooled. The QUOROM (Quality of Reporting of Meta-analyses) flowchart (Figure 8) summarises reasons for study exclusions. A total of 2206 non-duplicate references were identified, of which 176 were reviewed for inclusion, and 21 were included in the effectiveness review.

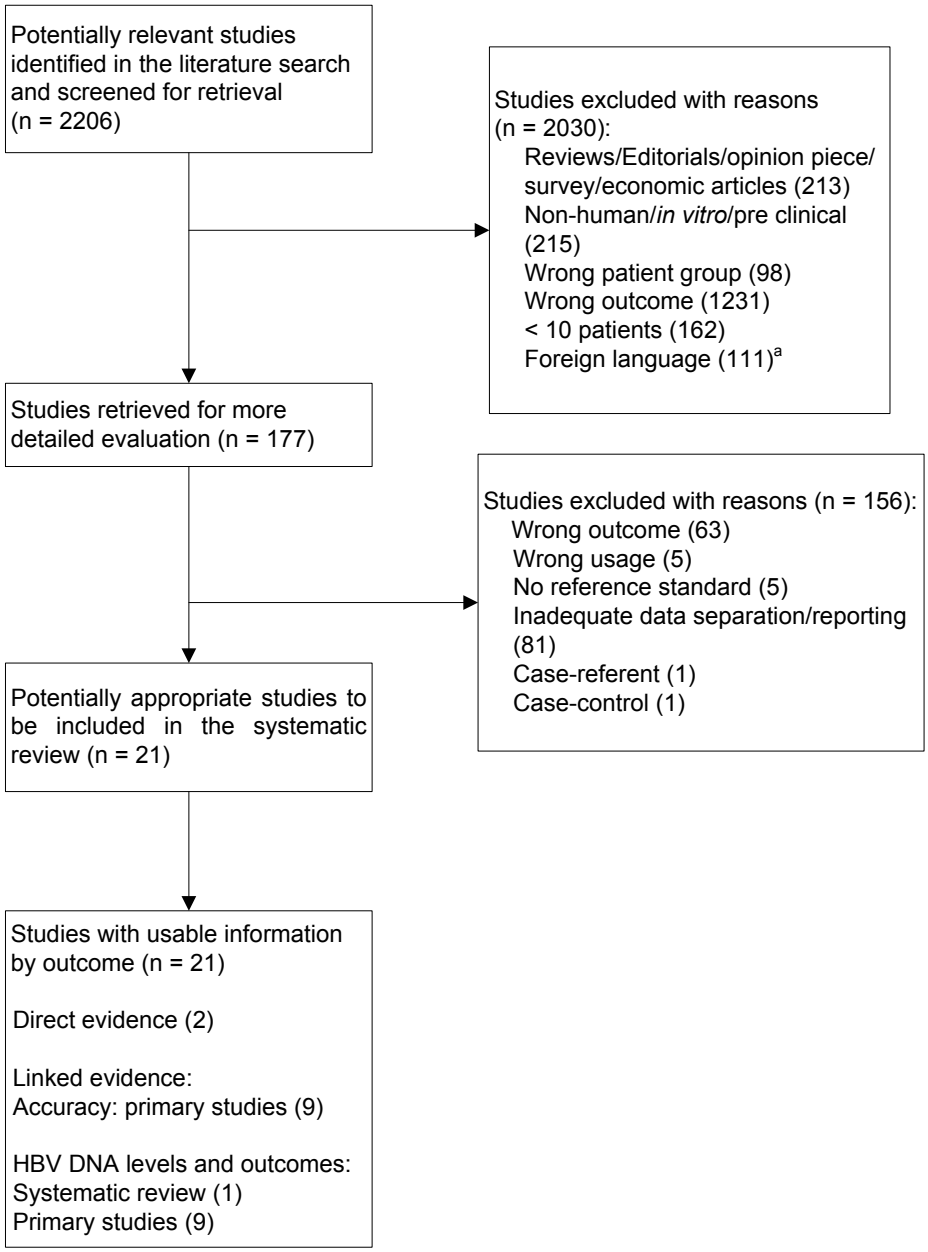

Figure 8 QUOROM flowchart: Identification and selection of studies from the HBV DNA testing literature review

Note: Translation and review of these studies was not possible due to time constraints of the assessment process

It was found that many studies' data and separation reporting was inadequate for this review. To attempt to address data reporting deficits, the authors of 53 studies were contacted to elicit further information to inform assessment of HBV DNA test accuracy. No suitable data were provided from this process.

Data extraction

A *pro forma* was applied that included parameters to accommodate data collation of trial and study population characteristics, tests used and outcomes reported. This follows procedures for data collection outlined in the *Cochrane Reviewers' Handbook* (Higgins et al 2005).

Statistical methods

Methodological considerations

Direct evidence supporting the value of HBV DNA testing relative to current clinical practice, when used in the relevant patient group, is required to justify reimbursement under Medicare. This should ideally be in the form of studies reporting effects on patient-centred health outcomes. Alternatively, evidence of greater diagnostic accuracy than the comparator(s), along with linked evidence of management change and that treatment will affect health outcomes is required.

Evidence of an effect on management change is a key component where an additional diagnostic test is to be used in the clinical pathway. The most appropriate design to investigate the effects on management change is a pre-test post-test case series study. Where a pre-test management plan is not reported, study outcomes do not fully represent patient management change, and outcomes may be biased.

The ideal design for a comparative accuracy study of diagnostic tests permits each test to be performed in a population with a defined clinical presentation, in a consecutive series. The study should be an independent, blinded comparison with a valid reference standard (NHMRC 2005).

To determine the relationship between a risk factor and clinical outcome, a Cox proportional hazards model can be applied to assess the relative risk. Multivariate-adjusted analysis allows exclusion of potential confounding factors.

Diagnostic performance

Evaluating accuracy of new diagnostic tests requires comparison of the novel approach with its comparators and a reference standard. The reference standard is the best available proxy for the true status of a disease or condition. The new diagnostic test and its comparators can be compared independently with the reference standard to assess sensitivity, specificity, accuracy, diagnostic odds ratio and likelihood ratios.

In this setting, sensitivity is defined as the proportion of all patients with a specified condition whose results are positive, and specificity as the proportion of all patients, who do not have the specified condition, who test negative. Test accuracy is reflected by the proportion of patients whom the test correctly identified as positive or negative. A plot of sensitivity against 1 minus specificity is known as a receiver operating characteristic

(ROC) curve. The closer the area under the curve (AUC) is to 1.0, the more accurate the test.

The positive predictive value is the proportion of all patients with positive test results who have the condition in focus. The negative predictive value is the proportion of all patients with negative test results who do not have the condition. Both positive and negative predictive values are prevalence dependent. Caution should be taken when interpretating these measures. In the context of this assessment, where HBV DNA testing may be used to predict response to antiviral therapies, the negative predictive value of the HBV DNA test indicates ability to identify patients who would not respond to treatment. The negative predictive value of HBV DNA testing, where the test is used to monitor patients undergoing antiviral drug treatment, is considered highly clinically relevant (advice from advisory panel).

The diagnostic odds ratio is an expression of the odds of positive test results among patients with the specified condition, compared with those who do not have the condition. A diagnostic odds ratio of 100 provides convincing evidence of the test's ability to discriminate between presence and absence of the condition.

The likelihood ratio of a positive test is the probability that positive test results would occur among people who have the condition, opposed to those who do not. The likelihood ratio of a negative test is the probability that a negative test result would occur among people who have the condition, opposed to those who do not. A positive ratio of >10 and a negative ratio <0.1 provide compelling diagnostic evidence. A positive likelihood ratio of >5 and a negative likelihood ratio of <0.2 provide strong diagnostic evidence (Medical Services Advisory Committee 2005). Bayes' theorem indicates that the post-test odds of a condition are equal to the pre-test odds of the condition multiplied by the likelihood ratio. The post-test probability of a condition can be determined for any given pre-test probability using this approach.

Appraisal of the evidence

Appraisals of evidence were conducted at three stages.

Stage 1: Appraisal of the applicability and quality of studies included in the review

Stage 2: Appraisal of the precision, size and clinical importance of the primary outcomes used to determine the safety and effectiveness of the test

Stage 3: Evidence consolidation for analysis and development of recommendations about the index test's net benefit in Australian clinical practice.

Appraisal of the quality and applicability of individual studies

The quality and applicability of included studies was assessed by applying pre-specified criteria according to the study design (**Appendix D**).

Ranking the evidence

Studies that evaluated direct impact of the test or treatment on patient outcomes were ranked according to the study design by applying levels of evidence designated by the National Health and Medical Research Council (NHMRC) (Table 10).

Table 10 NHMRC levels of evidence for effectiveness

Level of evidence	Study design
I	Evidence obtained from a systematic review of level II studies
II	Evidence obtained from properly designed randomised controlled trials
III-1	Evidence obtained from well-designed pseudo-randomised controlled trials (alternate allocation or some other method)
III-2	Evidence obtained from comparative studies with concurrent controls: non-randomised experimental trials, cohort studies, case-control studies, or interrupted time series with a control group
III-3	Evidence obtained from comparative studies without concurrent controls: historical control studies, two or more single-arm studies, or interrupted time series without a parallel control group
IV	Evidence obtained from case series, either post-test or pre-test/post-test outcomes

Source: NHMRC 2005

Diagnostic accuracy studies were ranked according to NHMRC levels of evidence for diagnoses (Table 11).

Table 11 NHMRC levels of evidence for diagnosis

Level of evidence	Study design
I	Evidence obtained from a systematic review of level II studies
II	Evidence obtained from studies of test accuracy with: an independent blinded comparison with a valid reference standard, among consecutive patients with a defined clinical presentation
III-1	Evidence obtained from studies of test accuracy with: an independent blinded comparison with a valid reference standard, among non-consecutive patients with a defined clinical presentation
III-2	Evidence obtained from studies of test accuracy with: a comparison with reference standard that does not meet the criteria required for level II or III-1 evidence
III-3	Evidence obtained from diagnostic case-control studies
IV	Evidence obtained from studies of diagnostic yield (no reference standard)

Source: NHMRC 2005

Studies were also graded according to pre-specified quality and applicability criteria (Table 12).

Table 12 Grading system used to rank included studies

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy versus the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed and to avoid bias? High quality = no potential for bias based on pre-defined key quality criteria Medium quality = some potential for bias in areas other than those pre-specified as key criteria Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q1 high quality Q2 medium quality Q3 poor reference standard poor quality or insufficient information

Ideally, comparative accuracy of diagnostic tests is derived from studies that perform all tests on all study participants. In this assessment, studies were classified as 'CX' (other comparison) where both tests were not performed for all study participants.

An applicable patient population was considered to reflect the research question for each indication in review. To be considered applicable, studies must be free from spectrum bias in patient selection: all consecutive patients with the appropriate clinical presentation should be included in the analysis. Patient populations applicable to the research question but with known spectrum bias were considered to offer limited applicability.

Study quality was determined by a number of predefined factors.

Verification bias is a key concept used to determine study quality, and occurs when a valid reference standard cannot be applied to all study participants. Verification bias is avoided when only data from participants with valid reference standards are analysed. The reference standard is defined as the best available proxy for the true status of a disease or condition. Differential verification bias occurs when different reference standards are applied to confirm positive and negative index test results. Studies conducted in consecutive series of participants, without potential for verification bias, were classified as providing high quality evidence.

A further factor affecting the study quality is selection bias. Studies are subject to selection bias when patient inclusion is based on receiving the index test or reference standard. To avoid selection bias, the accuracy of HBV DNA testing should be reported in a consecutive series of patients who meet the criteria to receive the index test (ie, have a defined clinical presentation). These criteria should be based on pre-test characteristics of the patients. The disease status of all patients should be verified by a high quality, valid reference standard.

The study should provide sufficient data to reconstruct a 2 × 2 table to check calculation of diagnostic accuracy outcomes and extract the numbers of true positive, false positive, true negative and false negative results. It is a requirement of this process that a diagnostic test be compared with a reference standard. Where long term outcomes are considered as reference standard this may not be possible due to unavailability of long term outcome data. In this circumstance, a test can be compared with other recognised indicators of disease status, although the diagnostic test under assessment may be a more accurate predictor of long term outcome than other tests.

When a comparator test is also considered to be a reference standard, and a diagnostic test is potentially superior to the existing comparator reference standard, a valid estimate of the accuracy of the index test would not be established. In this circumstance, there is a requirement for direct evidence to support index test effectiveness on patient outcomes (MSAC 2005).

Expert advice

An advisory panel with expertise in diagnostic virology, including hepatitis, was established to evaluate the evidence and provide advice to MSAC from a clinical perspective. In selecting members for advisory panels, MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations and consumer bodies for nominees. Membership of the advisory panel is provided at **Appendix B**.

Results of assessment

Is it safe?

Specimens to be used in HBV DNA testing are collected using general procedures for sampling blood. Collection of samples for HBV DNA testing carries a low safety risk.

However, as HBV DNA testing is not listed on the Medicare Benefits Schedule laboratories which perform this test do not need to be accredited. This may have potential implications for patient safety as HBV DNA testing is compulsory for patients to receive therapies listed on the PBS.

Is it effective?

The strategy used to identify the body of evidence to establish effectiveness of serum HBV DNA testing is outlined in Figure 9.

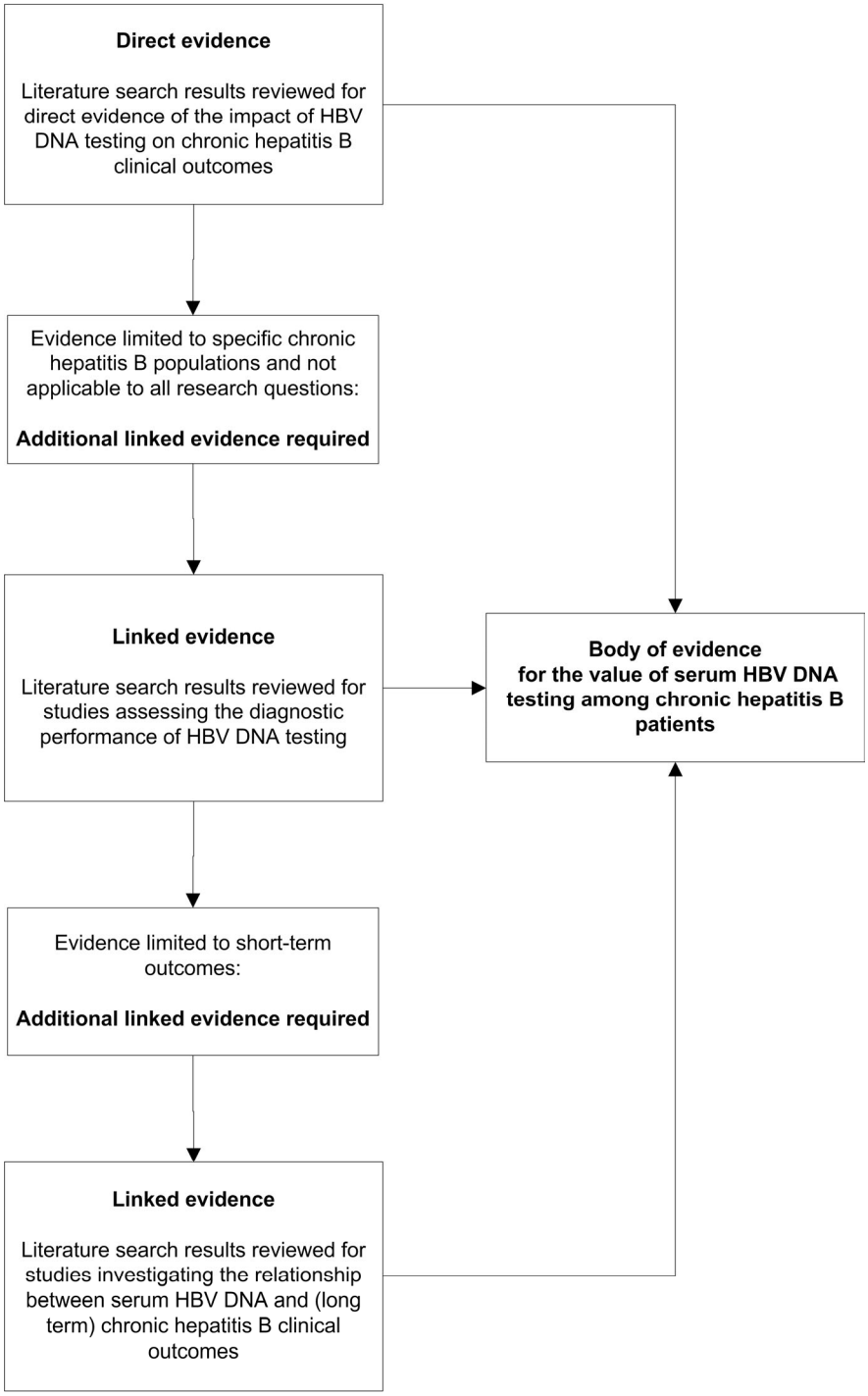

Figure 9 Strategy to identify evidence supporting effectiveness of serum HBV DNA testing

Abbreviation: HBV DNA, hepatitis B virus deoxyribonucleic acid

Direct evidence

Summary of direct evidence

HBV DNA testing and the monitoring of patients with chronic hepatits B not receiving antiviral therapy

Chan et al (2002) published evidence indicating that HBV DNA testing improves outcomes by reducing mortality risk among HBsAg positive renal transplant patients. HBV DNA testing was used to identify transplant patients with increasing DNA levels. Lamivudine was administered pre-emptively, regardless of alanine aminotransferase (ALT) levels.

Patient survival was similar to HBsAg negative patients when the pre-emptive strategy of HBV DNA testing and lamivudine therapy was readily available throughout the post-transplant period. When this strategy was unavailable to all transplant patients, survival declined and the relative risks of death and liver-related death increased. Compared with HBsAg negative renal transplant patients, the relative risk of death among transplant patients was 9.7 (95% CI: [4.7, 19.9], $p<0.001$); the relative risk of liver-related death was 68.0 (95%CI: [8.7, 533.2], $p<0.0001$).

Although it was indicated that HBV DNA testing can enhance clinical outcomes, results are not considered applicable in Australian clinical practice, because HBsAg positive patients were treated with lamivudine to prevent reactivation of viral replication during immunosuppressive therapy.

HBV DNA testing and the monitoring of patients with chronic hepatitis B receiving antiviral therapy

Lampertico et al (2005) assessed outcomes of adefovir dipivoxil therapy among HBeAg negative chronic hepatitis B patients who were lamivudine resistant. Adefovir was administered either at the time point of genotypic resistance (3-6 $\log_{10}$ copies/mL, normal ALT) or phenotypic resistance (>6 $\log_{10}$ copies/mL, high ALT). Because detection of genotypic resistance is possible only by measuring serum HBV DNA levels, comparison of adefovir treatment outcomes between the genotypic and phenotypic resistance cohorts enabled assessment of HBV DNA testing on patient outcomes.

When adefovir was co-administered with lamivudine, patients in the genotypic resistance cohort had significantly greater response compared with the phenotypic resistance cohort: the proportion of patients with undetectable serum HBV DNA levels at three months of adefovir therapy was 100 per cent and 46 per cent, respectively ($p<0.001$). Normalisation of ALT levels was time dependent in the phenotypic resistance cohort. ALT levels were normal throughout the study period among the genotypic cohort treated with adefovir at initial lamivudine resistance onset.

This suggests that ALT testing alone may not be the most effective way of detecting initial development of lamivudine resistance. Regular HBV DNA testing offers better monitoring of patients undergoing lamivudine monotherapy, which has potential to alter patient management and to deliver short term outcome improvements. Adefovir treatment initiation was based on availability, not ALT levels alone. Patients in the phenotypic resistant cohort may not be entirely comparable with those monitored by ALT levels alone.

Direct evidence was considered to be limited because the studies were not randomised controlled trials and treatment decisions were driven by availability of antiviral therapy rather than HBV DNA test results.

Does HBV DNA testing improve health outcomes?

Studies by Chan et al (2002) and Lampertico et al (2005) informed assessment of the additional value of HBV DNA testing compared with current clinical practice. Chan et al (2002) assessed impact of HBV DNA testing on health outcomes among HBsAg positive kidney allograft recipients. Lampertico et al (2005) investigated the impact of HBV DNA testing in monitoring patients undergoing lamivudine therapy.

No studies were found whose specific objective was to assess additional benefit provided by HBV DNA testing on health outcomes, compared with current clinical practice, and none that assessed serum HBV DNA testing on health outcomes when used as a part of initial patient assessment.

HBV DNA testing and monitoring patients with chronic hepatitis B not receiving antiviral therapy

Chan et al (2002) investigated HBV DNA testing on HBsAg positive kidney allograft recipients. HBV DNA testing sought to identify increasing DNA levels among transplant patients; lamivudine was administered pre-emptively, with or without elevation of ALT levels. Clinical outcomes were compared with a group of patients who underwent transplantation before lamivudine therapy became available (January 1996) to assess HBV DNA testing. When clinically indicated, patients in the transplant group were treated with lamivudine when it became available. Study characteristics are presented in Table 13.

Table 13 Study characteristics: assessment of the impact of HBV DNA testing among kidney transplant patients

Study	Study design	Patients (N)	Other tests	Test characteristics	Treatment	Study quality
Chan et al (2002) China	Cohort study (1983–2000) Prospective data collection from January 1996 Enrolment not reported	Period I cohort (1983–1995): HBsAg positive transplant patients[a] (n = 52) Period II cohort (1996–2000): HBsAg positive *de novo* transplant[a] patients (n = 15) HBsAg negative cohort (1983–2000): (n = 442) Exclusions: anti-hepatitis C positive (antibodies significant alcohol consumption; cirrhosis on biopsy; decompensated liver disease)	ALT; alkaline phosphatase; γ-glutamyl transpeptidase; and bilirubin[b] Prothrombin time measured when clinically indicated; HBeAg/anti-HBeAg status assessed at baseline and repeated yearly or when clinically indicated; anti-hepatitis D when clinically indicated; liver biopsy at baseline (consenting patients only— not obligatory criterion for lamivudine treatment) Further details of other tests nor reported	HBV DNA test: Digene Hybrid Capture II assay. Sensitivity limit of 1.4 x 10^5 copies/mL[b] Comparator test: ALT (Details not reported)	Lamivudine (100 mg/day) Begun if one of following criteria met: 1. HBV DNA level > 2.83 x 10^8 copies/mL among patients with normal ALT levels 2. HBV DNA level > 2.83 x 10^7 copies/mL among patients with increasing ALT levels and/or liver biopsy specimen showing significant hepatitis Resistance to lamivudine: defined by reappearance of HBV DNA in at least two consecutive samples among patients whose HBV DNA had previously become undetectable	Possible selection bias: Cohort study, patient selection not randomised Possible non-consecutive patient enrolment Applicability: Applicability of HBV DNA testing regimen to Australian clinical practice unknown

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Acceptance criteria for renal transplant patients did not change between 1983 and 2000

[b] Test schedule: 1. Every two to four weeks a) for the first 12 months after transplantation; b) for four months after pulse steroid and/or anti-lymphocyte therapy for acute rejection; c) when serial HBV DNA levels show increasing trend; d) during treatment with lamivudine. 2. Every four months in clinically stable long term renal allograft recipients on low-dose immunosuppressive medications.

The survival of HBsAg positive patients was compared among patients who underwent transplantation before (period I cohort) and after lamivudine became available (period II cohort, *de novo* transplantation) to assess the impact of HBV DNA testing combined with pre-emptive lamivudine treatment. The results reported by Chan et al (2002) are presented in Table 14.

Of the 52 patients from the period I cohort, 14 died before January 1996 (73% survival). Liver complications accounted for the deaths of eight of the 14 patients (57.1%). All 12 of the 15 patients from the period II cohort who were studied in the between-group comparisons[2] survived. It was not reported whether the survival difference between period I and II patients was statistically significant (p value not reported).

Of the 38 patients remaining from the period I cohort, 15 (39.5%) satisfied criteria for pre-emptive lamivudine treatment (Table 14). Of the 12 patients in the period II cohort, 11 (91.7%) satisfied criteria for pre-emptive lamivudine treatment, indicating a higher requirement for lamivudine therapy compared with the period I cohort ($p = 0.002$).

Lamivudine treatment was begun based on HBV DNA levels alone for seven (46.7%) and five (45.5%) patients from the period I (transplantation before lamivudine availability) and period II cohorts (lamivudine available at time of transplantation) respectively (p value not reported). Abnormal alanine aminotransferase (ALT) was also noted among remaining patients who underwent lamivudine treatment in both cohorts.

The duration of lamivudine treatment was not significantly different between period I and II patients ($p = 0.118$). There was no difference in treatment duration before HBV DNA levels became undetectable or ALT normalised between period I and II patients ($p = 0.643$ and $p = 0.846$, respectively) (Table 14).

The relative risks of death and liver-related death were also compared between each period I and period II cohort and with a cohort of HBsAg negative patients who underwent renal transplantation between 1983 and 2000 (Table 14). Compared with HBsAg negative renal transplant patients, the relative risk of death in period I patients was 9.7 (95% CI: [4.7, 19.9], $p<0.001$); the relative risk of liver-related death was 68.0 (95% CI: [8.7, 533.2], $p<0.0001$). Chen et al (2002) reported that survival of the period II cohort (*de novo* transplantation patients) was similar to HBsAg negative patients, but statistical data were not reported (p value not reported). HBeAg status at the time of transplantation or at last follow up was not related to patient survival ($p = 0.660$ and $p = 0.797$, respectively); however, it was unclear whether this analysis applied only to period I1 patients or to all HBsAg positive patients included in this study.

Chen et al (2002) provided some evidence that HBV DNA testing improved outcomes among HBsAg positive renal transplant patients (recipients). When the pre-emptive strategy of combined HBV DNA testing and lamivudine therapy was readily available throughout the post-transplant period (period II cohort), patient survival was similar to HBsAg negative patients. Conversely, when the pre-emptive strategy was not available to all transplant patients (period I cohort), survival was reduced and the relative risks of death and liver-related death increased.

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[2] Of the 15 patients from the period II cohort, three were late referrals who received lamivudine treatment as salvage therapy after developing liver decompensation. These three patients were excluded from between group comparisons by the study authors

HBV DNA test results indicated initiation of lamivudine therapy in almost half of patients in both cohorts. This suggests that in the absence of HBV DNA testing, a significant proportion of patients would not receive appropriate therapy if treatment decisions were based on elevated ALT levels alone. These patients would be treated only if ALT levels became elevated.

Study quality

A randomised controlled trial comparing outcomes between patient cohorts of those receiving HBV DNA testing, and another where testing was withheld, would provide the ideal study setting to determine the impact of HBV DNA testing among HBsAg positive renal transplant patients. This would be unethical because all patients who could benefit from lamivudine treatment would not be identified on the basis of ALT levels alone—HBV DNA levels can change without corresponding changes in ALT levels. Chan et al (2002) avoided this ethical dilemma by comparing outcomes before and after lamivudine availability. This approach may have introduced bias because there was no randomisation to select patients who did not have HBV DNA testing and pre-emptive lamivudine therapy. It was not reported whether patient enrolment was consecutive. Outcomes were compared between renal transplant patients during different time periods. It was possible that outcome variations between period I and II cohorts occurred because of differences between patient populations. Renal allograft survival was similar between cohorts, suggesting that that there were no differences in renal outcomes that impacted on liver-related outcomes. Data collection from January 1996 was prospective.

There was an indirect comparison of relative risk of death and liver-related death between period I and II cohorts. Outcomes from both cohorts were compared with HBsAg negative renal transplant patients between 1983 and 2000. Direct comparison between cohorts would have provided a more robust analysis that yielded a more accurate assessment of HBV DNA testing for HBsAg positive renal transplant patients.

The study's design did not permit direct assessment of additional value provided by HBV DNA testing over ALT testing. If elevated ALT levels alone were used to inform lamivudine initiation, almost half of the patients treated with lamivudine in this study would not have been treated. The consequences of not using HBV DNA testing to monitor patients were indicated by poor survival among period I cohort patients. It is unknown whether period I cohort outcomes would have differed had lamivudine therapy been available at the time of transplantation, and elevated ALT levels only used to inform treatment initiation.

Table 14 Outcomes of lamivudine treatment among renal transplant recipients

Study	Cohort[a]	Reasons for initiating lamivudine treatment	Treatment duration before HBV DNA became undetectable (weeks)	Treatment duration before ALT normalisation (weeks)	Outcomes			Study quality
					Survival[e]	Relative risks compared with HBsAg negative renal transplant patients		
						Death	Liver related death	
Chan et al (2002) China	**Period I** (n = 52) (1983 to 1995)	Based on HBV DNA alone (n = 7); abnormal ALT also noted among remaining patients (n = 8) 15[b] patients received pre-emptive lamivudine treatment [c]	6.4 +/– 2.1 (range 3–10)	14.5 +/– 11.5 (range 1–39)	73%	9.7 (95% CI:[4.7, 19.9] $p < 0.001$)	68.0 (95% CI:[8.7, 533.2] $p<0.0001$)	Possible selection bias: Cohort study, patient selection not randomised. Possible non-consecutive patient enrolment Applicability: HBV DNA testing regimen not applicable to Australian clinical practice where all HBsAg positive renal transplant patients receive prophylactic lamivudine therapy
	Period II (n = 15) *De novo* transplant patients (1996 to 2000)	Based on HBV DNA alone (n = 5); abnormal liver biochemistry also noted among remaining patients (n = 6) 11[d] patients received pre-emptive lamivudine treatment[c]	6.4 +/– 4.0 (range 3–14)	15.6 +/– 12.2 (range 5–38)	100%	Similar to HBsAg negative patients (result not reported)	Similar to HBsAg negative patients (result not reported)	

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Renal allograft survival was similar between cohorts and HBsAg negative patients, irrespective of lamivudine therapy status

[b] Of the 52 period I patients, 14 died before January 1996. Of the remaining 38 patients, 15 satisfied criteria for pre-emptive lamivudine treatment

[c] Period I patients: Lamivudine treatment started 71 +/– 27 months (range 15 to 118) after transplantation; period II patients: Lamivudine treatment started 71 +/– 27 months (range 1 to 18) after transplantation. Duration of lamivudine therapy was 36.3 +/– 11.4 months and 32.6 +/– 13.3 months for period I and period II patients respectively ($p = 0.118$)

[d] Of the 15 period II patients, 3 were late referrals for whom lamivudine was a salvage therapy following development of liver decompensation. These patients were excluded from between group comparisons

[e] For period I patients this value for survival refers to the period before January 1996; 14 of 52 patients died. Two other patients (not treated with lamivudine) died from non-liver related causes after January 1996

Applicability

HBV DNA testing was carried out every two to four weeks or every four months in the study by Chan et al (2002)[3]. Lamivudine treatment was begun if HBV DNA level was:

1. $>2.83 \times 10^8$ copies/mL among patients with normal ALT, or
2. $>2.83 \times 10^7$ among patients with increasing ALT levels or liver biopsy confirming hepatitis.

Results reported by Chan et al (2002) were not applicable to Australian current clinical practice. In Australia, all HBsAg positive renal transplant patients undergo prophylactic lamivudine treatment regardless of serum HBV DNA levels.

HBV DNA testing and the monitoring of patients with chronic hepatitis B receiving antiviral therapy

Results reported by Lampertico et al (2005), who applied HBV DNA testing to assess antiviral treatment response, informed assessment of HBV DNA testing over current clinical practice (Table 15). Lampertico et al (2005) assessed outcomes of adefovir dipivoxil therapy among hepatitis B e antigen (HBeAg) negative chronic hepatitis B patients who were lamivudine resistant. Adefovir was administered either at the time point of genotypic resistance (HBV DNA level rising) or phenotypic resistance (ALT level rising). The authors defined genotypic resistance as the presence of moderate HBV DNA levels (3–6 $\log_{10}$ copies/mL) and persistently normal ALT levels; and phenotypic resistance as presence of high levels of both HBV DNA (>6 $\log_{10}$ copies/mL) and ALT. The authors did not define normal ALT level.

All study participants were treated with lamivudine and monitored for signs of drug resistance from study initiation. Monitoring included bi-monthly quantitative HBV DNA testing. Study participants who developed lamivudine resistance were assigned to one of two cohorts. Cohort I included those who developed genotypic resistance to lamivudine when adefovir was not available and continued on lamivudine monotherapy. Genotypic resistance had become phenotypic resistance in all patients in this cohort by the time adefovir was available. Adefovir was available and administered at genotypic resistance to cohort II patients. Adefovir was added to lamivudine treatment in both cohorts. Comparison of adefovir treatment outcomes between the genotypic and phenotypic resistance cohorts informed assessment of HBV DNA testing on patient outcomes. In the second cohort, exposing lamivudine resistance would only happen by detecting phenotypic resistance, such as increasing ALT levels. Because decisions to begin adefovir treatment were based on treatment availability, not ALT level, the phenotypic resistant cohort in this study may not ne comparable with a group whose ALT levels were monitored. Table 16 shows adefovir treatment outcomes.

The results presented in Table 16 indicate that detecting genotypic resistance using HBV DNA testing among HBeAg negative patients undergoing lamivudine monotherapy improves treatment outcomes. When adefovir was co-administered with lamivudine,

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[3] Test schedule:
1. Every two to four weeks: a) for the first 12 months after transplantation; b) for four months after pulse steroid and/or anti-lymphocyte therapy for acute rejection; c) when serial HBV DNA levels show increasing trend; d) during treatment with lamivudine
2. Every four months for clinically stable long term renal allograft recipients receiving low-dose immunosuppressive medications

patients in the genotypic resistance cohort had a significantly greater response compared with the phenotypic resistance cohort: the proportion of patients with undetectable serum HBV DNA levels at three months was 100 per cent and 46 per cent, respectively ($p<0.001$). Serum ALT levels among the genotypic resistance cohort were also normal at lamivudine resistance onset and remained normal throughout the study period (24 months of adefovir treatment reported). In contrast, normalisation of ALT levels in the phenotypic resistance cohort was time-dependent (Table 16). The two-year clearance rate of serum HBV DNA was higher (100%) among the genotypic resistance cohort compared with the phenotypic cohort (78%) (p values not reported). There was no resistance to adefovir treatment (Table 16) reported in either cohort.

Lampertico et al (2005) provided evidence that pre-treatment HBV DNA levels can predict response to adefovir. Median pre-treatment serum HBV DNA levels for patients in the genotypic resistance and phenotypic resistance cohorts were 4.5 $\log_{10}$ copies/mL (range, 3.4–5.9) and 7.3 $\log_{10}$ copies/mL (range, 4.3–9.3) respectively ($p<0.001$). Pre-treatment levels of ALT were also significantly different between cohorts. Median pre-treatment serum ALT levels of patients in the genotypic cohort and phenotypic cohorts were 38 IU/mL (range, 20–70) and 145 IU/mL (range 42–2870) respectively ($p<0.001$). Although the time period between the development of genotypic and phenotypic resistance was relatively short, results indicated that adefovir treatment at genotypic resistance had more favourable outcomes because serum HBV DNA levels were lower than at the time of phenotypic resistance. Hepatitis B outcomes were poorly reported. It could not be determined if the benefits of detecting lamivudine resistance at the genotypic stage translated to longer term benefits in relation to cirrhosis and hepatocellular carcinoma.

Results reported by Lampertico et al (2005) indicated that adefovir therapy was more effective among lamivudine resistant patients when administered at the time of genotypic resistance (Table 16). By inference, this provided evidence that regular HBV DNA testing for patients undergoing lamivudine monotherapy improves health outcomes. The study also showed that adefovir therapy was less effective when administered at the time of phenotypic resistance. This can be inferred to suggest that lamivudine-treated patients who do not undergo regular HBV DNA testing could potentially have less favourable outcomes. These results suggested that ALT testing alone may not be the most effective means of detecting initial development of lamivudine resistance. Adding regular HBV DNA testing offers improvement for monitoring patients undergoing lamivudine monotherapy with potential for short term outcome improvements.

Table 15 Study characteristics: Assessment of additional value offered by HBV DNA testing among lamivudine treated patients

Study	Study design	Patients (N)	Other tests	Test characteristics	Treatment	Study quality
Lampertico et al (2005) Italy	Prospective; not reported if consecutive; cohort study (1997 to 2003)	HBeAg negative during previous 12 months; ≥18 years; HBsAg carriers; with histological or clinical diagnosis of chronic hepatitis or cirrhosis. Unresponsive to, or did not meet, criteria for interferon therapy **Lamivudine resistance phase:** Phenotypic[a] resistant cohort (n = 46), genotypic[b] resistance cohort (n = 28)	HbeAg; anti-HBeAg; histological or clinical assessment of chronic hepatitis B or cirrhosis	**HBV DNA test:** Versant 3.0 (bDNA), Bayer. Sensitivity limit of 2000 copies/mL or 3.3 $\log_{10}$ copies/mL. Serum HBV DNA assayed every 2 months **Comparator tests:** ALT (standard laboratory procedures, further details not reported)	Lamivudine (100 mg/day); adefovir 10 mg (orally) added to lamivudine monotherapy *Adefovir availability:* at time of phenotypic resistance (n = 46); adefovir added 33 months[c] (average) after lamivudine initiation at time of genotypic resistance (n = 28); adefovir added 41 months[c] (average) after lamivudine initiation	Potential for bias: patients who were treated using adefovir at different stages of lamivudine resistance were not randomised Non-consecutive recruitment: only included patients who developed lamivudine resistance Comparator test not interpreted independently of HBV DNA test Reduced applicability: less frequent HBV DNA testing than in Australian clinical practice

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Phenotypic resistance defined as high levels of HBV DNA (>6 $\log_{10}$ copies/mL) and high ALT levels (NR by study)

[b] Genotypic resistance defined as moderate levels of HBV DNA (3-6 $\log_{10}$ copies/mL) and persistently normal ALT levels (NR by study)

[c] Because genotypic resistance is identified before phenotypic resistance it is suspected that the timing of adefovir addition to therapy has not been reported correctly in this study and that adefovir treatment was begun at 33 months for the genotypic resistant cohort and at 41 months for the phenotypic resistant cohort

Table 16 Adefovir treatment outcomes among lamivudine resistant patients

Study	Cohort	Pre-adefovir treatment features (median, range)		Adefovir treatment outcomes				Study quality
		Serum HBV DNA (log_{10} copies/mL)	Serum ALT (IU/mL)	Response[a]	Serum ALT normalisation[b]	Two year clearance rate of serum HBV DNA	Onset of adefovir resistance[c]	
Lampertico et al (2005) Italy	**Genotypic resistance** (n = 28) Adefovir initiated 41 months[d] (average) after lamivudine Genotype D: 94% (of 17 tested)	4.5 (3.4–5.9)	38 (20–70)	100%	100% (throughout the study period)	100%	0%	Potential for bias: patients who received adefovir at different stages of lamivudine resistance were not randomised Non-consecutive recruitment: only included patients who developed lamivudine resistance Comparator test not interpreted independently of HBV DNA test Reduced applicability: less frequent HBV DNA testing in Australian clinical practice
	Phenotypic resistance (n = 46) Adefovir initiated 33 months[d] (average) after lamivudine Genotype D: 92% (of 39 tested)	7.3 (4.3–9.3)	145 (42–2870)	46%	**Time-dependent:** month 3 = 37% (p<0.0001) month 6 = 50% (p<0.001) month 24 = 93% (p not significant)	78%	0%	

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Patients (%) with undetectable serum HBV DNA after three months

[b] Patients (%) with normal ALT levels

[c] Patients (%) with greater than 1 log rebound of serum HBV DNA compared with on-treatment (adefovir addition) nadir. None of 10 patients with detectable serum HBV DNA at the end of the study had adefovir-related mutations, rtN236T AND rtA181V

[d] Because genotypic resistance was identified before phenotypic resistance it ispossible that timing of adding adefovir was not been reported accurately in this study, and that adefovir treatment commenced at 33 months for the genotypic resistant cohort and at 41 months for the phenotypic resistant cohort

Study quality

The phenotypic resistance cohort described by Lampertico et al (2005) is comparable with a group of patients monitored without HBV DNA testing. HBV DNA testing was available and genotypic resistance was detected, but adefovir was not made available until phenotypic resistance developed. Decisions to provide adefovir therapy was determined by test availability, not test results, to assess development of phenotypic resistance.
It appears possible that serum ALT levels among this cohort may not be comparable with patients undergoing treatment using adefovir in response to rising ALT levels alone. If detection of phenotypic resistance, such as by serum ALT levels, was considered to be the comparator to HBV DNA testing, there was no blinding between cohorts.
The serum ALT levels among the phenotypic cohort participants were not interpreted independently of HBV DNA levels—increased HBV DNA levels would have alerted treating clinicians to which patients' ALT was likely to change. These considerations make the phenotypic cohort less comparable with a cohort of patients undergoing lamivudine therapy who were not monitored using HBV DNA testing. It could not be determined whether reported outcomes for the phenotypic resistance cohort in this study provided a conservative estimate of outcomes where HBV DNA testing was unavailable.

There was potential for bias in this study which was not designed with the objective of determining whether HBV DNA testing improves health outcomes among patients undergoing lamivudine monotherapy. Bias may have been introduced because the cohorts who were treated using adefovir at different stages of lamivudine resistance (genotypic resistance and phenotypic resistance) were not randomised. It seems that patient recruitment was non-consecutive, which further enhanced bias potential.
Lampertico et al (2005) applied prospective recruitment—all patients who underwent lamivudine treatment were not included—only those who developed resistance.
This design or reporting omission did not allow assessment to be made about the impact of serum HBV DNA testing used to monitor all patients undergoing lamivudine therapy, irrespective of resistance status. Outcomes for patients who did not develop resistance were not reported, so comparisons could not be made.

Applicability

The antiviral therapies considered by Lampertico et al (2005) are applicable to Australian clinical practice. Lamivudine and adefovir are licensed in Australia, but government funding for adefovir is available only for lamivudine resistant patients. It was not reported whether pre- or post-treatment liver biopsies were obtained from study participants. Pre-treatment liver biopsy is required for access to therapies through the Australian Commonwealth Government Highly Specialised Drug Section 100 scheme for the treatment of chronic hepatitis B.

The two-monthly regimen of serum HBV DNA testing reported by Lampertico et al (2005) may reduce the applicability of the results to Australian clinical practice.
HBV DNA testing for HBeAg negative patients is conducted every three months in Australian clinical practice. Serum HBV DNA testing every three months is considered to be the optimal regimen in Australia for patients undergoing lamivudine therapy (advisory panel).

Lampertico et al (2005) conducted their study in Italy, where people with hepatitis B virus are more likely to have HBeAg negative disease, which is a progression from positive status (Bell et al 2005). The study's HBeAg negative patient population had extensive fibrosis or cirrhosis. Non-consecutive patient selection may have contributed

to a bias to introduce higher than expected levels of fibrosis and cirrhosis. The findings of this study related to HBeAg negative patients with advanced liver disease or cirrhosis.

Linked evidence

Linking evidence requires aggregating test accuracy and impact of the test on patient management with evidence for treatment effectiveness (MSAC 2005). This approach was necessary because evidence concerning the direct impact of HBV DNA testing on health outcomes was limited.

Lampertico et al (2005) provided some evidence supporting that HBV DNA testing use can lead to improved health outcomes, a linked evidence approach was required because:

1. This study applied to HBeAg negative patients with advanced liver disease or cirrhosis undergoing antiviral therapy. Evidence was also required to support effectiveness of HBV DNA testing among HBeAg positive patients, initial assessments, and monitoring those not undergoing antiviral therapy.

2. The study did not provide evidence that HBV DNA testing provides longer term clinical improvement outcomes. Evidence is required to indicate that changes in serum HBV DNA levels correlate with changes in clinical outcomes.

3. The quality of evidence presented was considered inadequate to inform assessment of HBV DNA testing in the absence of a linked approach.

The study by Chan et al (2002) was considered to provide direct evidence supporting the impact of HBV DNA testing on patient outcomes, but was limited to HBsAg positive kidney allograft recipients. This study had limited applicability to HBV DNA testing in wider clinical practice, and confirmed need for a linked evidence approach. The quality of evidence presented was considered inadequate to assess HBV DNA testing in the absence of a linked approach.

HBV DNA test accuracy: Summary of evidence

HBV DNA testing and initial assessment and monitoring of patients not undergoing antiviral therapy

Serum HBV DNA and differentiating inactive carriers from chronic hepatitis B patients

Manesis et al (2003) and Seo et al (2005) assessed the value of HBV DNA testing to differentiate inactive HBeAg negative carriers from HBeAg negative active chronic hepatitis B patients. Manesis et al (2003) reported that serum HBV DNA with a cut point of 30,000 copies/mL had the best diagnostic performance to differentiate active and carrier patient status. There was potential for patient misclassification: sensitivity (identification of patients with active chronic hepatitis B) was 89.6 per cent, meaning that patients could be misclassified as HBeAg negative carriers. This study also reported evidence suggesting that HBV DNA testing adds additional diagnostic performance to differentiation by IgM anti-Hbc complex measurement. In the other study a suitable serum HBV DNA cut point was hard to determine without misclassifying patients: at HBV DNA test thresholds of 4.5 and 5 log copies/mL, 23 per cent and 18 per cent of inactive carriers would be misclassified as active chronic hepatitis B patients respectively. The additional value of HBV DNA testing was not reported in this study. Both studies were considered poor quality as the HBV DNA test was interpreted with knowledge of the reference standard.

Serum HBV DNA and liver histology

Lindh et al (2000) found that increased serum HBV DNA levels were associated with extensive liver damage, especially among HBeAg negative patients. No association was found between serum HBV DNA levels and liver damage among HBeAg positive patients. Peng et al (2003) provided modest evidence indicating that higher serum HBV DNA levels were associated with increased liver damage in HBeAg negative patients. Both studies were considered to offer poor quality evidence because HBV DNA test results were interpreted with knowledge of the reference standard. Additional value provided by HBV DNA testing was not reported by either study.

Serum HBV DNA and sustained HBeAg seroconversion

Chan et al (2003) provided evidence that serum HBV DNA levels of $>10^5$ copies/mL at or following seroconversion are predictive of HBeAg reversion. No tests designated as comparators for this assessment were reported.

HBV DNA testing and monitoring of patients undergoing antiviral therapy

HBV DNA testing and predicting response to lamivudine

Buti et al (2001) found that a negative HBV DNA test at month three of therapy was both moderately sensitive and specific as an indicator of sustained response. This study was considered to present poor quality evidence because the reference standard was the clinical outcome of sustained treatment response which was measured by the index test.

Zollner et al (2001) reported sequential measurement of HBV DNA levels during lamivudine therapy for the test's potential value as a predictor of HBeAg seroconversion and emergence of drug resistance. HBV DNA testing giving a value of <log 2 copies/mL at month 12 of therapy was reported to be the optimal time to predict seroconversion or resistance to lamivudine. Patients with HBV DNA levels above the detection limit at 12 and 15 months did not have seroconversion, indicating no response to therapy. Serum HBV DNA below the detection limit at month 15 of therapy was a strong predictor that sensitivity to lamivudine was maintained.

HBV DNA testing and predicting response to interferon treatment

Lindh et al (2001) sought to determine if pre-treatment HBV DNA levels could predict sustained virological response among interferon-treated patients who had prior prednisolone (priming). When a serum HBV DNA threshold value of log 8.7 copies/mL was used, there was limited value in differentiating non-sustained responders from sustained responders: the PPV of serum HBV DNA level <log 8.7 copies/mL to predict sustained response was 67 per cent; the NPV of >log 8.7 copies/mL to predict non-sustained response was 67 per cent; and the sensitivity and specificity of log 8.7 copies/mL to predict sustained response and non-sustained were 60 per cent and 75 per cent, respectively. Study quality was considered to be poor because HBV DNA testing was also part of the reference standard.

Van der Eijk et al (2006) evaluated HBV DNA testing as a predictor of response among HBeAg positive chronic hepatitis B patients treated with interferon-α. Log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 12 was better able to predict the proportion of patients who would not respond to treatment: specificity equals 61 per cent. Incomplete specificity meant that misclassification of patients as potential treatment responders could occur if the criteria of log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 12 were used in clinical practice.

None of the studies which were considered to provide evidence for the accuracy of HBV DNA testing in monitoring treatment response reported data which enabled an assessment of the value of HBV DNA testing in addition to other tests (eg ALT, serology, liver histology).

None of the studies considered to provide evidence for the accuracy of HBV DNA testing reported data which facilitated reconstruction of diagnostic performance values. These values could not be confirmed

Is HBV DNA testing accurate?

An ideal study model to determine effectiveness of HBV DNA testing for chronic hepatitis B diagnosis would include measurement of serum HBV DNA levels among a prospectively recruited consecutive cohort of patients. These patient data would be cross-referenced to histological analyses of liver biopsy specimens or chronic hepatitis B clinical outcomes, such as cirrhosis, hepatocellular carcinoma, seroconversion, or sustained viral replication suppression. The reference standard, the best available method of determining true disease status, would be liver biopsy or chronic hepatitis B clinical outcomes. HBV DNA test results would be compared with the reference standard in an independent, blinded approach. It would also be necessary cross-reference the proportional change from baseline HBV DNA levels with liver biopsy results or chronic hepatitis B clinical outcomes where serum HBV DNA testing was used to monitor patients regardless of therapy regimen.

To confirm test accuracy data, where reporting was inadequate in studies considered for inclusion in this assessment, authors of 53 studies were contacted to seek further information. No additional data were obtained from this activity.

HBV DNA testing and the initial assessment and monitoring of patients not receiving antiviral therapy

Studies by Chan et al (2003), Lindh et al (2000), Manesis et al (2003), Peng et al (2003), and Seo et al (2005) informed assessment of the value of serum HBV DNA levels in initial assessment and monitoring of patients not undergoing antiviral therapy. Study characteristics are presented in Table 17.

Table 17 Study characteristics: Evaluating the predictive value of serum HBV DNA testing for initial assessment and monitoring of patients not undergoing antiviral therapy

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator test(s) characteristics	Reference standard	Study quality
Chan et al (2003) China	Cohort, direction unclear Enrolment not reported (Time period not reported)	Two groups of treatment naïve patients: 1. HBeAg positive at study entry, followed by sustained seroconversion for at least 6 months with normal ALT (n = 33) Sustained seroconversion: n = 20: 11 M, 9 F; median age 29 (range 12–39) HBeAg reversion: n = 13, 11 M, 2 F; median age 39 (range 13–68) 2. HBeAg negative, anti-HBeAg positive at study entry (n = 40) Persistent HBeAg negative: n = 29, 12M, 17F; median age = 41 (range 21–65) HBeAg reversion: n = –11, M 8,F 3, median age = 49 (range 37–67)	Tests before study entry not reported	Realtime PCR (TaqMan standardised to EUROHEP gentotype D, HBV standard) Sensitivity range = 10^2 to 10^9 copies/mL	Not reported	Clinical outcomes: Sustained seroconversion (HBeAg negative, anti-HBeAg positive) or reversion to HBeAg positive Details of assessment of sustained seroconversion or HBeAg reversion not reported	P1 Q3 *Applicability*: Unclear Criteria for patient inclusion not reported *Quality:* Poor Direction unclear Potential selection bias Inadequate reporting of reference standard

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator test(s) characteristics	Reference standard	Study quality
Lindh et al (2000) Sweden	Prospective, cohort, non-consecutive	Chronic hepatitis B patients attending regular check-up invited to participate (irrespective of clinical signs of disease) Excluded: HCV or HDV coinfected patients excluded (2 patients had previous interferon treatment 6 years before) n = 160 (105 M, 55 F) Mean age = 34 (range 16–66) HBeAg positive: n = 36 (mean age 30 years), HBeAg negative: n = 124 (mean age 40 years)	Tests before study entry not reported	Amplicor HBV monitor (Roche) Detection range = 10^3 to 10^7 copies/mL (measured at time of liver biopsy)	ALT (details not reported, Indexed ALT[a], ALTi used for analysis)	Liver biopsy: details NR Histological scoring: HAI (Knodell method) HAIinf (inflammatory score) and HAIfib (fibrosis score) Scoring blinded	P1 Q2 *Applicability*: Applicable *Quality*: Medium Valid reference standard, blinded to test results Potential for selection bias: non-consecutive recruitment
Manesis et al (2003) Greece	Direction unclear Consecutive cohort (1997–1999)	HBeAg negative chronic hepatitis B patients; HBsAg and HBeAg negative ≥6 months before study entry Excluded: HDV, HCV or HIV co-infected and patients with decompensated liver disease and/or hepatocellular carcinoma (n = 196) Classified as HBeAg negative chronic hepatitis B if ALT[b] activity increased and liver histology compatible with chronic hepatitis B Classified as HBeAg negative inactive carriers if had persistent normal ALT[b] during follow-up	HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBeAg by enzyme immunoassay HDV, HCV, HIV serology by enzyme immunoassay Liver biopsies scored for histological grade and stage according to Ishak score	PCR; Amplicor HBV Monitor (Roche) LLD = 400 copies/mL	IgM anti-HBc index by semi quantitative ELISA (Abbot) ALT, AST (Abbot spectrum auto analyser)	HBeAg carrier status Classified as HBeAg negative chronic hepatitis B if ALT activity increased and liver histology compatible with chronic hepatitis B Classified as HBeAg negative inactive carriers if had persistent normal ALT during follow-up	P1 Q3 *Applicability*: Applicable HBV DNA test regimen considered applicable to Australian clinical practice *Quality*: Poor Index test interpreted with knowledge of reference standard Some potential for selection bias: direction unclear, consecutive enrolment

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator test(s) characteristics	Reference standard	Study quality
Peng et al (2003) China	Prospective, non-consecutive cohort; test-based enrolment (patients with liver biopsy specimens <1.0 cm not enrolled) (1998 to 2000)	HBsAg positive; anti-HBs negative; no antiviral immunosuppressive therapy during period ≥6 months before study. No immunocompromising diseases. Hepatitis A, E, C or D virus co-infected or other liver disease patients excluded (n = 743) HBeAg positive = 64.1%; HBeAg negative = 35.9% HBeAg positive group (27.1+/– 8.3 years) vs. HBeAg negative group (32.7+/– 15.3 years) (p<0.001) Gender ratio: HBeAg positive vs HBeAg negative group: 413/63 vs 234/33 (M/F) (647 M, , 96 F)	HBsAg; anti-HBs	Dot-blot hybridisation-based, in-house assay. Semi-quantitative ranges: <20, 20–100, 100–200, 200–500, 500–1000 and >1000 pg/mL	NR	Liver biopsy: Surecut needles (16G X 70–90 mm), specimens formalin fixed, paraffin embedded and stained with hematoxylin, eosin and reticulin. Slides read blindly Histological scoring: HAI (Knodell method) HAIinf (inflammatory score) and HAIfib (fibrosis score)	P2 Q2 *Applicability*: Limited In-house assay *Quality*: Medium Valid reference standard, blinded to test results Potential selection bias: non-consecutive enrolment
Seo et al (2005) Japan	Retrospective, cohort, Enrolment not reported (1989–2002)	Chronic hepatitis B patients : HBeAg negative inactive carriers (with persistently normal ALT levels) (n = 22) HBeAg negative chronic hepatitis B (persistent or intermittent ALT elevation) (n = 26) Patients followed up for mean 51.5 months (range 5–157 months) and seen every 1–3 months	HBsAg; anti-HBs, HbeAg, anti-HBeAg enzyme immunoassay (Dianabot) ALT measurement not reported	PCR; Amplicor HBV Monitor (Roche) Detection range = 10^3 to 10^7 copies/mL	NR	HBeAg carrier status: HBeAg negative inactive: HBeAg negative and normal ALT HBeAg negative active: HBeAg negative and persistent on intermittent ALT elevation	P1 Q3 *Applicability*: Applicable *Quality*: Poor Index test interpreted with knowledge of reference standard Potential selection bias: patient selection criteria unknown, retrospective analysis

Abbreviations: ALT, alanine aminotransferase; ALTi, indexed ALT; AST, aspartate aminotransferase; EUROHEP, European concerted action on viral hepatitis; HAI, histological activity index; HAIinf, histological activity index fibrosis score HBV DNA, hepatitis B virus deoxyribonucleic acid; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus s antigen; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; LLD, lower limit of detection

[a] ALTi = ALT divided by upper reference value (URV; 0.8 μkat^{-1} for males and 0.6 μkat^{-1} for females)

[b] Patients with abnormal ALT levels at baseline (>upper limit of normal) followed by monthly ALT determination., with liver biopsy if increased ALT on at least twice. Patients with normal ALT followed for at least 24 months: ALT tested every three months for first two years and every six months thereafter

Serum HBV DNA and sustained HBeAg seroconversion

Chan et al (2003) aimed to identify a serum HBV DNA level to predict sustained disease remission or HBeAg reversion.

Remission occurred in 65 per cent of patients who were HBeAg positive at study entry (n = 33), and who subsequently seroconverted. The authors applied receiver operating characteristic (ROC) curve analysis (see page 23) to assess the value of HBV DNA levels at seroconversion to predict HBeAg reversion. The reported area under curve (AUC) (see page 23) for this ROC curve was 0.73 (95% CI: [0.53, 0.93]; $p = 0.03$). HBeAg reversion occurred in 28 per cent of a patient group who were HBeAg negative and anti-HBeAg positive at study entry (n = 40). Using HBV DNA levels at the time of study entry to predict HBeAg reversion, ROC curve analysis resulted in an AUC of 0.83 (95% CI: [0.67, 0.98]; $p = 0.002$). These data suggest that HBV DNA levels may be moderately accurate to predict HBeAg reversion. Chan et al (2003) did not however report data to confirm these analyses.

Interval likelihood ratios were also reported for the ability of different serum HBV DNA levels to predict HBeAg reversion. Analysis results are presented in Table 18.

Table 18 Serum HBV DNA levels and predicting HBeAg reversion

Study	Patient group	Serum HBV DNA (Log10)	Proportion of patients (%)		Interval likelihood ratio	Study quality
			Sustained seroconversion	HBeAg reversion		
Chan et al (2003) China	HBeAg positive at study entry, seroconverted	<4 [a]	55.0	15.4	0.3	P? Q3 *Applicability*: Unclear Criteria for patient inclusion not reported *Quality:* Poor Direction unclear Potential selection bias Inadequate reporting of reference standard
		4–5 [a]	40.0	38.5	1.0	
		>5 [a]	5.0	46.2	9.2	
	HBeAg negative, anti-HBeAg positive at study entry	<4 [b]	65.5	18.2	0.3	
		4–5 [b]	24.1	18.2	0.8	
		>5 [b]	10.3	53.8	5.2	

Abbreviations: HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid
[a] Serum HBV DNA levels at time of seroconversion
[b] Serum HBV DNA levels at time of study entry

The results presented in Table 18 indicate that serum HBV DNA levels of $>10^5$ copies/mL at the time of seroconversion are predictive of HBeAg reversion (interval likelihood ratio = 9.2). Likewise, serum HBV DNA levels of $>10^5$ copies/mL after seroconversion are also predictive of HBeAg reversion (interval likelihood ratio = 5.2). The additional value of HBV DNA testing could not be assessed because there were no tests considered to be comparators for this assessment reported by this study.

Results reported by Chan et al (2003) should be interpreted with caution. The reported clinical outcomes were considered to be the reference standard for this assessment. Chan et al (2003) did not report how sustained seroconversion or HBeAg reversion was assessed and if clinical outcomes were interpreted blind to HBV DNA levels. It was also

unclear whether this study was prospective or retrospective. There is potential for selection bias because patient enrolment was not reported. The applicability of this study is unclear for the reason that patient inclusion criteria were not reported. This study was considered poor quality.

According to NHMRC levels of evidence for diagnostic studies (see page 24), the study by Chan et al (2003) was considered to present level III–2 evidence.

Serum HBV DNA and liver histology

Studies by Lindh et al (2000) and Peng et al (2003) informed assessment of the value of serum HBV DNA testing to predict liver histology.

Lindh et al (2000) compared levels of serum HBV DNA and ALT with liver histology activity index (HAI) in treatment regimens for naïve chronic hepatitis B patients attending regular check-ups. They found no association between serum HBV DNA levels and liver histological activity among HBeAg positive patients. Increasing serum HBV DNA level was associated with higher histology activity index inflammation and histology activity index fibrosis scores ($p<0.0001$) among HBeAG negative patients. The authors reported predictive values of serum HBV DNA and indexed ALT (ALTi) for minimal and severe inflammation among HBeAg negative patients. The results of this analysis are presented in Table 19.

Table 19 Value of serum HBV DNA and ALT to predict liver inflammation among HBeAg negative patients

Study	Predictive value		Serum HBV DNA cut-off (copies/mL)			ALTi[a] cut-off			Study quality
			10^4	2 x 10^5	10^7	0.5	1.0	2.0	
Lindh et al (2000) Sweden	Predicting HAlinfl >3	PPV (%)	25	68	100	28	44	67	P1 Q2 *Applicability*: Applicable *Quality*: Medium Valid reference standard, blinded to test results Potential for selection bias: non-consecutive recruitment
		NPV (%)	84	87	81	97	88	82	
	Predicting HAlinfl >6	PPV (%)	14	36	83	14	20	42	
		NPV (%)	97	95	93	100	94	93	

Abbreviations: ALTi, indexed alanine aminotransferase; HAlinfl, histology activity index inflammation score; HBV DNA, hepatitis B virus deoxyribonucleic acid; NPV, negative predictive value; PPV, positive predictive value

[a] Indexed ALT (ALTi) is ALT divided by upper reference value; 0.8 μkat^{-1} for males and 0.6 μkat^{-1} for females

Data presented in Table 19 show that the positive predictive values for histology activity index inflammation scores >3 and >6 escalate as serum HBV DNA and ALTi cut-offs were increased. This suggests that increased serum HBV DNA and ALT levels are predictive of increased liver inflammation among HbeAg negative patients. The negative predictive values indicate the proportion of patients with histology activity index inflammation scores ≤3 and ≤6 below each cut-off value for serum HBV DNA and ALTi. There is little variation between these values indicating that liver damage (as assessed by histology activity index inflammation scores) may not necessarily increase as serum HBV DNA and ALTi levels increase. Positive predictive value and negative predictive value relate to different groups of patients—those with histology activity index inflammation scores >3 or >6 and those with histology activity index inflammation scores ≤3 and ≤6. Positive and negative predictive values are prevalence dependent—analysis results (Table 19) should be interpreted cautiously—they may not apply to other chronic hepatitis B populations. Inadequate data reporting meant that the additional value of HBV DNA testing over other tests could not be determined.

The prospective study by Lindh et al (2000) was considered to be medium quality and applicable to Australian clinical practice. Liver biopsy was considered to be an appropriate reference standard for this assessment. Liver biopsy histology activity index was scored blind to test results. Test results were interpreted with knowledge of the reference standard to determine the predictive value of HBV DNA testing. Inadequate data reporting did not permit confirmation of predictive values. There was potential for selection bias because patient recruitment was non-consecutive.

According to NHMRC levels of evidence for diagnostic tests (see page 24), the study by Lindh et al (2000) is considered to present level III–1 evidence.

Peng et al (2003) studied serum HBV DNA levels that were cross-classified to liver histology. This study's objective was to determine the clinical and histological characteristics of HBeAg negative and positive chronic hepatitis B patients. The study was not designed to assess the diagnostic performance of HBV DNA testing as an indicator of chronic hepatitis B. The characteristics of this study are described in Table 17. Peng et al (2003) did not report data that informed assessment of the additional value of HBV DNA testing.

The study by Peng et al (2003) considered a population who were prospectively recruited, non-consecutive cohort of chronic hepatitis B patients 64 per cent of whom were HBeAg positive and the balance HBeAg negative. Peng et al (2003) reported serum HBV DNA levels, cross-classified to histological assessment of liver biopsy samples by both histological activity index inflammatory and histological activity index fibrosis scores. Cross-classification allowed assessment to be made of the diagnostic performance of serum HBV DNA testing. In this analysis, when histological activity index inflammatory or histological activity index fibrosis scores were considered as reference standards, cut-off scores of ≥9 and ≥3, respectively, were considered positive for chronic hepatitis B.

Positive and negative likelihood ratios were calculated to assess the diagnostic performance of serum HBV DNA testing. Each range of serum HBV DNA values was considered the cut point for chronic hepatitis B positive. This analysis assessed the likelihood of developing chronic hepatitis B among patients who test positive (or negative) for different serum HBV DNA values. Analysis results are presented in Table 20.

There is a trend toward increased negative likelihood ratio values among HBeAg positive patients when lower serum HBV DNA levels are considered as threshold values for chronic hepatitis (Table 20). (Negative likelihood ratio values are held to be greater than positive likelihood ratio values). These results suggest that there is an increased likelihood of chronic hepatitis B among patients whose serum HBV DNA test results are below threshold values. Thus, lower levels of serum HBV DNA in HBeAg positive patients may be associated with increased liver damage.

Conversely, for each serum HBV DNA threshold value in HBeAg negative patients, positive likelihood ratio is greater than negative likelihood ratio indicating an increased likelihood of chronic hepatitis B among patients with HBV DNA values above the threshold. When the threshold is set at an HBV DNA level of 100–200 pg/mL, the positive and negative likelihood ratios of 4.18 and 0.48 respectively, indicate that this may be the most appropriate level to indicate presence of chronic hepatitis B. Overall, the results of this analysis among HBeAg negative patients indicate that higher levels of serum HBV DNA are associated with increased liver damage.

The study by Peng et al (2003) was considered to offer limited applicability and medium quality. Applicability was limited because the HBV DNA assay applied and reported was developed in-house and performance may be dissimilar to assays available in Australia. No evidence was reported to indicate whether the limits of detection and linearity were tested against current internationally recognised HBV DNA standards such as EUROHEP. Although the study was conducted in China, the patient population was considered applicable because a recent prospective cohort study of Australian chronic hepatitis B patients reported that 65 per cent had Asian ethnicity (Bell et al 2005).

Because there was potential for selection bias, quality was considered medium. Patients were recruited based on a suitable reference standard (liver biopsy specimen not <1.0 cm). It was significant that liver biopsy slides were read blind. Test results were interpreted by the authors with knowledge of the reference standard to inform determination of the predictive value of HBV DNA testing.

In compliance with NHMRC levels of evidence for diagnostic studies (see page 24), the study by Peng et al (2003) was considered to present level III–1 evidence.

Table 20 Diagnostic performance of a hybridisation-based serum HBV DNA assay

Patients	Reference standard	Serum HBV DNA levels (pg/mL[a])											
		<20		20–100		100–200		200–500		500–1000		>1000	
		LR+	LR–	LR+	LR–	LR+	LR–	LR+	LR–	LR+	LR–	LR+	LR–
HBeAg positive	Histological activity index inflammatory score	1.00	0.00[b]	0.75	4.84	0.44	2.86	0.24	1.61	0.41	1.10	0.35	1.03
	Histological activity index fibrosis score	1.00	0.00[b]	0.70	3.83	0.39	2.36	0.27	1.41	0.49	1.07	0.77	1.01
HBeAg negative	Histological activity index inflammatory score	1.00	0.00[b]	2.15	0.07	4.18	0.48	1.21	0.98	0.26	1.06	NR	NR
	Histological activity index fibrosis score	1.00	0.00[b]	1.65	0.17	2.31	0.60	1.61	0.97	0.00	1.02	NR	NR

Abbreviations: HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NR, not reported

[a] 1 pg/mL = 3 x 105 copies/mL

[b] When the value for true negative = 0, this has been adjusted to 0.1 to allow a meaningful LR– to be calculated

Serum HBV DNA and differentiating inactive carriers from chronic hepatitis B patients

In an analysis of patients consecutively examined during routine chronic hepatitis B monitoring, Manesis et al (2003) investigated the predictive ability of baseline measurements of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc) and HBV DNA levels to differentiate hepatitis B e antigen (HBeAg) negative chronic hepatitis B patients from HBeAg negative inactive carriers. The predictive ability of a combined IgM anti-HBc and HBV DNA index was also investigated. Analysis results are presented in Table 21 and Table 22.

The ability of several baseline tests to correctly classify active chronic hepatitis B and inactive carriers was measured at cut points appropriate for each test. These data informed determination of positive predictive value, negative predictive value, specificity and sensitivity to differentiate active chronic hepatitis B from inactive carriers (Table 21 and Table 22). It was considered that Manesis et al (2003) applied HBeAg negative carrier status (HBeAg negative and persistent normal ALT during follow-up) as the reference standard for analyses.

Results indicate that serum HBV DNA with a cut point of 30 000 copies/mL has the best sensitivity (89.6%), specificity (100.0%), positive predictive value (100.0%) and negative predictive value (81.6%) to discriminate between active chronic hepatitis B and inactive HBeAg negative patients (Table 21). The combined serum HBV DNA IgM anti-HBc index had slightly higher sensitivity (95.5%) but lower specificity (91.9%).

Manesis et al (2003) also investigated the diagnostic performance of baseline tests among a subset of patients with normal baseline ALT/AST levels (Table 22). This analysis included 25 active chronic hepatitis B patients with normal ALT/AST levels at baseline, but who subsequently developed abnormal ALT during follow-up. HBV DNA with a cut point of 30 000 copies/mL had the best diagnostic performance to discriminate between active chronic hepatitis B and inactive HBeAg negative patients in this patient subset. Compared with serum HBV DNA with a cut point of 30 000 copies/mL, the combined serum HBV DNA IgM anti-HBc index was more sensitive (92.0% vs 80%) but had lower specificity (91.9% vs 100.0%).

Results reported by Manesis et al (2003) suggested that a baseline serum HBV DNA levels with a cut point of 30 000 copies/mL could differentiate HBeAg negative active chronic hepatitis B patients from HBeAg negative inactive carriers. IgM antiHBc tests used in addition to HBV DNA testing at this cut point resulted in a modest improvement in sensitivity and a decrease in specificity. Serum HBV DNA testing at a cut point of 30 000 copies/mL with or without IgM antiHBc testing is not completely sensitive, and thus, potential for misclassification of patients as HbeAg inactive carriers.

This study was considered to present poor quality evidence. Although selection bias was minimised by enrolling patients consecutively, the index test was interpreted with knowledge of the reference standard, which potentially biased diagnostic performance results. Reported values of HBV DNA testing diagnostic performance could not be confirmed. This study was considered applicable to Australian clinical practice. The regimen of serum HBV DNA testing was considered to be appropriate.

In compliance with the NHMRC levels of evidence for diagnostic studies (see page 24), the study by Manesis et al (2003) is considered level III–2 evidence.

Table 21 Diagnostic performance of baseline tests to differentiate HBeAg negative chronic hepatitis B from HBeAg negative inactive carriers

Study	Baseline test	HBeAg negative chronic hepatitis B (n = 134)	HBeAg negative inactive carriers (n = 62)	Correct classification (%)	Positive predictive value (%)	Negative predictive value (%)	Sensitivity (%)	Specificity (%)	Study quality
Manesis et al (2003)	ALT (> vs ≤ upper limit of normal)	109/25	0/62	87.2	100.0	73.1	81.3	100.0	P1 Q3 *Applicability:* Applicable HBV DNA test regimen considered applicable to Australian clinical practice *Quality:* Poor Index test interpreted with knowledge of reference standard Some potential for selection bias: direction unclear, consecutive enrolment
	AST (> vs ≤ upper limit of normal)	92/42	0/62	78.6	100.0	59.6	68.7	100.0	
	IgM anti-HBc (≥ vs <0.2000)	104/30	5/57	82.1	95.4	65.5	77.6	91.9	
	HBV DNA (≥ vs <30 000 copies/mL)	120/14	0/62	92.9	100.0	81.6	89.6	100.0	
	HBV DNA (≥ vs <100 000 copies/mL)	117/17	0/62	91.3	100.0	78.5	87.3	100.00	
	HBV DNA-IgM anti-HBc index (positive[a] vs negative)	128/6	5/57	94.4	96.2	90.5	95.5	91.9	

Abbreviations: ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; AST, aspartate aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid; IgM, immunoglobulin M

[a] Positive = serum HBV DNA ≥ 30 000 copies/mL or IgM anti-HBc ≥ 0.200. All other cases considered negative

Table 22 Diagnostic performance of baseline tests to differentiate HBeAg negative chronic hepatitis B from HBeAg negative inactive carriers when baseline ALT and AST values are normal

Study	Test	HBeAg negative chronic hepatitis B (n = 25)	HBeAg negative inactive carriers (n = 62)	Correct classification (%)	Positive predictive value (%)	Negative predictive value (%)	Sensitivity (%)	Specificity (%)	Study quality
Manesis et al (2003)	IgM anti-HBc (≥ vs <0.2000)	14/11	5/57	81.6	73.7	83.8	56.0	91.6	P1 Q3 *Applicability:* Applicable HBV DNA test regimen considered applicable to Australian clinical practice *Quality:* Poor Index test interpreted with knowledge of reference standard Some potential for selection bias: direction unclear, consecutive enrolment
	HBV DNA (≥ vs <30 000 copies/mL)	20/5	0/62	90.8	100.0	88.6	68.0	100.0	
	HBV DNA (≥ vs <100 000 copies/mL)	17/8	0/62	90.8	100.0	88.6	68.0	100.0	
	HBV DNA-IgM anti-HBc index (positive[a] vs. negative)	23/2	5/57	92.0	82.1	96.6	92.0	91.9	

Abbreviations: HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Positive = serum HBV DNA ≥30 000 copies/mL or IgM anti-HBc ≥0.200. All other cases considered negative

In a retrospective analysis of patients who underwent routine chronic hepatitis B monitoring, Seo et al (2005) compared serum HBV DNA levels between HBeAg negative inactive carriers with HBeAg negative chronic hepatitis B patients. The aim was to determine the HBV DNA level that could distinguish these groups. Serum HBV DNA levels used to differentiate inactive carriers from chronic active hepatitis patients were analysed. HBeAg carrier status (HBeAg negative and active or inactive disease) was considered to be the reference standard. The results of this analysis were used to determine the diagnostic performance of HBV DNA levels to differentiate these patient groups. These results are presented in Table 23.

When serum HBV DNA levels measured at one time point were used, the correct classification of patients as inactive carriers (sensitivity) increased as the serum HBV DNA cut point was increased (Table 23). At lower cut points of 4.5 and 5 log copies/mL, 23 per cent and 18 per cent respectively of inactive carriers would be misclassified as active chronic hepatitis B patients. The correct classification of patients with active chronic hepatitis B (specificity) was poor at the lower cut points, and was very low at the highest cut point, <6 (Table 23). Increasing the cut point for serum HBV DNA means that active chronic hepatitis B patients could be misclassified as inactive carriers. When serum HBV DNA levels were measured at two time points, the misclassification of inactive carrier patients at the lower cut points was somewhat reduced (Table 23). The misclassification of active chronic hepatitis B patients at the higher cut points was also reduced (Table 23).

These results indicate that differentiation of status between HBeAg negative inactive carriers and HBeAg negative active chronic hepatitis patients made on the basis of serum HBV DNA testing alone is not possible, and suggests that results of DNA testing should be considered with other tests (such as serology, serum ALT levels). It was not possible to assess the additional value of HBV DNA testing in this study because data regarding other test results were absent. It is likely that fluctuation in HBV DNA levels in both active and inactive carriers was responsible for HBV DNA testing's low ability to differentiate these patient groups.

This study was considered to present poor quality evidence. There was potential for selection bias because criteria for patient selection were not reported, and the analysis was retrospective. HbeAg carrier status (inactive or active, chronic) was considered the reference standard in this study. Assessment of HBV DNA levels to distinguish inactive carriers from patients with chronic active disease was not performed independently of patients' true carrier status. The values reported for the diagnostic performance of HBV DNA testing could not be confirmed. This study was considered to be applicable to Australian clinical practice: the regimen of HBV DNA testing (test and frequency) was considered appropriate.

According to the NHMRC levels of evidence for diagnostic tests (see page 24), the study by Seo et al (2005) was considered to present level III–2 evidence.

Table 23 Diagnostic performance of serum HBV DNA levels for differentiating between inactive carriers and chronic chronic hepatitis B patients

Study	Serum HBV DNA cut-off level (log copies/mL)	Serum HBV DNA measurement at one time point[a]		Serum HBV DNA measurement at two time points[b]		Study quality
		Sensitivity	Specificity	Sensitivity	Specificity	
Seo et al (2005)	<4.5	77	50	80	71.4	P1 Q3 Applicability: *Applicable* Quality: *Poor* Index test interpreted with knowledge of reference standard Potential selection bias: patient selection criteria unknown, retrospective analysis.
	<5	82	45	90	71.4	
	<5.5	91	32	100	57.1	
	<6	100	14	100	35.7	

Abbreviations: HBeAg, hepatitis b e antigen; HBV DNA, hepatitis b virus, deoxyribonucleic acid

[a] Measured at the end of follow-up (patient monitoring): Mean duration = 51.5 months (range 5–157 months), patients were seen every 1–3 months, or more often if clinically indicated. HBeAg negative inactive patients (n = 22); HBeAg negative active patients (n = 26)

[b] Mean time between 2 measurements was 4 +/– 2 months. HBeAg negative inactive patients (n = 10); HBeAg negative active patients (n = 14)

HBV DNA testing and monitoring of patients receiving antiviral therapy

Buti et al (2001), Zollner et al (2001), Lindh et al (2001), and van der Eijk et al (2006) (see Table 24) evaluated the predictive value of HBV DNA testing for patients undergoing lamivudine (Buti et al and Zollner et al) and interferon (Lindh et al and van der Eijk et al) therapy.

HBV DNA testing and predicting response to lamivudine therapy

Buti et al (2001) (see Table 25) investigated the predictive value of HBV DNA testing to assess response to lamivudine and reported findings about the ability of negative HBV DNA test results at month three of therapy for early prediction of maintained response to therapy. The study found that negative HBV DNA test results at month three of therapy is a moderately sensitive indicator of sustained response (sensitivity = 73%). Positive HBV DNA test results at month three were found to be a moderately specific indicator of lack of sustained response (specificity = 88%). Assessment of the additional value of HBV DNA testing was not possible because the results of serologic markers and ALT were not reported at month three.

According to NHMRC criteria, this study quality was considered poor and offered limited applicability. The performance of HBV DNA testing in predicting sustained treatment response is likely to be biased as sustained treatment response was also measured by HBV DNA testing: the index test was also the reference standard. Inadequate data reporting did not allow the diagnostic performance values to be confirmed. Applicability is considered limited: patient selection criteria were not reported and outdated HBV DNA tests with poor lower limits of detection were used.

In compliance with NHMRC levels of evidence for diagnostic test studies (see page 24), the study by Buti et al (2000) is considered level III–2.

Table 24 Characteristics of studies evaluating the predictive value of serum HBV DNA testing among patients undergoing antiviral therapies

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator/ other test(s) characteristics	Reference standard	Study quality
Buti et al (2001) Spain	Prospective, cohort; open-label trial, no placebo group Enrolment not reported (time period not reported)	HBV DNA positive chronic hepatitis B patients Lamivudine 100 mg/day >1 year (range 12–24 months) (N = 35) **Characteristics at baseline:** Mean age = 46.1 (range 24–62 years) Serum ALT (mean 133 U/L, range 49–364 U/L) HBeAg positive = 15 (M 25, F 10)	Pre-treatment liver biopsy in 31 patients (details NR)	**Study entry:** Chemoluminescent bDNA assay (Quantiplex, Chiron) LLD = 7.0×10^5 **At month three of treatment:** Real-time PCR assay, LLD = approximately 1×10^3 genome copies/mL	HBsAg (Abbot-Auszyme Mc); HBeAg and anti-HBe detected by radioimmunoassay (Diasorin)	Maintained treatment response: defined as undetectable serum HBV DNA throughout therapy period (≤1 year) (Non-response = persistence of serum HBV DNA or reappearance after initial clearance	P2 Q3 *Applicability*: Limited Patient selection criteria unknown HBV DNA test outdated, LLD poor *Quality*: Poor Index test is also reference standard Inadequate data reporting
Lindh et al (2001) INTREPED study group: Denmark, France, Spain, Sweden, UK	Direction unclear Enrolment not reported Patients sampled from RCT (1987–1990)	HBeAg positive chronic hepatitis B patients: Placebo followed by interferon n = 47 Interferon after prednisolone n = 51 (2 weeks prednisolone at 0.6 mg/kg/d , then 1 week at 0.45 mg/kg/d, then 1 week at 0.25 mg/kg/d, with 2 weeks rest before interferon) (Interferon at 10 µg for 1 week induction, then 10 µg three times per wk for 11 weeks)	NR	Amplicor HBV monitor (Roche) Linear detection range = 10^3 to 10^7 copies/mL Serum samples taken before prednisolone treatment, before interferon, at end of treatment and on follow-up (mean 204 days after interferon cessation)	HBeAg, anti-HBeAg by radio immunoassay or enzyme immunoassay Indexed aminotransferase value: AST or ALT (if no AST) in relation to ULN. Test details not reported	Treatment response/non-response Sustained response defined as HBV DNA level $<10^6$ copies/mL at follow-up with at least 1.5 log reduction in viral load compared with pre-interferon treatment	P2 Q3 *Applicability*: Limited; prednisolone pretreatment HBV DNA test regimen *Quality*: Poor Index test part of reference standard

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator/ other test(s) characteristics	Reference standard	Study quality
van der Eijk et al (2006) Netherlands, Germany, Belgium, United Kingdom, Denmark, France, Spain, Italy, Greece (16 centres)	Retrospective analysis of data from prospective RCT[a]: After 16 week course of interferon-α (10 million units, 3 x per week) HBeAg positive patients at week 16 were randomised—no further treatment or prolonged treatment for further 16 weeks (time period NR)	Patients: aged 18–70 years; HbsAg positive ≥6 months; HBeAg positive and HBV DNA positive (hybridisation methodology); AST or ALT elevation (3 x in 3 months before study entry); liver biopsy evidence of chronic hepatitis (during 6 month period before study enrolment). Completion of study regimen and sufficient serum samples required (n = 133) **Characteristics (N = 133 at baseline)** Age (median): 34 (range 16–70) Male:female: 97:36 Ethnicity: Caucasian 80%; Asian 16%; other 5% Cirrhosis: 16% HBV DNA (log copies/mL) (median): 8.7 (range 4.1–10.0) AST (ULN) (median): 1.5 (range 0.55–16.7) Genotype: A 46%; B 5%; C 12%; D 32%; other 6%	HbsAg; HBeAg; HBV DNA (hybridisation methodology); AST; ALT; liver biopsy; anti-HDV, HCV, or HIV; serum levels of haemoglobin, platelets and leukocytes	Real-time PCR assay (TaqMan, calibrated according to EUROHEP HBV DNA standards[b])	HBeAg: AxSYM HBe 2.0 (Abbott)	Sustained responders/non-responders Sustained response at wk 52[c]: defined as loss of HBeAg, (<0.27 PEI U/mL), with a decrease in HBV DNA $<10^5$ copies/mL at week 52. Non responders were patients who did not fulfil these criteria	P2 Q3 *Applicability*: Limited HBV DNA testing frequency different from Australian practice *Quality*: Poor Retrospective analysis; index test also part of reference standard

Study	Study design	Patients (N)	Prior tests	HBV DNA test characteristics	Comparator/ other test(s) characteristics	Reference standard	Study quality
Zollner et al (2001) Germany	Prospective, cohort, no placebo, longitudinal Consecutive	Treatment naïve chronic hepatitis B patients, HCV negative Lamivudine 100 mg/day Median duration = 12 months (range 6–31 months) (n = 28) (25M:3W) Mean age = 45 years (range = 17–69) **Characteristics at baseline:** Mean log HBV DNA viral load = 6.71/Ml (range = 3.47–7.69) HBeAg positive = 24 (86%)	Test before study entry NR	Real time PCR (light cycler DNA master SYBER GreenI Roche) detection limit 10^2 genomes/mL, dynamic range. ≤10^9 genomes/mL (EUROPHEP standard calibrated) HBV DNA measured every three months	HBsAg, HBeAg, anti HBeAg, anti-HBsAg, anti-hbc — all by EIA (axysm, Abbot)	Clinical outcome **Seroconversion:** HBeAg, anti HBeAg (axysm, Abbot) **Lamivudine resistance:** Definition unclear, possibly HBV DNA levels above detection limit and continuously detectable HBeAg	P1 Q3 *Applicability*: Applicable *Quality*: Poor Index test and reference standard not independently interpreted Inadequate reporting of reference standard

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; EUROHEP, European concerted action on viral hepatitis; HAI, histological activity index; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus s antigen; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; INTREPED, evaluating interferon treatment with or without prednisolone priming; LLD, lower limit of detection; NR, not reported; PCR, polymerase chain, ULN, upper limit of normal
reaction; RCT, randomised controlled trial

[a] Janssen et al (1999)

[b] Heerman et al (1999) J. Clin Microbiol 37(1):68–73

[c] Definition of virologic response recommended by NIH workshop on chronic hepatitis B: Lok et al (2001) Gastroenterology 120(7): 1828–1853

[d] Treatment discontinued if both resistant HBV (not defined by study authors) with rising ALT levels detected

Table 25 Predictive ability of negative HBV DNA test for maintained therapy response

Study	Diagnostic performance measure				Study quality
	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	
Buti et al (2000)	73	88	74	95	P2 Q3 *Applicability*: Limited Patient selection criteria unknown HBV DNA test outdated, LLD poor *Quality*: Poor Index test is also reference standard Inadequate data reporting

Abbreviations: NPV, negative predictive value; PPV, positive predictive value

In the study by Zollner et al (2001) the sequential measurement of HBV DNA levels during lamivudine therapy was assessed for its value in predicting HBeAg seroconversion and the emergence of drug resistance. In those patients who had detectable HBeAg at baseline (n = 24), only those patients whose viral load decreased below the detection limit of serum HBV DNA of <log 2 copies/mL lost HBeAg. The PPV and NPV of HBV DNA <log 2 copies/mL for several time points for prediction of HBeAg seroconversion and development of lamivudine resistance was then determined by Zollner et al (2001). The results of this analysis are presented in Table 26.

Table 26 Predictive value of serum HBV DNA below detection limits for various time points

Study	Seroconversion				Lamivudine resistance[a]			Study quality
	HBV DNA <log 2 copies/mL at month	PPV[b] (%)	NPV[c] (%)	*p* value[d]	PPV[b] (%)	NPV[c] (%)	*p* value[d]	
Zollner et al (2001)	3	100	76	ns	66	35	ns	P1 Q3 *Applicability*: Applicable *Quality*: Poor Index test and reference standard not independently interpreted Inadequate reporting of reference standard
	6	50	85	ns	82	47	ns	
	9	50	91	ns	82	47	ns	
	12	54	100	0.017	83	50	ns	
	15	54	100	0.017	100	53	0.023	

Abbreviations: HBV DNA, hepatitis B virus deoxyribonucleic acid; NPV, negative predictive value; ns, not significant; PPV, positive predictive value

[a] Continuously detectable HBeAg and serum HBV DNA copy numbers above the limit of detection

[b] PPV: provided for the probability for the loss of e-antigen and maintenance of lamivudine sensitive HBV with copy numbers below the limit of detection, respectively

[c] NPV: provided for the probability of continuously detectable e antigen and emergence of lamivudine resistance with copy numbers above the limit of detection respectively. The HBV DNA assay used in this study was Real time PCR (light cycler DNA master SYBER GreenI Roche) detection limit 10^2 genomes/mL, dynamic range. ≤10^9 genomes/mL

[d] *p*-values determined to establish whether PPV and NPV values are statistically significant

The results of this analysis suggest that HBV DNA levels below the detection limit (<log 2 copies/mL) after three months of lamivudine therapy is suggestive of HBeAg seroconversion. This result was not statistically significant, although a real effect may not be seen because patient numbers were limited (n = 28). Patients with HBV DNA levels above the detection limit at 12 and 15 months did not have seroconversion (NPV = 100%), indicating that these patients did not respond to therapy. From the perspective of a treating clinician this indicates that patients who maintain serum HBV DNA levels above the detection limit at these time points will not respond to lamivudine therapy (as assessed by seroconversion). Serum HBV DNA below the detection limit at month 15 of therapy was strongly predictive of maintenance of sensitivity to lamivudine.

The results of the analysis (Table 27) should be interpreted cautiously. PPV and NPV are prevalence dependent, so these results may not apply to other chronic hepatitis B populations undergoing antiviral therapy.

Zollner et al (2001) used a ROC curve analysis to determine the optimal time point to predict loss of HBeAg and emergence of lamivudine resistance. This analysis found that 12 months was optimal time point for predicting seroconversion with a sensitivity of 100 per cent, specificity of 69 per cent, PPV of 54 per cent and NPV of 100 per cent. Month 12 was also the optimal time point to predict lamivudine resistance, with sensitivity of 78 per cent, specificity of 59 per cent, PPV of 83 per cent and NPV of 50 per cent. Data were not reported that enabled ROC curve analyses to be confirmed.

This study is considered poor quality. For this assessment the clinical outcomes of HBeAg seroconversion and lamivudine resistance were considered to be the reference standards for HBV DNA testing. However, in order to determine the optimal time point for prediction of seroconversion and lamivudine resistance serum HBV DNA levels were not interpreted blind of these clinical outcomes, that is, serum HBV DNA levels were not used to predict outcome independently of the outcome itself. Definition of the reference standard (clinical outcome of lamivudine resistance) was not adequately reported.

This study was considered applicable to Australian clinical practice. HBV DNA test sensitivity and frequency of testing was considered to be applicable to HBV DNA test use in Australia.

According to NHMRC levels of evidence for diagnostic studies (see page 24), the study by Zollner et al (2001) is considered level III–2.

HBV DNA testing and predicting response to interferon treatment

Two studies were identified where the serum HBV DNA testing was assessed for predicting response to interferon treatment (Lindh et al 2001; van der Eijk et al 2006). The characteristics of these studies are presented in Table 24.

In the study by Lindh et al (2001) the predictive ability of pre-treatment HBV DNA levels to predict sustained virological response was assessed for patients treated with interferon and prior prednisolone. Lindh et al (2001) reported the diagnostic performance of pre-treatment serum HBV DNA of log 8.7 copies/mL. The PPV of serum HBV DNA level <log 8.7 copies/mL to predict sustained response was 67 per cent; the NPV of >log 8.7 copies/mL to predict non-sustained response was 67 per cent. The sensitivity and specificity of log 8.7 copies/mL to predict sustained response and non-sustained were 60 per cent and 75 per cent, respectively. These results indicate that this threshold value of pre-treatment serum HBV DNA has limited predictive value in differentiating non-sustained responders from sustained responders. It was not possible to determine the additional value of HBV DNA testing over other tests in predicting interferon response.

The results reported by Lindh et al (2001) should be interpreted with caution. For this assessment, the index test (HBV DNA test) was also part of the reference standard (sustained response). This would bias test performance in favour of the test. Assessment of whether the HBV DNA test could predict response was not done independently of the reference standard. The enrolment of patients was not reported, introducing further potential for selection bias. For these reasons this study is considered poor quality. It was not possible to recalculate the diagnostic performance values reported in this study. The applicability of this study to Australian clinical practice may be limited because of prednisolone priming before interferon treatment.

In compliance with the NHMRC levels of evidence for diagnostic studies (see page 24), the study by Lindh et al (2001) is considered level III–2 evidence.

Van der Eijk et al (2006) evaluated HBV DNA testing as a predictor of response or non-response among HBeAg positive chronic hepatitis B patients treated with interferon-α. The study compared quantitative measurement of serum HBV DNA with quantitative measurement of HBeAg. Inadequate data reporting did not enable determination of the additional value of HBV DNA testing over HBeAg testing. Clinical outcome, defined by

sustained response (or non-response) to interferon was considered the reference standard for this assessment. The characteristics of this study are presented in Table 24.

This study was a retrospective analysis of an RCT by Janssen et al (1999) designed to establish whether prolonged treatment with interferon–α has increased efficacy. Patients recruited to this study were HBeAg positive. The objectives of the study by van der Eijk et al (2006) were to determine whether treatment response could be predicted by quantitative assessment of serum HBV DNA or HBeAg.

For both serum HBV DNA and HBeAg testing, van der Eijk et al (2006) determined whether treatment response/non-response could be predicted by the following test criteria:

1. Baseline serum HBV DNA or HBeAg levels

2. Decrease between baseline and week 8 or 12 levels of serum HBV DNA or HBeAg

3. The combination of baseline level and decrease between baseline and week 8 or 12 levels of serum HBV DNA or HBeAg

For each test criterion the area under the curve (AUC) was determined from receiver operating characteristic (ROC) plots—this analysis allows the comparative diagnostic performance of different tests or test criteria to be assessed (see page 23).
The proportion of sustained responders correctly identified by the test (ie true positives) was plotted against the proportion of non-responders predicted to be responders by the test (ie false positives). The authors reported that this was done for each possible threshold value for test criteria described above. ROC plots could not be reconstructed using data reported in the study. Results of ROC plot analysis were reported for the following test criteria.

When testing for baseline values in combination with decrease from baseline to week 12 was compared for HBV DNA and HBeAg, the AUC were 0.87 and 0.76 respectively. This result is statistically significant ($p<0.05$) and provides some evidence that HBV DNA testing is a more accurate predictor of treatment response than HBeAg testing among HBeAg positive patients.

ROC plot analysis was also used to investigate whether testing for HBV DNA before week 12 would be a better predictor of treatment response: ROC curves were plotted for log HBV DNA at baseline in combination with decrease between baseline and week 8 versus HBV DNA at baseline in combination with decrease between baseline and week 12. For the combination of baseline testing and decrease between baseline and follow-up tests the AUC were 0.85 for testing at week 8 and 0.86 for week 12 testing.This minor difference was not statistically different ($p = 0.60$) (the study authors did not provide any explanation for the minor difference in AUC value for the predictive value of HBV DNA at baseline in combination with decrease between baseline and week 12 in this analysis compared with the value determined in the comparison with HBeAg testing).

For each test criterion the following measures of diagnostic performance were also determined:

1. Positive predictive value (PPV): percentage observed sustained responders out of all predicted by the test to respond

2. Negative predictive value (NPV): percentage observed non-responders of all predicted by test to be non-responders

3. Sensitivity (true positive rate): percentage sustained responders correctly predicted by the test

4. Specificity (false positive rate): percentage non-responders correctly predicted by the test

5. False negative rate: percentage observed sustained responders not predicted by the test

6. False positive rate: percentage observed non-responders predicted to be sustained responders by the test.

Van der Eijk et al (2006) reported only results of serum HBV DNA testing (Table 27). The test criteria presented in Table 27 were selected by the study investigators to maximise the number of sustained responders correctly predicted by test criteria: ie the true positive rate (or sensitivity) = 100 per cent. The authors reported that this approach was used to maximise the predictive value for non-responders: ie to maximise the negative predictive value (NPV) of the test. The presented NPV values presented in Table 27 are all (apart from one exception) 100 per cent. It is not clear why the authors reported the data presented in Table 27 for log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 8: the intention was to establish cut-off points with sensitivity of 100 per cent. It is possible that the reported sensitivity of 93 per cent was the optimal sensitivity for the range of baseline values and decrease between baseline and week 8 that were observed in the study.

As measures of diagnostic performance, NPV and PPV generally have poor transferability between study populations as these measures are prevalence dependent: if the test criteria used in this study are applied to predict interferon-α responders and non-responders in another population, differences in patient characteristics could result in differences in predictive ability.

Of the test criteria chosen by the study investigators to optimise the NPV and sensitivity, the log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 12 had a better ability to predict the proportion of patients who would not respond to treatment (ie highest specificity). Translated to clinical practice this result suggests that this test criterion has the potential to maximise the correct identification of patients least likely to benefit from interferon-α treatment. Specificity of this test criterion was 61 per cent. Due to the absence of complete specificity, there was margin for patient misclassification: if the criteria of log HBV DNA at baseline >8 and <1.0 log decrease between baseline and week 12 were used in clinical practice to predict non-responders, it is possible that patients could be incorrectly classified as potential treatment responders.

The study by van der Eijk et al (2006) is considered to be of limited applicability and poor quality. The applicability is limited as HBV DNA testing in Australia is not conducted as frequently as the study. The quality of this study is poor for reasons related to HBV DNA test use as part of the reference standard. If definition of a sustained response according to HBV DNA levels is not itself an accurate measure of final outcome (eg improvement in liver histology, reduced risk of cirrhosis etc), then using HBV DNA levels to predict response to treatment has the potential to overestimate the predictive ability of the test. Because of the retrospective nature of this analysis, the test criteria were interpreted with knowledge of a component of the reference standard. Inadequate data reporting meant that it was not possible to reconstruct the analyses reported in this study.

In compliance with the NHMRC levels of evidence for diagnostic studies (see page 24), the study by van der Eijk et al (2006) is considered level III–2 evidence.

Table 27 Prediction of treatment response by serum HBV DNA testing

Test criteria[a]		Measure of diagnostic performance (%)						Study quality
		NPV	PPV	Sensitivity[b]	Specificity[c]	FN rate	FP rate	
Log HBV DNA at baseline and decrease between baseline and week 12	Log HBV DNA at baseline >8 and <1.0 log decrease	100	46	100	61	0	39	P2 Q3 *Applicability*: Limited HBV DNA testing frequency different from Australian practice *Quality*: Poor Retrospective analysis; index test also part of reference standard
	Log HBV DNA at baseline >9 and <2.5 log decrease	100	38	100	46	0	54	
Log HBV DNA at baseline and decrease between baseline and week 8	Log HBV DNA at baseline >8 and <1.0 log decrease	96	43	93	60	7	40	
	Log HBV DNA at baseline >9 and <1.5 log decrease	100	38	100	46	0	54	

Abbreviations: HBV DNA, hepatitis B virus deoxyribonucleic acid ;FN, false negative; FP, false positive,; NPV, negative predictive value; PPV, positive predictive value

[a] Study authors defined these test criteria as 'abnormal test', which is understood to mean 'test negative' ie test predicts non-responders

[b] Sensitivity = Proportion of sustained responders correctly predicted by the test

[c] Specificity = Proportion of non-responders correctly predicted by the test

Do serum HBV DNA levels correlate to clinical outcomes?

Evidence that changes in HBV DNA levels correlate with changes in long term clinical outcomes was considered necessary to inform the linked evidence approach applied in this assessment. The accuracy studies discussed did not provide adequate evidence that using the HBV DNA test provides or predicts improvements in long term clinical outcomes.

The results of the literature search were reviewed again and studies using an appropriate statistical analysis (page 23) to compare serum HBV DNA levels with outcomes were retrieved.

Summary of evidence for the relationship between serum HBV DNA levels and clinical outcomes

Systematic review
The relationship between serum HBV DNA levels and clinical outcomes was investigated. Although the impact of test use on clinical outcomes was not investigated, there is evidence to support that HBV DNA levels can predict outcome and indicate treatment efficacy. Serum HBV DNA levels (both baseline and change from baseline) were correlated with liver histology and HBeAg seroconversion.

Serum HBV DNA and hepatocellular carcinoma risk
The REVEAL-HBV study reported that increased HBV DNA levels at baseline were an independent risk factor for hepatocellular carcinoma. After adjusting for other hepatocellular carcinoma risk factors, participants with serum HBV DNA of $\geq 1.0\times10^6$ copies/mL and $300–9.9\times10^3$ copies/mL had hazard ratios of developing hepatocellular carcinoma of 6.1 (95% CI: [2.9, 12.7]; $p<0.01$) and 1.1 (95% CI: [0.5, 2.3]; $p = 0.86$) respectively. The findings of the REVEAL-HBV study were the same for HbeAg negative and positive people, but most participants in this study were HBeAg negative. Participants with increased serum HBV DNA levels, who did not have cirrhosis, also had an increased risk of developing hepatocellular carcinoma.

The increased risk of developing hepatocellular carcinoma with increased HBV DNA levels was evident in this study: increased serum HBV DNA level at study entry was associated with increased cumulative incidence of hepatocellular carcinoma

Most findings from the REVEAL-HBV study were based on serum HBV DNA measured at a single time point. Persistently elevated serum HBV DNA levels were also shown to increase risk of hepatocellular carcinoma: of participants with serum HBV DNA levels of 10,000 to 99,999 copies/mL at study entry, those with serum HBV DNA levels of ≥100 000 at follow-up, had a statistically significant increase in hepatocellular carcinoma risk (multivariate-adjusted HR = 3.5; 95% CI: [1.4, 9.2]; $p = 0.01$). Okhubo et al (2001) found that when hepatocellular carcinoma was diagnosed, serum HBV DNA levels were an independent prognostic factor for survival. Patient survival increased when serum HBV DNA levels were low.

Harris et al (2003) found that spontaneous HBV DNA seroconversion is a prognostic factor for hepatocellular carcinoma death. Patients who spontaneously became HBV DNA negative (stable or unstable) increased risk of hepatocellular carcinoma death. Low serum HBV DNA levels at baseline were associated with a reduced risk of hepatocellular carcinoma death.

Serum HBV DNA and cirrhosis risk
The REVEAL-HBV study also investigated the relationship between serum HBV DNA levels and cirrhosis. Serum HBV DNA levels were shown to be an independent risk factor for cirrhosis. After adjusting for age, sex, smoking, alcohol consumption, HBeAg status and ALT level, participants with serum HBV DNA of $\geq 1.0\times10^6$ had a relative risk of cirrhosis of 6.5 (95% CI: [4.1, 10.2]; $p<0.001$). This risk was reflected in cirrhosis incidence. Increased baseline serum HBV DNA levels were associated with increased cumulative incidence of cirrhosis.

Serum HBV DNA and hepatocellular carcinoma prognosis in cirrhotic patients
Ishikawa et al (2001) and Mahmoud et al (2005) examined the association between serum HBV DNA levels and hepatocellular carcinoma development among patients with HBV-related cirrhosis. Elevated serum HBV DNA was the strongest prognostic factor for development of hepatocellular carcinoma among these patients.

Serum HBV DNA and hepatocellular carcinoma recurrence after curative resection
Kubo et al (2000) and Kubo et al (2003) assessed the value of HBV DNA testing as a prognostic factor for hepatocellular carcinoma recurrence after surgical resection. It was uncertain if patient cohorts overlapped. Results indicated that high serum HBV DNA levels were significant prognostic indicators of hepatocellular carcinoma recurrence. These analyses were conducted using serum HBV DNA levels taken at a single time-point before surgery. It is possible that fluctuations in serum HBV DNA levels post-hepatocellular carcinoma resection, which may contribute to recurrence, were not considered.

Serum HBV DNA and liver histology
Yuen et al (2004) found a weak correlation between serum HBV DNA levels and liver histology in anti-HBeAg positive patients (HBeAg negative). It was suggested that liver damage severity increases as serum HBV DNA levels escalate. No correlation was found between serum HBV DNA levels and liver histology among HBeAg positive patients.

Systematic review

A systematic review by Mommeja-Marin et al (2003) investigated the relationship between serum HBV DNA levels and clinical outcomes. The characteristics of this systematic review are presented in Table 28, and summarised results are presented in Table 29.

The change in median serum HBV DNA level from baseline correlated strongly with the median change in histologic activity index (HAI) from baseline ($r = 0.96$, $p = 10^{-6}$) for treated and untreated patients. This correlation was also present in the subset analyses of HBeAg positive patients ($r = 0.98$, $p = 6\times10^{-6}$) and nucleoside analogue treated patients ($r = 0.98$, $p = 0.0003$).

The absolute level of serum HBV DNA level and the change in median serum HBV DNA level from baseline were both weakly correlated with the proportion of patients with normal ALT levels (see Table 29). Subgroup analyses showed that these correlations were stronger in studies using nucleotides analogues (Table 29).

The absolute level of HBV DNA and incidence of HBeAg seroconversion was strongly correlated in studies where patients were treated with nucleotides analogues ($r = 0.92$, $p = 0.001$). There was a moderate correlation between change in HBV DNA level from baseline and incidence of HBeAg seroconversion ($r = 0.72$, $p = 0.0002$). Subgroup analysis showed that this correlation was slightly better in the cirrhosis group ($r = 0.87$, $p = 5\times10^{-5}$). The absolute level of serum HBV DNA was also correlated with the incidence of HBeAg seroconversion ($r = 0.72$, $p = 0.02$).

Absolute HBV DNA level was weakly correlated for all end point comparisons in the subgroup of studies where liquid hybridisation assays had the lower limit of detection

corrected (Table 29). The authors did not report whether a similar subgroup analysis was performed for studies where PCR HBV DNA assays were used.

The authors reported that the correlation between loss of HBsAg and HBV DNA change from baseline was not assessable. Analysis correlating absolute HBV DNA levels and loss of HBsAg found that two of 367 (0.5%) patients with viral loads greater than 1000 copies/mL lost HBsAg, and 25 of 74 (33.8%) patients with viral loads less than 1000 copies/mL ($p<0.0001$). The authors reported that this analysis relied almost entirely on interferon trials, and commented that these findings may not be directly applicable to nucleosides.

The authors also reported a correlation between histologic necroinflammatory index and baseline HBV DNA levels among untreated patients ($r = 0.78$, $p = 0.0001$). The authors reported that this correlation was primarily influenced by one study of patients with low viral load levels. A correlation between HAI and serum HBV DNA levels at the end of treatment was also reported patients ($r = 0.71$, $p = 0.003$). A weak correlation between HBV DNA levels and the percentage of patients with normal ALT was reported ($r = 0.62$, $p = 0.0004$).

The change in serum HBV DNA level from baseline had a stronger correlation with the change of HAI score from baseline ($r = 0.96$, $p = 10^{-6}$) than with either the proportion of patients with normal ALT levels ($r = 0.5$, $p = 0.06$) or the incidence of HBeAg seroconversion ($r = 0.72$, $p = 0.0002$). Findings were similar in the nucleoside analogue treated subgroup (Table 29). These results could potentially be interpreted as providing indirect evidence that changes in serum HBV DNA levels are more indicative of changes in the extent of active liver disease than ALT or HBeAg seroconversion. Comparisons of this kind should be performed in the same patient population.

This systematic review by Mommeja-Marin et al (2003) provides evidence that serum HBV DNA levels (baseline and changes from baseline) correlate to short term outcomes (eg change in HAI score and incidence of HBeAg seroconversion). Significantly changes in serum HBV DNA levels correlate with the incidence of HBeAg seroconversion—which is one of the aims of antiviral therapy. Reported evidence suggests that changes in serum HBV DNA illustrate treatment efficacy. No evidence was presented to indicate that improvement in short term outcomes are provided HBV DNA testing itself, or that short term benefits translate to improvements in long term outcomes. Comparison of the results of the subgroup analyses should be interpreted with caution as differences in the strength of correlation could result from the variation in the patient populations of studies included in this systematic review.

Application of the NHMRC quality criteria (**Appendix D**) classifies this systematic review as medium quality: importantly heterogeneity was explored by subgroup analyses; but no quality assessment was reported for included studies. Validity was reduced as no subgroup analysis was performed for PCR HBV DNA assays.

Table 28 Characteristics of a systematic review evaluating the relationship between serum HBV DNA levels and clinical outcomes

Systematic review	Objective	Search strategy	Inclusion/exclusion criteria	Methodology	Study quality/Comment[a]
Mommeja-Marin et al (2003)	To investigate the relationship between viral load level or suppression and treatment response	Medline (1996–2002) Search terms: hepatitis B; HBV; and HBV DNA	Inclusion criteria: Published in English; reported HBV DNA levels; specified other end-points (ALT, serology, & histology); reported medians, means or data for estimation of median; prospective patient enrolment; >25 chronically infected patients; non-immuno-depressed; viral loads reported at start and/or end of therapy (including placebo, if applicable). Studies in children and cirrhotic patients also included. Exclusion criteria: Post-liver transplantation studies; HIV and/or hepatitis C co-infection. (26 studies met final selection criteria)	Data abstraction by two separate (blinded) reviewers Viral loads reported as pg/mL were converted to copies/mL Some study characteristics summarised. Results not summarised for individual studies An assessment of study quality was not undertaken Sources of heterogeneity were explored by subgroup analysis Results presented as correlation co-efficients: exponential and linear regressions compared to determine best data fit; statistical significance explored by *p* value calculation	Medium quality: Heterogeneity explored by subgroup analyses No quality assessment reported for included studies Medium validity: Subgroup analysis not performed for PCR HBV DNA assays

Abbreviations: ALT, Alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Systematic review appraised by applying the quality criteria described in **Appendix D**

[b] Conversion: 1 pg/mL = 3 x 10^5 copies/mL+

Table 29 Summary of results of a systematic review evaluating the relationship between serum HBV DNA levels and clinical outcomes

	HBV DNA versus end points	Coefficient of correlation and statistical significance between HBV DNA and end points by subgroups						
		Overall	HBeAg positive	HBeAg negative	Cirrhosis	Nucleoside analogue treated	Interferon treated	Liquid hybridisation assay LOD corrected
Absolute level	HAI score at baseline	0.78 (p = 0.0001)	0.61 (p = 0.05)	0.95 (p = 0.0004)	ND	NA	NA	0.65 (p = 0.002)
	HAI score at end of treatment	0.71 (p = 0.003)	0.71 (p = 0.01)	0.66 (p = 0.3)	ND	0.76 (p = 0.006)	0.65 (p = 0.08)	0.67 (p = 0.01)
	Proportion of patients with normal ALT levels	0.62 (p = 0.004)	0.66 (p = 0.008)	0.71 (p<0.05)	0.8 (p = 0.003)	0.82 (p = 0.001)	0.55 (p = 0.02)	0.61 (p = 0.005)
	Incidence of HBeAg seroconversion	0.72 (p = 0.002)	NA	NA	ND	0.92 (p = 0.001)	ND	0.55 (p = 0.3)
Change in median from baseline	HAI score, median change from baseline	0.96 (p = 10^{-6})	0.98 (p = 6 x 10^{-6})	ND	ND	0.96 (p = 0.0003)	ND	NA
	Proportion of patients with normal ALT levels	0.5 (p = 0.06)	ND	ND	ND	0.76 (p = 0.003)	ND	NA
	Incidence of HBeAg seroconversion	0.72 (p = 0.0002)	NA	NA	0.87 (p = 0.00005)	0.7 (p = 0.05)	ND	NA

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis e antigen; HBV DNA, hepatitis B deoxyribonucleic acid; LOD, lower limit of detection; NA, not applicable; ND, not done

Primary studies

Two studies were identified which assessed the relationship between HBV DNA levels and long term clinical outcomes (Chen et al 2006; Iloeje et al 2005). These studies reported different outcomes from the REVEAL-HBV study (Risk Evaluation of Viral Load and Associated Liver Disease/Cancer-in Hepatitis B Virus)—a Taiwanese population-based, long term prospective study. This population represents a natural history cohort, as participants did not undergo HBV antiviral treatment. These treatments were not reimbursed under the universal national health insurance scheme in Taiwan until 2003. The characteristics of the REVEAL-HBV study are presented in Table 30.

Four further studies were identified which assessed the relation between serum HBV DNA levels and the development of HBV-associated hepatocellular carcinoma (Harris et al 2003; Ishikawa et al 2001; Mahmood et al 2005 and Okhubo et al 2001). The characteristics of these studies are presented in Table 30.

Two further studies were identified which assessed the value of HBV DNA testing as a prognostic factor for recurrence after resection for HBV-associated hepatocellular carcinoma (Kubo et al 2000; Kubo et al 2003). The characteristics of these studies are presented in Table 30.

In study by Yuen et al (2004) the relationship between serum HBV DNA levels and liver histology was investigated. The characteristics of this study are presented in Table 30.

REVEAL-HBV study

The studies by Chen et al (2006) and Iloeje et al (2006) reported the risk of hepatocellular carcinoma and cirrhosis in relation to serum HBV DNA levels respectively. The characteristics of the REVEAL-HBV study are shown in Table 30. Multivariate-adjusted logistic regression analyses were used to account for other variables which are also potential prognostic factors for hepatocellular carcinoma and cirrhosis. Results from the REVEAL-HBV study are shown in Table 31.

Chen et al (2006) found that increasing baseline serum HBV DNA level is associated with increased cumulative incidence of hepatocellular carcinoma (see Table 31). Patients with undetectable serum HBV DNA (<300 copies/mL) had a cumulative incidence of hepatocellular carcinoma of 1.3 per cent. In contrast patients with serum HBV DNA of $\geq 1.0 \times 10^6$ had a cumulative incidence of 14.9 per cent. Most participants (80%) in this study were HBeAg negative with normal ALT levels and no liver cirrhosis: these participants had a cumulative incidence of hepatocellular carcinoma of 13.5 per cent and 0.7 per cent when baseline HBV DNA levels were $\geq 1.0 \times 10^6$ and <300 copies/mL respectively.

Similarly, Iloeje et al (2005) reported that increasing baseline serum HBV DNA level is associated with increased cumulative incidence of cirrhosis (see Table 31). Patients with undetectable serum HBV DNA (<300 copies/mL) had a cumulative incidence of cirrhosis of 4.5 per cent. In contrast, patients with serum HBV DNA of $\geq 1.0 \times 10^6$ had a cumulative incidence of 36.2 per cent.

Table 30 Characteristics of studies used to evaluate the relation between serum HBV DNA levels and clinical outcomes

Study	Study design	Patients (N)	HBV DNA test Characteristics	Other test(s) characteristics	Study quality/comment
REVEAL-HBV study group Results reported by Chen et al (2006) & Iloeje et al (2006) Taiwan	Prospective cohort (population-based) Non-consecutive (recruited 1991–1992)	89,293 residents (30–65 years) in 7 townships invited to participate: 23,820 enrolled—4155 were HBsAg +ve: of these 3851 had serum samples frozen at enrolment tested for DNA 198 excluded (195 anti-HCV seropositive, 3 lacked adequate sample for anti-HCV test) **Chen et al (2006):** final cohort n = 3653 [a] **Iloeje et al (2006):** a further 71 excluded from analysis (2 deaths within 6 mo of cohort entry – non-hepatic causes - 69 diagnosed with cirrhosis within 6 mo of enrolment): final cohort n = 3582	PCR (COBAS Amplicor; Roche) Lower limit of detection of 300 copies/mL (tested at baseline and every 6–12 months during follow-up examination)	HBsAG, HBeAg by radioimmunoassay,(Abbot); anti-HepC by radioimmunoassay (Abbott); ALT by serum chemistry auto-analyser (model 736; Hitachi) (tested at baseline and every 6–12 months during follow-up examination)	Prospective Potential for selection bias: Majority of invited individuals did not participate Non-consecutive enrolment Population considered applicable to Australian clinical practice
Harris et al (2003) China	Prospective cohort Non-consecutive (1992–2000)	HbsAg +ve, HBV DNA +ve (dot blot hybridisation), with 3 or more serum samples available for analysis , hepatocellular carcinoma-free at study entry (n = 114) (subjects selected from α-fetoprotein intervention arm of an early detection screening study)	Dot blot hybridisation, quantified by bio-imaging analyser system (Fuji medical systems) Sensitivity limit = 3 x 10^5 virions/mL	AFP by enzyme immunoassay Other tests NR	Prospective Potential for selection bias: non-consecutive patient selection Potential for spectrum bias: patients selected from screening study Treatment not applicable to Australian clinical practice HBV DNA test outdated
Ishikawa et al (2001) Japan	Cohort Direction unclear Non-consecutive (Time period unclear)	HbsAg +ve cirrhotic patients, excluded patients with non-viral cirrhosis (n = 65)	TMA and hybridisation protection assay (Details NR) Serum samples taken at time of first diagnosis of cirrhosis	HbsAg by enzyme immunoassay (Dainabot) HbeAg, anti-HBeAg, by enzyme immunoassay; anti-HBc by reversecell Ab kit (Yamanouchi) Serum samples taken at time of first diagnosis of cirrhosis	Direction unclear Potential for selection bias: non-consecutive patient selection Reduced applicability to Australian clinical practice: less sensitive HBVDNA test

Study	Study design	Patients (N)	HBV DNA test Characteristics	Other test(s) characteristics	Study quality/comment
Kubo et al (2000) Japan	Direction unclear Non-consecutive (1990–1998)	HBV DNA positive patients, negative for anti-HCV, who had curative[b] liver resection for hepatocellular carcinoma. (n = 40)	Branched DNA assay (Chiron Amplex) (Serum samples for test obtained before surgery)	Anti-HCV by ELISA; serum HCV RNA by PCR, HBeAg, anti-HBeAg, HBsAg,and anti-HBsAg by enzyme immunoassay (International Reagents Corp) (Serum samples for test obtained before surgery)	Potential selection bias: direction unclear, non-consecutive enrolment Applicability to Australian clinical practice potentially limited: less sensitive HBV DNA test
Kubo et al (2003) Japan	Direction unclear Non-consecutive (1989–2001)	HBV DNA positive patients, negative for anti-HCV, who had curative[b] liver resection for hepatocellular carcinoma. (n = 52)	TMA-HPA (Chugai diagnostics): sensitivity range = 3.7 –8.7 LGE/mL[c] Branched DNA assay (Quantiplex); detection limit 0.7 mEq/mL (7×10^5 copies/mL) (Serum samples for test obtained before surgery)	Anti-HCV by ELISA ; HBeAg, HBsAg by enzyme immunoassay (International Reagents Corp) (Serum samples for test obtained before surgery)	Potential selection bias: direction unclear, non-consecutive enrolment Applicability to Australian clinical practice potentially limited: less sensitive HBV DNA test
Mahmood et al (2005) Japan	Direction unclear Enrolment NR (Cirrhosis diagnosis period 1996–2003)	Patients diagnosed with HBV related cirrhosis by liver biopsy and/or peritoneoscopy. HbsAg positive, anti-hep C negative at study commencement. Cirrhotic patients with possible hepatocellular carcinoma association at the time of hepatocellular carcinoma diagnosis were excluded from (n = 91; 23 patients were treated using interferon[d])	Amplicor Monitor (Roche)	HBsAg, anti-HBsAg by chemiluminescent enzyme immunoassay (Fuji); HBeAg, anti-HBeAg by enzyme-linked immunosorbent assay (Abbot) ALT (test details NR)	Potential selection bias: direction unclear and enrolment NR. HBV DNA test applicable to Australian clinical practice
Okhubo et al (2002) Japan	Direction unclear Enrolment NR (recruited 1983–1998)	Patients (74) with HBV-associated hepatocellular carcinoma, HBsAg +ve and anti-HCV –ve (selected from 404 patients identified with hepatocellular carcinoma histopathologically or clinically) Excluded: HBsAg –ve, antiHCV +ve patients Observation began at time of hepatocellular carcinoma diagnosis and ended at death or end of Oct 1999	Transcription-mediated amplification method, details NR Detection limit[e] = 3.7 LGE/mL (Serum sample taken at time of hepatocellular carcinoma diagnosis)	HBsAg, HBeAg, and anti-HbeAg assayed by commercially available radio-immunoassay kits (Dainabot); Anti-HCV, enzyme-linked immunoabsorbent assay (Ortho Diagnostics) ALT assay methods NR (Serum sample taken at time of hepatocellular carcinoma diagnosis)	Potential for selection bias: direction unclear Did not investigate whether hepatocellular carcinoma treatment impacted on survival Limited Applicability: HBV DNA test not applicable to Australian clinical practice

Study	Study design	Patients (N)	HBV DNA test Characteristics	Other test(s) characteristics	Study quality/comment
Yuen et al (2004) China	Direction unclear cohort (2001–2003)	Chronic hepatitis B patients undergoing liver biopsy; no antiviral therapy; HbsAg +ve ≥6 months ; excluded patients with concomitant liver diseases including hepatitis C or D, alcoholic liver disease, autoimmune hepatitis, Wilson's disease, primary biliary cirrhosis and drug induced hepatitis. (n = 94)	Cobas Amplicor, HBV Monitor test (Roche) Lower limit of detection = 200 copies/mL (HBV DNA tested in serum samples taken 2 weeks before liver biopsy)	HBsAg, HBeAg, anti- HBeAg by microparticle enzyme immunoassay (Abbot) ALT test details NR Liver histology graded by criteria of Knodell et al (1981). Histology assessed blind to liver biochemistry and HBV DNA test results	Direction unclear Potential selection bias – only included patients with liver biopsies Blinding between liver histology and test results Patient population considered applicable

Abbreviations: AFP, α-fetoprotein tests; ALT, alanine aminotransferase; ELISA, enzyme linked immunoassay; F, female; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCV, hepatitis C virus; HDV, hepatitis D virus; LGE, logarithm of genome equivalent; M, male; NR, not reported; PCR, polymerase chain reaction; REVEAL-HBV, The Risk Evaluation of Viral Load and Associated Liver Disease/Cancer-in Hepatitis B Virus; RNA, ribonucleic acid; TMA, transcription mediated assay

[a] Chen et al (2006) examined the impact of changing HBV DNA level on hepatocellular carcinoma risk in a subset of these participants (n = 1619) who had serum HBV DNA level of ≥10 000 copies/mL at study entry. Follow-up serum samples were taken at the last follow-up examination or at the follow-up examination preceding the diagnosis of hepatocellular carcinoma

[b] Curative resection defined as complete resection of all macroscopically evident tumour. Absence of tumour cells along the parenchymal transection line confirmed histologically

[c] Kubo et al (2003) reported that this range is equivalent to 5×10^3 to 5×10^8 copies/mLof serum HBV DNA

[d] Patients were treated with either interferon-α or interferon-β at 6 MU/day for 4 weeks: proportions undergoing interferon-α or interferon- β therapy not reported

[e] Okhubo et al (2002) reported that the detection limit of the branched DNA assay (0.7 milliequivalent per mL) corresponds to 5.8 LGE/mL by the TMA method

Table 31 Relationship between serum HBV DNA levels and long term clinical outcomes: results from the REVEAL-HBV study group

Study	Patient characteristics		Long term outcomes	Study quality/comment
Chen et al (2006) REVEAL-HBV study group Taiwan	n = 3653 Average follow-up = 11.4 years Males = 62% Age >39 years = 67% Never drank alcohol = 87% HbeAg +ve = 15%; ALT ≥45 IU = 6% No liver cirrhosis = 98%	**Cumulative hepatocellular carcinoma incidence stratified by baseline HBV DNA Level (copies/mL)** Undetectable (<300) = 1.30% 300–9.9 x 10^3 = 1.37% 1.0– 9.9 x 10^4 = 3.57% 1.0–9.9 x 10^5 = 12.17% ≥1.0 x 10^6 = 14.89%	**Hazard ratio[b] of hepatocellular carcinoma (95% CI) stratified by baseline HBV DNA level (copies/mL)** Undetectable (<300) = 1.0 (reference) 300–9.9 x 10^3 = 1.1 (0.5–2.3, p = 0.86) 1.0 – 9.9 x 10^4 = 2.3(1.1–4.9, p = 0.02) 1.0–9.9 x 10^5 = 6.6 (3.3–13.1, p <0.001) ≥1.0 x 10^6 = 6.1 (2.9–12.7, p <0.001) **Hazard ratio[b] of hepatocellular carcinoma (95% CI) by other variables** HbeAg –ve = 1.0 (reference) HbeAg +ve = 2.6 (1.6–4.2, p<0.001) ALT (IU) <45 = 1.0 (reference) ALT ≥45 = 1.5 (1.1–2.1, p = 0.64) Liver cirrhosis[c] = 9.1 (5.9–13.9, p<0.001)	Prospective Potential for selection bias: Majority of invited individuals did not participate. Non-consecutive enrolment Population considered applicable to Australian clinical practice.
Iloeje et al (2006) REVEAL-HBV study group Taiwan	n = 3582 Median follow-up = 11 years (range NR) Median age = 45 Males = 61.3%; Alcohol drinkers = 12%; HbeAg +ve = 15.2%; Median HBV DNA = 10^3 copies/mL (range NR) ALT ≥45 IU = 5.7% deaths during follow-up 8.7%	**Cumulative cirrhosis[a] incidence stratified by baseline HBV DNA Level (copies/mL)** Undetectable (<300) = 4.5% 300–9.9 x 10^3 = 5.9% 1.0–9.9 x 10^4 = 9.8% 1.0–9.9 x 10^5 = 23.5% ≥1.0 x 10^6 = 36.2%	**Relative risk[b] of cirrhosis (95% CI) stratified by baseline HBV DNA level (copies/mL)** Undetectable (<300) = 1.0 (reference) 300–9.9 x 10^3 = 1.4 (0.9–2.2, p value NR) 1.0 – 9.9 x 10^4 = 2.5 (1.6–3.8, p < 0.01) 1.0–9.9 x 10^5 = 5.6 (3.7–8.5, p < 0.01) ≥ 1.0 x 10^6 = 6.5 (4.1–10.2, p < 0.01) **Relative risk[b] of cirrhosis (95% CI) by other variables** HbeAg –ve = 1.0 (reference) HbeAg +ve = 1.7 (1.2–2.3, p < 0.01) ALT (IU) <45 = 1.0 (reference) ALT ≥45 = 1.5 (1.1 –2.1, p < 0.05)	

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid;. IU, international units; NR, not reported; PCR, polymerase chain reaction; REVEAL-HBV, Risk Evaluation of Viral Load and Associated Liver Disease/Cancer-in Hepatitis B Virus

[a] Cirrhosis diagnosed by high resolution real time ultrasound, with a quantitative scoring system from liver surface appearance, liver parenchymal texture, intrahepatic blood vessel size, splenic size. All patients had baseline screening ultrasound; ultrasounds also performed every 6–12 months at follow-up. Ultrasound blinded to HBV DNA testing

[b] Multivariate-adjusted hazard ratio and relative risk determined by Cox proportional hazard regression analysis

[c] Diagnosed with ultrasonography within 6 months of study entry

Chen et al (2006) reported that increasing serum HBV DNA levels were strongly associated with an increased (multivariate-adjusted) hazard ratio of developing hepatocellular carcinoma (see Table 31). After adjusting for other hepatocellular carcinoma risk factors (age, sex, smoking, alcohol consumption) participants with serum HBV DNA of $\geq 1.0\times10^6$ copies/mL and $300–9.9\times10^3$ copies/mL had hazard ratios of developing hepatocellular carcinoma of 6.1 (95% CI: [2.9, 12.7]; $p<0.01$) and 1.1 (95% CI: [0.5, 2.3]; $p = 0.86$) respectively. The adjusted hazard ratio for developing hepatocellular carcinoma for HBeAg positive participants was 2.6 (95% CI: [1.6, 4.2]; $p<0.001$) and for participants with serum ALT ≥45 IU/mL was 1.1 (95% CI: [0.7, 1.7]; $p = 0.64$).

Chen et al (2006) also reported an adjusted hazard ratio of developing hepatocellular carcinoma for participants with liver cirrhosis of 9.1 (95% CI: [5.9, 13.9]; $p<0.001$). This indicates that liver cirrhosis at study entry was a significant risk factor for developing hepatocellular carcinoma. However, when participants with no liver cirrhosis, who were HBeAg negative, with normal serum ALT were compared with participants with serum HBV DNA levels of <300 copies/mL the adjusted hazard ratios for serum HBV DNA levels of $300–9.9\times10^3$ copies/mL was 1.4 (95% CI: [0.5, 3.8]; $p = 0.56$); $1.0–9.9\times10^4$ copies/mL was 4.5 (95% CI: [1.8, 11.4]; $p = 0.001$); for $1.0–9.9\times10^5$ copies/mL was 11.3 (95% CI; [4.5, 28.4]; $p<0.001$) and for $\geq 1.0\times10^6$ copies/mL was 17.7 (95% CI: [6.8, 46.3]; $p<0.001$). These data show that in the absence of liver cirrhosis, participants with serum levels of HBV DNA $>10^5$copies/mL had an increased risk of developing hepatocellular carcinoma.

Similarly, Iloeje et al (2006) found that the relative risk of cirrhosis (multivariate-adjusted) was also strongly associated with increasing serum HBV DNA levels (see Table 31). After adjusting for age, sex, smoking, alcohol consumption, HBeAg status and ALT level, participants with serum HBV DNA of $\geq 1.0\times10^6$ had a relative risk of cirrhosis of 6.5 (95% CI: [4.1, 10.2]; $p<0.001$). For comparison multivariate-adjusted relative risks of cirrhosis for participants who were HbeAg positive or had serum ALT ≥45 IU/mLwere 1.7 (95% CI: [1.2, 2.3]; $p<0.01$) and 1.5 (95% CI: [1.1, 2.1]; $p<0.05$) respectively. These data suggest that baseline serum HBV DNA levels are a stronger predictor of cirrhosis than either HBeAg status or ALT level.

Chen et al (2006) also examined the impact of changing HBV DNA level on hepatocellular carcinoma risk in a subset of study participants (n = 1619) who had serum HBV DNA level of ≥10 000 copies/mL at study entry. Follow-up serum samples were taken at the last follow-up examination or at the follow-up examination preceding the diagnosis of hepatocellular carcinoma. The results of this analysis are presented in Table 32.

Of the participants who had serum HBV DNA levels of 10 000 to 99 999 copies/mL at study entry, those with serum HBV DNA levels of ≥100 000 at follow-up, had a statistically significant increase in hepatocellular carcinoma risk (multivariate-adjusted HR = 3.5; 95% CI: [1.4, 9.2]; $p = 0.01$) (Table 32). Of the participants who had serum HBV DNA levels of ≥100 000 copies/mL at study entry, there was a statistically significant increase in hepatocellular carcinoma risk with follow-up HBV DNA levels ($p < 0.01$). This trend was not changed after additional adjustment for study entry HBeAg seropositivity, serum ALT level and presence of liver cirrhosis (Table 32). These results suggest persistently elevated serum HBV DNA levels lead to an increased risk of hepatocellular carcinoma.

The REVEAL-HBV study results reported by Chen et al (2006) and Iloeje et al (2006) provide evidence that serum HBV DNA levels are predictive of long term outcomes—increasing serum HBV DNA levels were associated with increased risk of cirrhosis and hepatocellular carcinoma. Chen et al (2006) also provide evidence that hepatocellular carcinoma risk increases with persistent elevation of HBV DNA. Although the REVEAL-HBV study does not provide evidence that serum HBV DNA testing itself impacts on long term outcomes, there is evidence that serum HBV DNA levels are a better predictor of long term outcomes than HBeAg seropositivity or serum ALT. Comparison with serum ALT levels is limited in the use of a single cut point for analysis of risks. To facilitate comparison with serum HBV DNA levels, reporting relative risks and hazard ratios for different ranges of ALT levels, rather than a single cut point, would have been more informative.

Chronic hepatitis B is a dynamic disorder with periods of intense viral replication interspersed with periods of relative quiescence. The REVEAL-HBV study is somewhat limited in that serum HBV DNA and other markers were not measured at regular time points throughout follow-up—although serum samples were obtained every six to 12 months during the study. Although Chen et al (2006) reported follow-up HBV DNA levels for a subset of participants, a more thorough analysis of HBV DNA levels throughout the entire follow-up period would have provided more information regarding the duration and extent of viral replication and its relation to hepatocellular carcinoma and cirrhosis risk.

The REVEAL-HBV DNA study was a population-based, long term prospective study, with follow-up considered long enough for outcomes to occur. There is potential for selection bias in this study as 73 per cent of individuals invited to take part chose not to participate. Recruitment was non-consecutive and included participants who may not reflect the true spectrum of chronic hepatitis B in the wider population. Only patients who had serum samples for HBV DNA analysis frozen at enrolment were included. This is likely to have resulted in minimal selection bias because 93 per cent of all HBsAg positive patients had serum samples for HBV analysis. Although this study was carried out in Taiwan, the patient population is considered applicable to Australian clinical practice: in a recent prospective cohort study of Australian chronic hepatitis B patients, 65 per cent had Asian ethnicity (Bell et al 2005). The HBV DNA test used in this study is applicable to Australian clinical practice.

The REVEAL-HBV included population represents a natural history cohort. Participants did not undergo HBV antiviral treatment because there was no reimbursement in Taiwan during the study period. This may reduce the applicability of the results to current clinical practice in Australia because suitable antiviral therapy would be available when clinically indicated.

Table 32 Risk of hepatocellular carcinoma by serum HBV DNA levels at study entry and last follow-up

Study	Level of serum HBV DNA (copies/mL)		Hazard ratio (95% CI) adjusted for:		Study quality/ comment
	At study entry	At follow-up[a]	Sex, age, cigarette smoking and alcohol consumption	HBeAg positive, liver cirrhosis, ALT level	
Chen et al (2006)	<10 000	Not tested	1.0	1.0	Prospective
REVEAL-HBV study group	10 000 to 99 999	<10 000	1.6 (0.7–3.9)	1.3 (0.5–3.1)	Potential for selection bias:
Taiwan	10 000 to 99 999	10 000 to 99 999	0.5 (0.1–3.6)	0.4 (0.1–3.2)	Majority of invited individuals did not participate
	10 000 to 99 999	≥100 000	3.5 (1.4–9.2)	2.9 (1.0–9.8)	
	≥100 000	<10 000	3.8 (1.7–8.4)	1.9 (0.8–4.4)	Non-consecutive enrolment
	≥100 000	10 000 to 99 999	7.3 (3.5–15.3)	4.3 (2.0–9.3)	Population considered applicable to Australian clinical practice
	≥100 000	≥100 000	10.0 (6.3–16.2)	5.3 (2.9–9.7)	

Abbreviations: ALT, alanine aminotransferase; CI, confidence intervals; HBeAg, hepatitis B e-antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; REVEAL-HBV, Risk Evaluation of Viral Load and Associated Liver Disease/Cancer-in Hepatitis B Virus
[a] Median time between study entry and follow-up serum samples was about 10 years

Factors associated with the prognosis of HBV-associated hepatocellular carcinoma

Harris et al (2003) and Okhubo et al (2002) examined the relationship between serum HBV DNA levels and hepatocellular carcinoma development. Ishikawa et al (2001) and Mahmoud et al (2005) investigated hepatocellular carcinoma occurrence in HBV-related cirrhotic patients and serum HBV DNA levels. Kubo et al (2000) and Kubo et al (2003) investigated the relationship between serum HBV DNA levels and recurrence after resection of HBV-related hepatocellular carcinoma.

Harris et al (2003) examined the relationship between serum HBV DNA levels and hepatocellular carcinoma in a prospective study of a non-consecutive group of patients (n = 114) selected from the α-fetoprotein intervention arm of an early detection screening. In this study the relationship between the clearance of high titre viremia and subsequent risk of hepatocellular carcinoma was investigated.

Serum samples for HBV DNA analysis were collected between 1992 and 1997. Hepatocellular carcinoma mortality was followed until 2000. During follow-up 54 patients (47.4%) spontaneously converted to HBV DNA negative (defined as one or more subsequent samples HBV DNA negative by dot blot hybridisation). Of these patients 27 (50%) were HBV DNA negative for at least two consecutive observations six months apart, without relapse—these patients were considered to have undergone stable high-titre HBV DNA conversion. The remainder of the patients who spontaneously seroconverted reverted to HBV DNA positive.

Using Cox proportional hazards model the relative risk of hepatocellular carcinoma death (controlled for age and sex) for stable HBV DNA seroconversion was 2.2 (95% CI: [0.9, 5.3]) and for unstable HBV DNA seroconversion was 1.6 (95% CI: [1.1, 7.4]).

It was unclear from this study whether this relative risk was determined compared withrisk of hepatocellular carcinoma death of patients who did not seroconvert; but, it is likely that this was the analysis undertaken. The relative risk of hepatocellular carcinoma death associated with any HBV DNA seroconversion (stable or unstable) was not significantly changed (RR: 2.8, 95% CI: [1.1, 7.4]) when other possible hepatocellular carcinoma risk factors (family history of hepatocellular carcinoma, acute hepatitis history, alcohol consumption, cigarette smoking) were accounted for. These data suggest that HBV DNA seroconversion (stable or unstable) is a prognostic factor for hepatocellular carcinoma death. Cumulative hepatocellular carcinoma-free survival was reduced when patients who seroconverted to HBV DNA negative at least once were compared with those who did not ($p = 0.02$).

Harris et al (2003) also reported that low HBV DNA load at baseline (<1.8 virions/mL) had a relative risk of hepatocellular carcinoma death of 0.51 (95%CI: [0.19, 1.4]), suggesting that low serum HBV DNA levels are associated with reduced risk of hepatocellular carcinoma death (but it was not clear what was compared with the relative risk). The relative risk of hepatocellular carcinoma death for other baseline serum HBV DNA levels was not reported. Application of a single point measure of HBV DNA at baseline does not capture the potential impact of fluctuating HBV DNA levels throughout the follow-up period.

The results of this study have the potential for bias: patient selection was non-consecutive and patients were selected from an early detection screening study. It was possible that patients with more severe chronic hepatitis B were included. The treatment and HBV DNA test are not considered applicable to Australian clinical practice.

In a prospective study Okhubo et al (2002) investigated factors associated with the prognosis of HBV-associated hepatocellular carcinoma. Patients (n = 74) positive for HbsAg and negative for anti-HCV were identified from a wider group of patients diagnosed with hepatocellular carcinoma. To identify factors involved in hepatocellular carcinoma prognosis univariate analysis was performed on variables present at the time of hepatocellular carcinoma diagnosis. The results of this analysis are presented in Table 33.

Serum HBV DNA level <3.7 LGE/mL and serum ALT <30 IU/L at the time of hepatocellular carcinoma diagnosis were reported to be associated with significantly longer survival. Clinical stage of hepatocellular carcinoma, the presence of a solitary tumour, and tumour size <2 cm were all significantly associated with longer survival (Table 33). Age, gender, alcohol intake, and history of blood transfusion did not appear to affect the cumulative survival of patients.

To establish independence of prognostic factors, multivariate analysis was carried out by stepwise logistic regression using the same variables as the univariate analysis. Serum HBV DNA <3.7 LGE/mL ($p = 0.0022$) and tumour size <2 cm ($p = 0.0106$) were identified as significant prognostic factors for HBV-associated hepatocellular carcinoma.

Because HBeAg positive patients with hepatocellular carcinoma had a poor prognosis compared withHBeAg negative patients and because all HBeAg positive patients had HBV DNA levels ≥3.7 LGE/mL, Okhubo et al (2002) also compared the cumulative survival after hepatocellular carcinoma diagnosis in HBeAg negative patients. This was compared in HBeAg negative patients with HBV DNA level ≥3.7 LGE/mL and those with levels <3.7 LGE/mL. The cumulative survival curves were different between the

two groups ($p = 0.0057$) suggesting that serum HBV DNA levels at the time of hepatocellular carcinoma diagnosis in HBeAg negative patients have an impact on survival time.

Results from this study suggest that serum HBV DNA levels at diagnosis of HBV-associated hepatocellular carcinoma are a prognostic factor for survival, but they should be interpreted with some caution: Serum HBV DNA was tested by an assay that limits applicability to Australian clinical practice. It was not reported whether serum HBV DNA levels were a prognostic factor independent of hepatocellular carcinoma treatment. These results have potential for bias: the study direction (prospective or retrospective) was unclear and patient enrolment was not reported.

Table 33 Factors associated with prognosis of HBV-associated hepatocellular carcinoma

Study	Patient characteristics	Variable	Median survival time (years)	*p*-value	Study quality/ comment
Okhubo et al (2002) Japan	Males = 74.3% Female = 25.7% Age range = 32–76 years Cirrhosis = 87.8% **Hepatocellular carcinoma diagnosis**: Histopathology n = 20 Ultrasonography, CT and hepatic arteriography n = 54 **Hepatocellular carcinoma treatment**: Surgical resection n = 13 Non-surgical[a] treatment n = 43 No treatment n = 18	**Serum HBV DNA** <3.7 LGE/mL(n = 15) ≥3.7 LGE/mL(n = 53) Not assessed (n = 6) **Serum ALT** <30 IU/L (n = 24) ≥30 IU/L (n = 48) Not assessed (n = 2) **Clinical stage** I (n = 39) II or III (n = 33) Not assessed (n = 2) **Liver tumour** Solitary (n = 37) Multiple (n = 37) **Tumour size** <2 cm (n = 28) ≥2cm (n = 46)	 5.8 1.2 4.9 0.9 3.2 0.9 4.3 0.7 4.9 1.0	 0.0002 0.0008 0.0007 0.0002 0.0008	Prospective Did not investigate whether hepatocellular carcinoma treatment impacted on survival Limited Applicability: HBV DNA test not applicable to Australian clinical practice

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid; IU, international units; LGE, logarithm of genome equivalent

[a] Transcatheter arterial embolisation, and/or percutaneous ethanol injection

Serum HBV DNA and hepatocellular carcinoma prognosis in cirrhotic patients

Two studies were identified where the relationship between serum HBV DNA levels and hepatocellular carcinoma occurrence in HBV related cirrhotic patients was investigated (Ishikawa et al 2001 and Mahmoud et al 2005)

Ishikawa et al (2001) examined several predictive factors for hepatocellular carcinoma, including serum HBV DNA and serum ALT, in a non-consecutive cohort of HbsAg positive compensated cirrhotic patients (n = 65). At the time of first diagnosis of cirrhosis, serum samples were taken for later analysis. During the observation period (mean 75.2 months), 28 patients (43.8%) developed hepatocellular carcinoma.

The cumulative hepatocellular carcinoma appearance rates were 1.5 per cent, 9.4 per cent and 18.9 per cent at years 1, 3 and 5, respectively. Univariate analysis identified three factors which influenced hepatocellular carcinoma: ALT ≥100 IU/mL; lactate dehydrogenase (LDH) ≥480 IU/L and serum HBV DNA ≥3.7 LGE/mL. Multivariate analysis indicated that these factors were independent significant risk factors for hepatocellular carcinoma. The results of this analysis are presented in Table 34.

The data presented in Table 34 suggest that serum HBV DNA ≥3.7 LGE/mL is the most prognostic factor for the development of hepatocellular carcinoma in HBsAg positive compensated cirrhosis patients. Serum ALT levels were also prognostic of hepatocellular carcinoma development.

This study has potential for bias: patient selection was non-consecutive and the direction of the study (prospective or retrospective) was unclear. It was possible that included patients represent a group with more severe chronic hepatitis B. The applicability of the results to Australian clinical practice may be limited because an older HBV DNA test was used.

Table 34 Predictive factors for hepatocellular carcinoma in cirrhotic chronic hepatitis B patients (Ishikawa et al 2001)

Study	Variable (no of patients)	Relative risk[a] (95% CI)	Study quality/comment
Ishikawa et al	**ALT**		Direction unclear
(2001)	≥100 IU/L (14)	4.525 (1.202–17.030)	Potential for selection bias: non-consecutive patient selection
	<100 IU/L (51)	1	
	LDH		Reduced applicability to Australian clinical practice: less sensitive HBVDNA test
	≥480 IU/L (30)	2.880 (0.881–9.412)	
	<480 IU/L (35)	1	
	HBV DNA		
	≥3.7 LGE/mL (46)	7.712 (1.511–39.365)	
	<3.7 LGE/mL (19)	1	

Abbreviations: ALT, alanine amino transferase; CI, confidence interval; LDH, lactate dehydrogenase; HBV DNA, hepatitis B virus deoxyribonucleic acid

[a] Relative risks calculated using Cox regression analysis

Mahmoud et al (2005) examined the association between serum HBV DNA and hepatocellular carcinoma occurrence in HBV related cirrhotic patients (n = 91). Of these patients, 23 (25.3%) developed hepatocellular carcinoma over seven years. Logistic regression analysis found that serum HBV DNA was the only statistically significant predictor of hepatocellular carcinoma occurrence (p<0.029). Serum ALT was somewhat predictive of hepatocellular carcinoma occurrence (p = 0.062). Multivariate analysis indicated that serum HBV DNA level was the only predictor of hepatocellular carcinoma occurrence in this study: odds ratio = 2.33 (95% CI: [5.6, 1.1]; p = 0.033).

The results of the study by Mahmoud et al (2005) indicate that serum HBV DNA is the most significant prognostic factor for hepatocellular carcinoma occurrence among patients with HBV related cirrhosis. It was unclear from this study whether the analysis was done on serum HBV DNA levels measured at a single time point at study entry or whether average serum HBV DNA levels during the follow-up period were used.

This study has potential for bias: patient selection was not reported and the direction of the study (prospective or retrospective) was unclear. It was possible that included patients represent a group with more severe HBV-related cirrhosis. The HBV DNA test used in this study is considered applicable to Australian clinical practice.

Serum HBV DNA and hepatocellular carcinoma recurrence after curative resection

In two further studies the relationship between serum HBV DNA level and recurrence after resection of HBV-related hepatocellular carcinoma was investigated (Kubo et al 2000; Kubo et al 2003). It was unclear from these studies whether the results reported by Kubo et al (2000) represent patients also included in the more recent study (Kubo et al 2003).

In the study by Kubo et al (2000) recurrence occurred in 19 (47.5%) of the 40 patients who had resection for hepatocellular carcinoma. Post-resection tumour free survival rate was significantly lower among patients with a high serum HBV DNA (≥0.7 mEq/mL) compared withpatients with lower HBV DNA levels ($p = 0.0025$). There was no tumour free survival at three years post-resection among patients with high serum HBV DNA. In contrast, the tumour free survival rate at nine years post-resection among patients with low serum HBV DNA was 64 per cent. In a multivariate analysis, a high serum HBV DNA level (≥0.7 mEq/mL) was found to be an independent risk factor for hepatocellular carcinoma recurrence after resection: relative risk= 5.13 (95%CI: [1.57, 16.67]; $p = 0.0069$). A positive surgical margin was also an independent risk factor for hepatocellular carcinoma recurrence after resection: relative risk= 2.14 (95%CI: [1.10, 6.80]; $p = 0.0296$).

In a similar study, Kubo et al (2003) also investigated the relationship between serum HBV DNA levels and hepatocellular carcinoma recurrence after resection. In this study of 52 patients, serum HBV DNA (by TMA-HPA assay) was ≥3.7 LGE/mL in 38 patients (group 1) and <3.7 LGE/mL in the remaining 14 patients (group 2). Hepatocellular carcinoma recurrence occurred in 24 (63.2%) of group 1 patients and two (14.3%) of group 2 patients. Tumour-free survival rate was significantly lower in group 1 patients with high serum HBV DNA levels (≥3.7 LGE/mL) in comparison with group 2 patients ($p = 0.007$). In a multivariate analysis, serum HBV DNA ≥3.7 LGE/mL was the most prognostic independent factor for recurrence after hepatocellular carcinoma resection: relative risk= 6.58 (95% CI: [1.52, 28.57]).

The results of the studies by Kubo et al (2000 & 2003) indicate that high serum HBV DNA levels are a significant prognostic indicator of hepatocellular carcinoma recurrence after resection. Analyses reported in these studies were calculated using serum HBV DNA levels taken before surgery. It appears possible that fluctuations in serum HBV DNA levels following surgical resection for hepatocellular carcinoma that may contribute to recurrence were not reported.

The studies by Kubo et al (2000 & 2003) have potential for bias: patient selection was non-consecutive and the direction of the study (prospective or retrospective) was unclear. It was possible that included patients represent a group with more severe chronic hepatitis B. The applicability of this study to Australian clinical practice may be reduced because a less sensitive HBV DNA test was used.

Relationship between serum HBV DNA levels and liver histology

The results of the study by Yuen et al (2004) are presented in Table 35 and Table 36. This study investigated the relationship between serum HBV DNA levels and liver histology of chronic hepatitis B patients. None of the patients in this study had treatment for HBV before liver biopsy.

This study included HBeAg positive patients and HBeAg negative patients. HbeAg positive patients (n = 43) had a significantly younger median age (37.2 years, range = 18.3–57.6) and a higher median serum HBV DNA level (1.1×10^9 copies/mL, range = 1.9×10^6–2×10^{13}) compared to HBeAg negative patients (n = 51; age: 45.2 years, range = 23.0–68.2; HBV DNA: 8.3×10^6 copies/mL; range = <200–1.4×10^9) ($p = 0.001$ and $p<0.001$, respectively).

HBV DNA levels were positively, but weakly ($r<0.5$), correlated with liver histology among HBeAg negative patients (Table 35). Serum ALT levels at liver biopsy were also weakly correlated with liver histology among HBeAg negative patients. There was no correlation between serum ALT levels during liver biopsy and HAI-F score. Similar serum ALT levels were reported among HBeAg positive patients (Table 35). Yuen et al (2004) investigated the association between serial ALT levels before biopsy and liver histologic scores. Their analysis provided some evidence that patients with ALT levels persistently lower than 1×ULN had less severe liver inflammation and fibrosis. The analysis was limited by low patient numbers in each subgroup.

Table 35 Relationship between serum HBV DNA, ALT levels and liver histology

Study	Patient characteristics	Variable	Correlation[b] with liver histology			Study quality/ comment
			Total HAI score	HAI-NI score	HAI-F score	
Yuen et al (2004) China	[a]HBeAg +ve = 43 (46.9%) [a]HBeAg –ve = 51 (54.3%) [a]Median ALT level, U/L = 91 (range = 21–602) [a]Median HBV DNA level, copies/mL = 1.9 x 10^7 (range = < 200–2.0 x 10^{13}) Median age, yr = 43.3 (range = 20–51) M = 74, F = 20	HBV DNA HBeAg negative	*r* = 0.37 (*p* = 0.008)	*r* = 0.31 (*p* = 0.014)	*r* = 0.33 (*p* = 0.017)	Direction unclear, possibly prospective Potential selection bias – only included patients with liver biopsies Blinding between liver histology and test results Patient population considered applicable
		ALT HBeAg negative	r = 0.31 (*p* = 0.028)	*r* = 0.35 (*p* = 0.011)	–	
		ALT HBeAg positive	*r* = 0.40 (*p* = 0.009)	*r* = 0.43 (*p* = 0.004)	–	

Abbreviations: CI, confidence intervals; F = female; HAI, histologic activity index, HAI-F, histologic activity index fibrosis; HAI, histologic activity index necroinflammation; HBeAg, hepatitis B e-antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; M = male

[a] At time of liver biopsy.

[b] Correlation between different continuous variables with skewed distribution was tested by Spearman's rank correlation. Two-tailed *p* value <0.05 considered statistically significant

For HBeAg negative patients, those with high grade necroinflammation had significantly higher median HBV DNA levels (1.8×10^7 copies/mL, range = 2.9×10^5–8.5×10^8) in comparison with patients with low grade necroinflammation (5.4×10^6, range = <200–

1.4×10^9) ($p = 0.009$). Patients with high grade fibrosis also had significantly higher median HBV DNA levels than patients with low grade fibrosis (1.8×10^7, range = 2900–1.4×10^9 vs 6.1×10^6, range <200–6.4×10^7 respectively; $p = 0.01$). The liver histologic scores for HBeAg negative patients in relation to serum HBV DNA levels are presented in Table 36.

For HBeAg positive patients there was no correlation between HBV DNA levels and HAI-NI, HAI-F and total HAI scores ($p = 0.91$, 0.88 and 0.93 respectively). No difference was found in median HBV DNA levels between patients with high-grade necro-inflammation (HAI-NI ≥7) and patients with low-grade necro-inflammation (HAI-NI <7) ($p = 1.0$). There was also no difference in median HBV DNA levels between patients with high grade fibrosis (HAI-F ≥3) and patients with low grade fibrosis (HAI-F <3) ($p = 0.39$).

Table 36 Relation between serum HBV DNA levels and liver histology scores in HBeAg negative patients

Study and country	Liver histology score	Serum HBV DNA levels (copies/mL)			Study quality/ comment
		<10^5	<10^6	<10^7	
Yuen et al (2004) China	**Necro-inflammation**				Direction unclear Potential selection bias—only included patients with liver biopsies Blinding between liver histology and test results Patient population considered applicable
	Median score (range)	2 (0–5)	3 (0–10)	2 (0–18)	
	No of patients with score ≤ 3	6 (85.7%)	9 (81.8%)	15 (57.7%)	
	Fibrosis				
	Median score (range)	0 (0–4)	0 (0–4)	1 (0–4)	
	No of patients with score = 0	6 (85.7%)	7 (63.6%)	11 (42.3%)	
	Median total HAI score (range)	2 (0–9)	3 (0–14)	4.5 (0–22)	

Abbreviations: HAI, histology activity index; HBV DNA, hepatitis B virus deoxyribonucleic acid

The cohort study by Yuen et al (2004) was of unclear direction and had potential for selection bias, as it only included patients who underwent liver biopsies. The reasons for these patients having liver biopsies were not reported. It could not be determined whether the included population was representative of patients with more severe chronic hepatitis B. The potential for selection bias should be considered.

This study found a weak correlation between serum HBV DNA levels and liver histology in HBeAg negative patients. There was also evidence suggesting that liver damage is more severe in HBeAg negative patients with increased levels of serum HBV DNA. No correlation was found between serum HBV DNA levels and liver histology in HBeAg positive patients. It was possible that the relationship between serum HBV DNA levels and liver damage was nonlinear, investigation would have been informative. It seems possible that the correlation was weak because serum HBV DNA levels are more sensitive indicators of viral replication than liver histology scores.

In this study serum HBV DNA levels were measured at one point (two weeks before liver biopsy) were compared with liver histology. To clearly establish the relation between liver histology and serum HBV DNA levels serial measurements of HBV DNA levels before biopsy are required. This would enable an assessment of the impact of sustained (low to high) or fluctuating levels of serum HBV DNA on liver histology. This study design was unethical because patients with high levels of viral replication were candidates for antiviral therapy. It is impractical to measure serum HBV DNA levels over a meaningful time scale before liver biopsy.

Does HBV DNA testing improve patient management?

To illustrate that changes in clinical decisions can be attributed to information provided by a diagnostic test, evidence is required to show that test information results in decisions which can involve stopping, starting or modifying treatment (MSAC 2005). For example, pre and post-test management studies can be used to provide this type of evidence (MSAC 2005).

No studies were identified which correspond to the study designs recommended in the MSAC guidelines. The study by Lampertico et al (2005) (see page 36) was considered to support that HBV DNA testing can alter management of patients undergoing lamivudine monotherapy. These changes may not be possible in the absence of HBV DNA testing. If ALT testing was the only method to detect lamivudine resistance, all patients identified by HBV DNA testing as genotypically resistant could potentially experience adefovir therapy delays, which may lead to suboptimal outcomes. Evidence was not identified to support that HBV DNA testing impacts change in patient management results for long term chronic hepatitis B clinical outcomes.

Does treatment result in improved health outcomes?

Licensed therapies in Australia for chronic hepatitis B include interferon-α, pegylated interferon, lamivudine, entecavir and adefovir dipivoxil. Government funding for adefovir is only available in cases where lamivudine-resistance has developed. Pre-treatment liver biopsy is required for access to therapies through the Australian Commonwealth Government Highly Specialised Drug Schedule 100 scheme for treatment of chronic hepatitis. HBV DNA testing is an essential part of the criteria to establish patient eligibility for treatment with interferon-α, lamivudine, entecavir or adefovir dipivoxil.

The PBS listing of these treatments indicates that the effectiveness of these therapies has been recognised. Evidence supporting treatment effectiveness for chronic hepatitis B was considered unnecessary for this assessment.

Appendix G presents examples of studies that illustrate treatment effectiveness of antiviral therapies.

What are the economic considerations?

Summary of economic considerations

Three research questions focusing on the the economic implications when HBV DNA testing is used were considered: 1. in the initial assessment of chronic hepatitis B patients before antiviral therapy; 2. for monitoring patients not undergoing antiviral therapy; and 3. to monitor patients undergoing antiviral therapy.

If HBV DNA testing is listed under the MBS scheme, each HBV DNA test is expected to cost Medicare Australia $130. This estimate was based on cost information for four HBV DNA test systems currently available in Australia (Digene Hybrid Capture II assay, Bayer Versant HBV 3.0 test, Roche COBAS TaqMan HBV test, and Qiagen (Artus) RealArt HBV PCR).

One HBV DNA test for each newly reported (incident) chronic hepatitis B infection in Australia is expected to result in a demand of about 6500 tests per year. Based on previous incidence data, the number of tests is not expected to change significantly in subsequent years.

There are about 8200 patients with chronic hepatitis B in Australia not undergoing antiviral therapy who are monitored by one HBV DNA test annually. This number is expected to be stable in subsequent years.

HBV DNA test use is expected to increase when used to monitor patients undergoing antiviral therapy. The number of patients treated with lamivudine and/or adefovir or interferon is expected to increase during subsequent years. This is expected to result in increased demand for HBV DNA testing. Consistent with current clinical practice in Australia, patients' recieving antiviral therapies such as lamivudine or adefovir are expected to be tested four times per year; patients undergoing interferon treatment are tested three times throughout a 12-month treatment period. The total number of HBV DNA tests required to monitor patients undergoing these antiviral therapies is estimated to be about 4700 to 5900 tests per year.

In total, about 20,000 HBV DNA tests per year are required. The expected cost to Medicare Australia is about $2.5 to $2.7 million per year.

The HBV DNA test is considered to have important potential impacts on patient management: 1. it can identify those patients least likely to benefit from antiviral therapy; 2. it can identify patients who do not require continuing antiviral therapy, so treatment can be terminated when HBV DNA tests become negative; and 3. enables earlier detection of resistance to antiviral therapy, facilitating change in patient management with the aim of avoiding disease progression or chronic hepatitis B complications. These impacts of HBV DNA testing are likely to result in benefits for other healthcare funders if HBV DNA testing is listed under the MBS scheme. A detailed assessment of the potential economic benefits of HBV DNA testing on long term chronic hepatitis B outcomes is not preseneted here: current evidence for impacts on long term outcomes following HBV DNA testing is not considered adequate for a reliable estimation of economic benefits.

The results of a recent study by Butler et al (2004), summarised in Table 37, reports the estimated average annual direct cost of managing a patient with chronic hepatitis B (chronic hepatitis B) in Australia. The study was performed as a retrospective chart analysis of 149 patients with varying stages of chronic hepatitis B, which were treated between 1995 and 2002 at four public teaching hospitals in NSW and Victoria. Costs for palliative care for chronic hepatitis B and hepatocellular carcinoma are based on observations in a palliative care unit. Cost components included were outpatient visits, outpatient pathology, outpatient imaging, drug treatment, inpatient admissions, and inpatient procedures. Indirect costs reflecting the value of lost production, and other costs such as time and travel for patients and carers, were not included. Differences in costs were tested to identify influencing factors such as age, gender, marital status, country of birth and duration of follow-up. They were not found to contribute to differences in costs.

Table 37 Direct cost of chronic hepatitis B in Australia

	Costs per patient (AUD)[1]			
Chronic hepatitis B subgroup (sample size)	**Mean ± SD**	**Median**	**Range**	**95% CI**
Non-cirrhotic chronic hepatitis B (n = 80)	1233	456	120–6295	939, 1544
Active disease (n = 38)	1778	884	125–6295	1212, 2374
Inactive disease (n = 42)	758	373	120–3247	519, 1045
Compensated cirrhosis (n = 20)	1394	1031	119–3176	975, 1797
Decompensated cirrhosis (n = 21)	11,961	5599	120–47,698	6993, 18,503
Liver transplantation (year 1)	144,392 ± 115,374	110,000	61,000–617,000	
Liver transplantation (year 2+)	23,160 ± 19,289	17,500	6700–54,700	
Hepatocellular carcinoma (n = 27)	11,753	7024	60–56,994	7385, 17,159
Palliative care	6307			4848, 8187

Source: Butler JR, Pianko S, Korda RJ, Nguyen S, Gow PJ, Roberts SK, Strasser SI, Sievert W (2004). 'The direct cost of managing patients with chronic hepatitis B infection in Australia'. *J Clin Gastroenterol*. 38: S187–S192. Reproduced with permission of Lippincott, Williams and Wilkins

Abbreviations: SD, standard deviation; AUD, Australian dollar

[1] Costs estimated for 2001

As shown in this study, the costs of managing chronic hepatitis B patients vary significantly between the early and advanced disease stages, showing markedly higher treatment costs for all advanced stages of chronic hepatitis B. These costs are valid before the introduction of specific anti-HBV therapies and an increasing usage of these services.

No other economic assessment of HBV DNA testing in Australia was identified.

Test costs

Major capital equipment

Capital costs are those required to purchase major medical facilities and equipment required to perform the service under evaluation. For this assessment, 'capital expenditure' refers to the outlay required to purchase the automated test systems used

for HBV DNA testing, such as luminometer, PC, monitor and software for automatic reading, and other associated equipment. Capital costs are typically investments made at a single point in time (often at outset); whereas running costs occur throughout the equipment's life cycle.

Capital costs for equipment and buildings are subject to depreciation, reflecting that their value decreases over time (because of technological and material wear). Depreciation can be calculated using one of three approaches— linear, progressive or degressive. Opportunity costs also contribute to overall capital costs. They represent costs of resource options that are no longer available, leading to selecting next-best and (frequently) less cost-effective options. To calculate the current value of an investment, all costs and benefits were discounted to their current values. Discounting is a method to adjust costs and benefits occurring at different points in time to their present values. The underlying principle for discounting is that costs and benefits arising in the future have a lower value than they would if they arose today.

In general, Medicare does not cover laboratory systems and their maintenance costs. Laboratories fund equipment purchases. Equipment may be provided as part of a reagent rental agreement. Some laboratories establish agreements with diagnostic technology manufacturers to purchase their product. Hardware costs are included in the price for the reagent.

The Applicant provided information that three of the four test systems available in Australia are available via reagent rental. For the fourth test system, Qiagen (Artus) RealArt HBV PCR, the reagent is available in different sets specifically tailored to different instruments. No major equipment costs were considered for the total test cost estimate.

Major equipment costs were calculated to indicate pathology lab expenditure needed for purchase of equipment to process HBV DNA samples (**Appendix H**).

Other equipment costs

The HBV DNA test cost for consumables and professional time was quoted at $70 to $110 per test in the application.

Test costs were re-calculated based on price information provided by the Applicant. Table 38 provides the results of this calculation. The costs for each of the available four test systems are shown separately, considering the number of necessary batch controls, maximum batch size, and assuming a true batch size of 20 tests. Labour costs were calculated based on an average time of four hours for a mid-range scientist plus one minute of a virologist per test for the interpretation of test results. Average equipment costs per HBV DNA test were then calculated assuming an equal market share for each of the available test systems and including a 17 per cent margin for pathology laboratories.

According to this calculation, the average test costs are $130 per HBV DNA test.

Table 38 HBV DNA test costs other than capital costs

Resource		Unit cost				Calculation	Comment
		Test system					
Reagent cost		**Digene Hybrid Capture II assay**	**Bayer Versant HBV 3.0 test**	**Roche Cobas TaqMan HBV test**	**Qiagen (Artus) RealArt HBV PCR**		
A	Purchase costs per kit	$4600.00	$6400.00	$4200.00	$4600.00		Provided by manufacturer
B	Number of tests per kit	96	96	48	96		Provided by manufacturer
C	Cost per test	$47.92	$66.67	$87.50	$47.92	C = A / B	
D	Number of controls needed per batch	12	12	3	6		Provided by manufacturer
E	Maximum number of test per batch	N/A	N/A	24	32		Provided by manufacturer
F	Assumed number of tests per batch	20	20	20	20		Assumption
G	Reagent cost per batch	$1533.44	$2133.44	$2,012.50	$1,245.92	G = (Number of required batches*C*D) + (C*F)	
H	Reagent cost per test	$76.67	$106.67	$100.63	$62.30	H = G / F	Including costs for controls
Labour costs							
I	Scientist to run sample	$6.05	$6.05	$6.05	$6.05		Four hours to process 20 samples as estimated by AP
J	Virologist to interpret test results	$1.89	$1.89	$1.89	$1.89		One minute per test
Total costs							
K	Total costs per test system	$84.61	$114.61	$108.57	$70.24	K = H + I + J	
L	Average costs per test	$94.51				L = ∑K / 4	
M	**85% Medicare fee**	**$110.57**				M = L * 1.17	17% margin added
N	**100% Medicare fee**	**$130.09**				N = (M * 100%) / 85%	

Abbreviation: AP, Advisory Panel; N/A, not applicable

Annotations: Hospital scientist, 4th year, NSW Award, Micropay job code 05.003.14: $999.60 pw +15% = $1149.54 / 38 hours a week = $30.25 per hour; APP: App 205,000 pa / (40 hours per week * 52 weeks) = $113.34 per hour

Ref: http://www.health.nsw.gov.au/policies/ib/2006/pdf/IB2006_023.pdf and http://www.health.nsw.gov.au/jobs/empcond/awards/HREA/hospital-scientists.pdf .

Ref: Salary for APP provided by Professor Syd Bell.

An assessment was made about how much the average costs per HBV DNA test depend on the number of tests per batch. The number of tests per batch was varied and the costs per test re-calculated in order to perform the assessment.

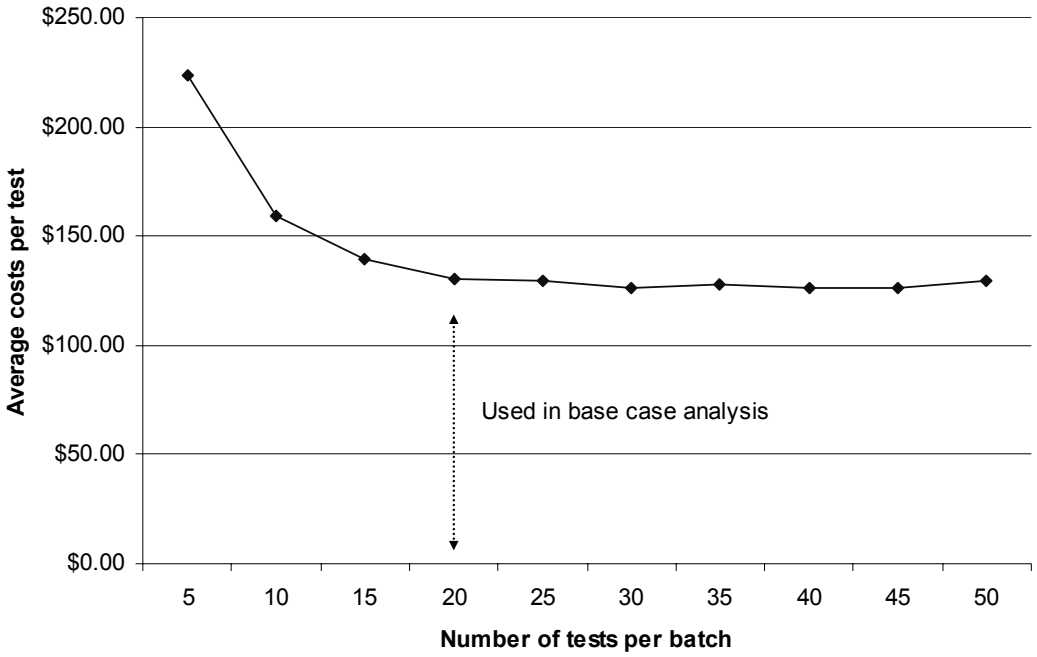

Figure 10 Average equipment costs per HBV DNA test, by number of tests per batch

The average equipment costs per HBV DNA test, soon reaches a steady state and does not significantly decrease if the number of tests per batch increases (Figure 10). This effect occurs because the maximum batch size for some of the test systems is restricted. For test systems with no restriction on the maximum batch size (Digene Hybrid Capture II Assay, Bayer Versant HBV 3.0), the costs per test would decrease further if the number of samples per batch increases.

Patient management costs

The following three research questions were defined for this assessment:

- Initial assessment and monitoring of chronic hepatitis B patients before or not undergoing antiviral therapy
- Monitoring chronic hepatitis B patients not undergoing antiviral treatment
- Monitoring chronic hepatitis B patients undergoing antiviral treatment.

Clinical pathways addressing these research questions were developed from Advisory Panel consultation.

Initial assessment and monitoring of chronic hepatitis B patients before or not undergoing antiviral therapy

According to the patient management flowcharts shown in Approach to assessment (see Figure 1 and Figure 2); the test would be added to the initial assessment of patients as DNA measurements are useful in assessing the extent of disease in the liver. The additional costs are $130.09 per patient (one test during initial assessment) should the HBV DNA test be listed under the MBS.

The HBV DNA test confirms the presence and course of HBV infections to inform treatment decisions. The test detects active disease in HBeAg negative and ALT normal patients and optimising treatment timing. In a sample of Australian patients described by Bell et al (2005), 47 per cent of all HBeAg negative patients did not exhibit elevated ALT levels at referral. HBeAg negative patients represent half of all patients with active viral replication.

The monitoring of chronic hepatitis B patients not on antiviral therapy would be increased if there were significant levels of viral replication associated as this places patients at a higher risk of developing complications or needing treatment. Patients with high viral load are more likely to have underlying disease that may be detected on biopsy and lead to treatment. At least one HBV DNA test would be performed to detect increasing DNA levels and determine treatment for all patients not undergoing drug treatment and presenting with elevated ALT levels. The test would add $130.09 to the general non-drug management costs.

Monitoring chronic hepatitis B patients undergoing antiviral therapy

Lamivudine and/or adefovir treatment

Patient management flowcharts for the treatment with lamivudine and/or adefovir (see Approach to assessment, Figure 3, Figure 4, and Figure 5) were translated into a decision tree as shown in **Appendix I**.

The HBV DNA test would be performed every three months together with the routinely performed ALT and HBeAg serology. It allows distinction among:

1. Patients presenting with normal ALT and low levels of HBV DNA (the HBV infection is well controlled and patients will continue antiviral treatment for a certain period (depending on HBeAg serology results) before switching to non-drug treatment monitoring)

2. Patients presenting elevated ALT levels, but who are HBeAg negative and show low levels of HBV DNA (the increased ALT levels are likely to be caused not by an HBV infection but other conditions, so patients could be taken off inadequate treatment

3. Patients presenting with normal ALT, HBeAg positive or negative, but increasing levels of HBV DNA. (Lamivudine monotherapy does not control disease due to either primary or secondary treatment failure[4]. Patients would stop lamivudine treatment and switch to either adefovir (if HBeAg negative) or interferon (if HBeAg positive and not contraindications).

[4] Secondary treatment failure = drug resistance

The proportion of patients inadequately treated with antivirals cannot be quantified. A comprehensive model for the course of HBV infection was beyond the scope of this assessment. The clinical flowcharts (see Approach to assessment) illustrate that it was not possible to identify patients being treated inadequately with ALT/HBeAg serology only. Lamivudine treatment could not be terminated before two follow-up measurements showing normal ALT and negative HBeAg serology were acquired.

Inadequately treated patients are exposed to unnecessary drug treatment and associated side effects. Both the treatment of side effects and the drug treatment are expenditures that could be avoided by identifying patients whose treatment protocols are inappropriate and making necessary amendments. Annual drug treatment costs with lamivudine reach $1558 per patient[5], with adefovir monotherapy $7604[6].

HBV DNA testing also allows detection of drug resistance among patients adequately treated with either lamivudine or adefovir. At present, drug resistance against lamivudine is more frequently reported compared to resistance rates against adefovir which are reported to be low (Locarnini et al 2004). A future increase in resistance to adefovir also seems to be possible. HBV DNA testing would not necessarily detect more cases of drug resistance but it could allow detecting (genotypic) resistance to antiviral treatment earlier than with ALT/HBsAg serology (which only can measure phenotypic resistance). The median time between genotypic and phenotypic resistance is reported to be four months (Hadziyannis et al 2000). Early diagnosis of drug resistance is important for patients to avoid severe liver damage from disease progression resulting from viral breakthrough. For example, severe exacerbations among patients with cirrhosis may result in organ failure; graft loss and death can occur in liver transplant patients (Locarnini et al 2004). It is not possible to quantify the benefit of earlier detection and improved long term health outcomes based on currently available evidence. Since the alternative antiviral drugs (adefovir, interferon 2a, interferon 2b) are more expensive than lamivudine, earlier switching to these drugs increases overall medication costs per patient. In the longer term, avoiding advanced stages of HBV infection, such as cirrhosis or hepatocellular carcinoma, would provide an economic benefit as they are shown to be more expensive (Butler et al 2004).

The additional (incremental) costs for monitoring in three month intervals would be $520.36 per patient, and a year of antiviral treatment (four times $130.09 per test).

Interferon

Patient management flowcharts for the treatment with interferon are presented in the Approach to assessment (see Figure 6 and Figure 7).

Where Interferon treatment is considered in HBsAg positive patients as first line treatment in patients with low HBV DNA and high ALT or as second line treatment after primary drug failure of lamivudine, about a third of all patients would be eligible for interferon treatment. The Advisory Panel indicated that in practice about 20 per cent of these patients are treated.

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[5] PBS item number 6257H: 28*100 mg = $119.50, recommended dosage = 100 mg per day

[6] PBS item number 6450L: 30*10 mg = $625.00, recommended dosage = 10 mg per day

An antiviral immune response with interferon can be reached in 20 to 30 per cent of patients treated with interferon-α 2a, and in about 40 per cent of patients treated with interferon-α 2b (within six months after treatment end), respectively (eMIMS 2006). According to the Advisory Panel, interferon treatment would be provided for a total duration of 12 months. Treatment would not be stopped where after six months of treatment, DNA levels are not found to be lowered. This indicates that the HBV DNA test allows continuous disease monitoring, but that treatment decisions would require review after 12 months at the earliest.

HBV DNA testing was considered to be performed at the beginning and the end of a six month treatment cycle and after 12 months of treatment. This would cost $390.27 per patient in addition to routine ALT and HBeAg monitoring.

Financial implications of a positive recommendation

The number of expected HBV DNA tests needed to be first estimated to calculate the financial implication of a positive recommendation. The financial implications of listing HBV DNA testing under the MBS are shown separately for Medicare Australia and other health funders.

Initial testing

HBV incident infections data from the National Notifiable Diseases Surveillance System were used to estimate uptake of HBV DNA testing for the initial assessment. In 2004, the national notification rate for confirmed incident cases was 1.4 per 100,000[7] and 1.2 per 100,000 in 2005, showing a declining tendency in recent years. (Yohannes et al 2006) Unspecified cases were reported at a rate of 29.1 per 100,000[8] (in 2004) also showing a declining tendency. In total in 2004, new HBV infections were reported at a rate of 30.5 per 100,000.

Based on demographic data, the Australian population size is expected to grow continuously during coming years (Australian Bureau of Statistics 2006). Considering a declining prevalence, but a growing population with continuous immigration from high-risk areas, it was assumed that the number of incident cases would remain stable at the current level of 6475 cases in 2006 (data for 2006 projected based on reported estimates 17 October 2006) (see Figure 11). These cases are considered to require a single initial HBV DNA test.

[7] Incident hepatitis B notifications: confirmed incident cases of HBV infection.
[8] Unspecified hepatitis B notifications: newly reported HBV infections that do not meet the confirmation criteria for incident HBV notifications.

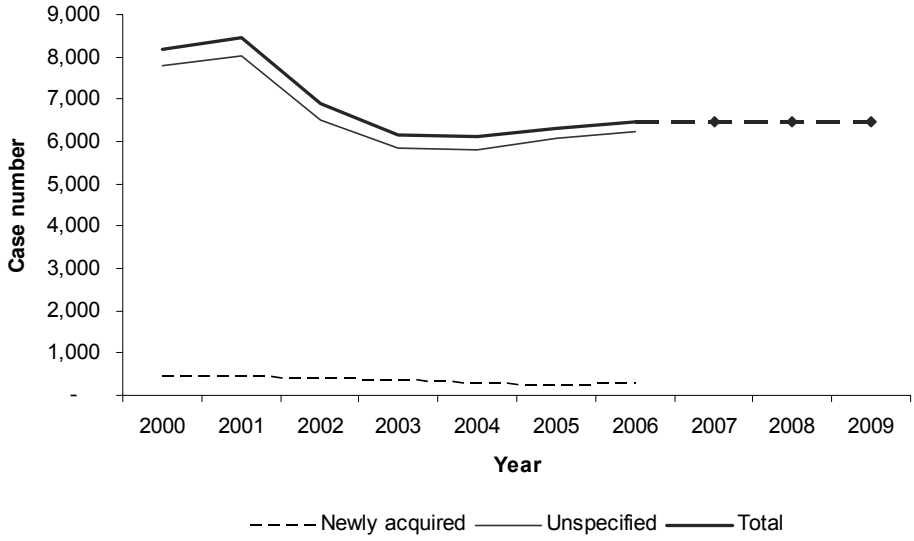

Figure 11 Forecast incident HBV cases, 2007–2010

Reference: Based on National Notifiable Diseases Surveillance System projected figures for 2006, forecast for 2007–2009

Monitoring chronic hepatitis B patients undergoing antiviral therapy

The number of patients undergoing antiviral drug treatment was assessed using Medicare statistics (Medicare Australia, 2006). The total PBS and RPBS benefits for lamivudine (PBS items 6257H, 6271C) and adefovir (6450L); interferon-α 2a (6210W, 6211X, 6212Y, 6213B) and interferon-α 2b (6246R, 6253D, 6218G, 6219H, 6254E, 6255F) were applied and converted into number of packs. Assuming ongoing treatment for the duration of one year, the number of packs was then translated into patient numbers by considering pack size and recommended dosage. In the case of interferon, this approach provides an overestimation of patients treated for chronic hepatitis B, as both interferon-α 2a and 2b are registered for the treatment of other conditions, such as chronic hepatitis and non-Hodgkin's lymphoma.[9]

The calculated patient numbers for lamivudine and/or adefovir are shown in Figure 12, for interferon in Figure 13.

[9] Also registered for: Hairy cell leukaemia; AIDS-related Kaposi's sarcoma; cutaneous T cell lymphoma; chronic hepatitis C; chronic myelogenous leukaemia (CML); excessive thrombocytosis associated with CML and other myeloproliferative disorders in patients >18 years; multiple myeloma, non-Hodgkin's lymphoma; advanced renal cell carcinoma, malignant melanoma.

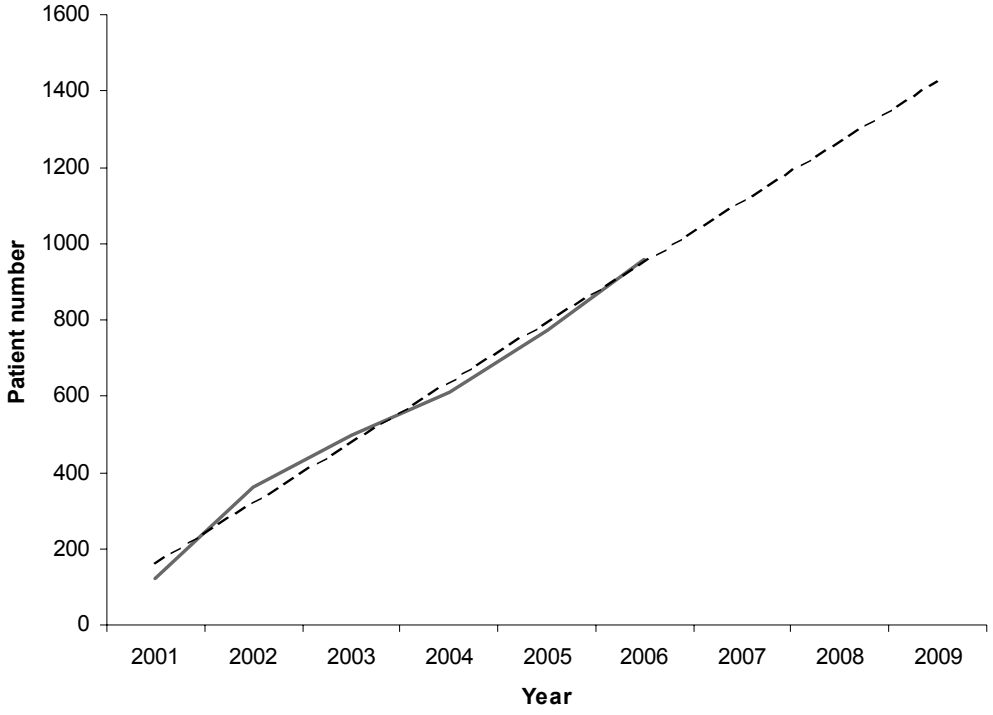

Figure 12 Forecast of patients treated with antivirals

It was assumed that patient numbers would follow past patterns—a linear rather than a damped trend—for antiviral treatment. It was calculated that about 957 patients would be treated using either lamivudine and/or adefovir in 2006 under the PBS or RPBS. Patient numbers are forecast to increase to 1107 (2007), 1265 (2008), and 1423 (2009).

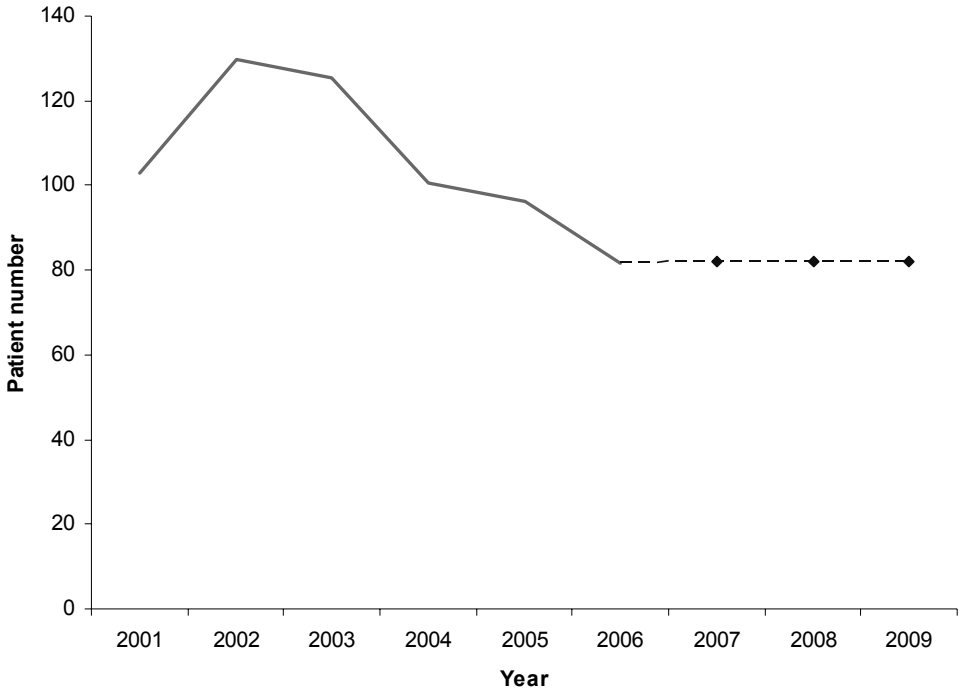

Figure 13 Forecast of patients treated with interferon-α 2a or 2b

In relation to interferon treatment, it is assumed that patient numbers will not follow past patterns—showing an exponential declining trend. This assumption is based on the recent changes in interferon listing and indications. The utilisation is rather expected to increase over time, as patients have been waited for pegylated interferon to become available for chronic hepatitis B treatment under the PBS scheme. Since 1 December 2006, Pegintron (Pegasys®) is available as monotherapy for chronic hepatitis B (6439X, 6449K). It was previously restricted to the treatment of chronic HCV only. PBS uptake data were not available to inform calculation of expected numbers of patients receiving interferon treatment, including pegylated interferon. Because alternate estimation is not possible, the number of patients was considered to be stable at the current level of about 82 patients a year (advice from the advisory panel).

Monitoring chronic hepatitis B patients not undergoing antiviral treatment

Based on HBV patients attending St Vincent's Hospital Melbourne, and drug treatment surveillance data from the Highly Specialised Drugs (HSD) Program presented by the National Centre in HIV Epidemiology and Clinical Research (Yohannes et al 2006), the number of patients in Australia attending liver clinics and who are not treated using drug therapy was calculated and tabulated (Table 39). This is considered as the number of patients who would not undergo antiviral treatment, but require regular monitoring. The calculation assumes that patients undergo drug treatment at the same frequency as provided by St. Vincent's Hospital Melbourne.

According to the application, testing is recommended for patients undergoing non-drug treatment should ALT levels become elevated. It was assumed that monitoring of HBV DNA is performed once a year in all 8142 patients.

Table 39 Estimated number of chronic hepatitis B patients not undergoing antiviral treatment that requires monitoring

	Item	Data	Source
A	HBV patients presenting to St. Vincent'sHospital Melbourne 2001–2005	850	Advisory Panel
B	HBV patients treated with antiviral drugs and interferon by St Vincent's Hospital Melbourne 2001–2005	365	Advisory Panel
C	Probability of undergoing drug-treatment[1]	43%	C = B / A
D	HBV patients in Australia undergoing drug treatment auspiced by the HSD program 2005[2]	6129	National Centre in HIV Epidemiology and Clinical Research 2006
E	Estimated number of HBV patients in Australia attending liver clinics	14,253	E = D / C
F	Estimated number of HBV patients in Australia attending liver clinics and not who are not being treated using drug therapy (requiring monitoring)	8124	F = E – D

Abbreviation: ALT, serum alanine aminotransferase

[1] As provided in St. Vincent's Hospital Melbourne liver clinic, assumed to be representative for other liver clinics as well

[2] Including lamivudine and adefovir, based on the number of prescriptions at the end of 2005, considering ongoing drug treatment

Medicare Australia

The aggregated financial impact of funding the HBV DNA test for Medicare Australia is shown in Table 40. Since Medicare Australia would meet the costs of the pathology test, the financial impact calculation is based on the estimated number of provided services and the cost of test equipment.

Table 40 Aggregated financial impact of HBV DNA test funding to Medicare Australia

		Year 1	Year 2	Year 3	Reference
Initial testing					
A	Number of incident cases	6475	6475	6475	Figure 11
B	Number of HBV DNA tests per year (initial assessments)	6475	6475	6475	One test during initial assessment
Monitoring antiviral treatment					
C	Number of patients on antiviral treatment under PBS and RPBS	1107	1265	1423	Figure 12
D	Number of HBV DNA tests/year for patients on antiviral treatment	4428	5060	5692	Four tests a year D = C * 4
E	Number of patients receiving interferon treatment under PBS and RPBS	82	82	82	Figure 13
F	Number of HBV DNA tests/year for patients receiving interferon treatment	246	246	246	Three tests during 12 months treatment F = E * 3
Monitoring patients not on antiviral treatment					
G	Number of patients presenting to liver clinics and not on drug treatment	8214	8214	8214	Table 39 One test per year
Totals					
H	Total number of HBV DNA tests	19,364	19,995	20,627	H = B + D + F + G
I	Cost per HBV DNA test	$130.09	$130.09	$130.09	Table 38
J	**Annual costs HBV DNA test**	**$2,519,063**	**$2,601,149**	**$2,683,366**	**J = H * I**

Current costs are not shown because HBV DNA testing would be provided as an additional test and would not replace any other test

Assuming that the HBV DNA test would reach an uptake rate of 100 per cent and is funded as a pathology test, the financial impact for Medicare Australia would be in the range of $2.65 to $2.7 million per year.

Other healthcare funders

The funding for the HBV DNA test would come from the hospital laboratory funding. The aggregated financial impact of HBV DNA testing is shown in **Appendix J**.

The PBS budget would also be negatively affected as patients identified with drug resistance to lamivudine would either switch to adefovir or interferon; both treatments are associated with markedly higher treatment costs per patient and year. The impact cannot be further quantified since the number of patients switching drug treatments cannot be estimated. On the other hand, the PBS budget could be positively affected as patients inadequately treated for HBV could stop drug treatment. Annual drug treatment costs with lamivudine reach $1558 per patient (PBS item number 6257H: 28*100 mg = $119.50, recommended dosage = 100 mg per day), with adefovir monotherapy $7604 (PBS item number 6450 L: 30*10 mg = $625.00, recommended dosage = 10 mg per day). By improving the management of chronic hepatitis B patients, such as by detecting drug resistance earlier, disease progression might be positively influenced and complications avoided. Hospital budgets would also be affected by listing the HBV test. These influences could not be evaluated because current evidence supporting a link between HBV DNA testing and long term outcomes is limited.

Conclusions

Effectiveness

Previously, measures of serum ALT and liver histology were considered suitable indicators of hepatitis B disease activity. Patients with chronic hepatitis B may have other reasons for elevated ALT and changes in liver histology, such as non-alcoholic fatty liver disease, or concurrent viral infections (hepatitis C). The serological marker HBeAg was previously considered to indicate active HBV replication, but some variants of the hepatitis B virus have a mutation that prevents serological detection of HBeAg. Thus, absence of HBeAg does not necessarily indicate that HBV replication has not occurred. This is so among patients who were previously HBeAg positive, because this mutation can develop during immune clearance of HBeAg. Differentiation of inactive HBeAg negative carriers from HBeAg negative active chronic hepatitis B patients is necessary to determine patients who require antiviral therapy.

Measurements of serum HBV DNA levels, as well as biochemical, serological and histological evaluations, have become widely considered as necessary to determine appropriate management for chronic hepatitis B patients. This is particularly true with respect to the introduction of and monitoring of antiviral therapy. It is important to monitor virological response during antiviral therapy because patients who may become, or are currently resistant to therapy, can be identified. Monitoring also identifies patients whose response to therapy is not optimal. Monitoring is an essential aspect of patient management to identify those who require other antiviral therapy options and to pinpoint patients least likely to benefit from therapy.

The intention of HBV DNA testing is to measure underlying hepatitis B virus replication, rather than its effects on markers, such as elevated liver enzymes. Because drug treatment outcomes are considered to be associated with pre-treatment HBV DNA levels, the test also allows clinicians to select the most appropriate drug treatment for patients. In addition, for HBeAg negative patients with non-elevated ALT levels, testing positive for HBV DNA provides access to some of the drugs listed on the PBS for the treatment of chronic HBV infection (adefovir, entecavir).

The aims of the current assessment were to determine the additional value of serum HBV DNA testing in: the initial assessment of patients before antiviral therapy; monitoring patients not undergoing antiviral therapy; and monitoring patients undergoing antiviral therapy. The aims were to assess the clinical effectiveness (diagnostic performance and the impact of diagnosis on changes in clinical management and changes in clinical outcomes) and cost-effectiveness of serum HBV DNA testing.

Because samples for HBV DNA testing can occur when blood is collected for other tests (eg ALT), it was considered unlikely that there will be any major safety issues for the patient with respect to the HBV DNA test itself—specimens are collected for the test using standard blood collection methods.

Direct evidence

Direct evidence for the impact of serum HBV DNA testing on chronic hepatitis B clinical outcomes was sought. Two studies were identified which allowed an assessment

of the impact of serum HBV DNA testing on chronic hepatitis B clinical outcomes. One study provided evidence that short term patient outcomes (virological response and ALT normalisation) are improved when HBV DNA testing is used in addition to ALT testing to alter the management of lamivudine resistant HbeAg negative patients. Long term follow-up of these patients is required to determine whether these short term benefits translate to long term improvements in chronic hepatitis B clinical outcomes.

In another study, the combined strategy of HBV DNA testing and lamivudine therapy improved health outcomes (patient survival; liver-related death) in HBsAg positive renal transplant patients. Results presented in this study did not allow an assessment of the additional value of HBV DNA testing. This study is considered not applicable to clinical practice in Australia, where all HBsAg positive patients are treated pre-emptively with lamivudine to prevent viral reactivation, irrespective of serum HBV DNA levels.

The direct evidence identified in both studies was considered limited: primarily because the studies were not randomised controlled trials and treatment decisions were driven by the availability of antiviral therapy, rather than the results of HBV DNA testing alone. Despite limitations in designs, results from both studies indicate that serum HBV DNA testing has potential to improve health outcomes among chronic hepatitis B patients.

Linked evidence: Accuracy studies

Although direct evidence for the impact of HBV DNA testing on chronic hepatitis B outcomes was identified in the literature review, a linked evidence approach was deemed necessary. This was primarily because the direct evidence was limited to specific chronic hepatitis B patient populations (HBsAg positive renal transplant patients, and HbeAg negative patients undergoing lamivudine therapy) and did not adequately address all the aims of this assessment. Assessment of HBV DNA testing in the absence of a linked evidence approach was considered inadequate because of direct evidence limitations.

HBV DNA testing and initial assessment and monitoring of patients not undergoing antiviral therapy

Five studies were identified which provided evidence indicating the accuracy of serum HBV DNA testing in initial assessment and monitoring of patients not undergoing antiviral therapy.

Two studies provided evidence that HBV DNA testing enables the differentiation of inactive HBeAg negative carriers from HBeAg negative patients with active chronic hepatitis B (eg elevated serum ALT). One of these studies provided evidence that HBV DNA testing adds additional diagnostic performance to the differentiation of these patients by serological testing (IgM anti-HBc complex measurement). Patient classification was based on serum HBV DNA levels alone, introducing potential for misclassification. Further research is necessary to establish additional value provided by HBV DNA testing to differentiate patient groups.

Two studies provide evidence that elevated serum HBV DNA levels are associated with increased liver damage. This was true of HBeAg negative patients, but not HBeAg positive patients. Although increased levels of serum HBV DNA are indicative of increased viral replication, which is considered to be associated with increased liver damage, Patients in the immune tolerant phase of chronic hepatitis B infection are characterised by high HBV DNA, HBeAg positivity, and low levels of liver

inflammation. This may explain the findings of these studies, where the association between serum HBV DNA levels and liver damage in HbeAg positive patients was not a straightforward linear relationship.

One further study assessed the role of HBV DNA testing in predicting HBeAg reversion among patients who had seroconverted (become HBeAg negative). This study provided evidence that serum HBV DNA levels of $>10^5$ copies/mL at the time of seroconversion are predictive of reversion.

These studies show that HBV DNA testing has potential value in: differentiating HBeAg negative inactive patients from HBeAg negative patients with active disease; identifying patients likely to revert to HBeAg positive after prior seroconversion; and is indicative of liver damage in HBeAg negative patients.

HBV DNA testing and monitoring of patients undergoing antiviral therapy

Four studies were identified which were considered to provide evidence for the value of HBV DNA testing in monitoring patients undergoing antiviral therapy.

Two studies provided evidence of the possible value of HBV DNA testing in monitoring patients undergoing lamivudine therapy. One study indicated that HBV DNA testing at month 3 of therapy has potential in predicting those patients who will or will not have sustained response to lamivudine therapy. Another study provided further evidence that HBV DNA testing can identify patients who will not respond to lamivudine therapy. This study also indicated that HBV DNA testing can also predict HBeAg seroconversion or resistance to lamivudine.

Lindh et al (2001) and van der Eijk et al (2006) provided evidence supporting the potential value of HBV DNA testing for monitoring patients undergoing interferon therapy. Lindh et al (2001) indicated that pre-treatment serum HBV DNA levels could differentiate patients who would have a sustained response to interferon from those patients who would not. Van der Eijk et al (2006) provided evidence supporting that combining serum HBV DNA level measurement when treatment is begun, and during treatment, had potential to identify patients who would not respond to interferon treatment.

Results from studies that considered treatment with lamivudine or interferon indicated that a significant role for serum HBV DNA testing may be to identify patients who would not respond to therapy. This may help to design more effective therapy regimens for these patients with potential to offer clinical and economic benefits.

Evidence from the linked approach was considered limited because results of HBV DNA testing were interpreted with knowledge of the reference standard (chronic hepatitis B clinical outcomes or liver histology). This has the potential to introduce bias, resulting in over-estimated accuracy of HBV DNA testing. The limitations of these studies are considered to arise because they are effectively studies researching the potential use of HBV DNA testing, but don't reflect how it is actually used in clinical practice, where it is used in addition to other tests. Indeed, all but one of these studies failed to provide evidence which allowed an assessment of the value of serum HBV DNA testing in addition to ALT, serology or liver histology. They were not considered to be accurate representations of HBV DNA test use in clinical practice. Despite these

considerations, the studies were considered to provide supportive, but limited, evidence of the potential value of serum HBV DNA testing in clinical practice.

Linked evidence: Serum HBV DNA levels and chronic hepatitis B clinical outcomes

The evidence from the accuracy studies was considered limited because impact of HBV DNA testing on long term clinical outcomes was not shown. Because these outcomes (cirrhosis, liver cancer) are long term, and widespread HBV DNA testing (combined with therapies requiring assessment of HBV DNA levels) is relatively recent, evidence may not become available for several years. The absence of this evidence required supplementation by studies that examined relationships between serum HBV DNA levels and long term outcomes. Although these studies did not directly assess the ability of HBV DNA testing to change outcomes, evidence was provided that HBV DNA levels can indicate long term outcomes.

Studies that reported serum HBV DNA levels and chronic hepatitis B outcomes were generally limited by reporting measurement of serum HBV DNA levels at one time point. This may not fully capture the dynamic nature of viral replication because periods of intense viral replication can be interspersed with phases of relative quiescence. The impact of fluctuating or sustained increased levels of serum HBV DNA was not assessed. Data from these studies suggest that HBV DNA level measurement at a single time point has potential to fulfil an important prognostic role.

One systematic review was identified which provided evidence that HBV DNA levels are predictive of outcome, illustrates treatment efficacy. Significantly, serum HBV DNA levels (baseline and change from baseline) were correlated with liver histology and HBeAg seroconversion. This indicates that serum HBV DNA levels are potentially important indicators of liver damage and predictors of HbeAg seroconversion.

In a long term population-based study (REVEAL-HBV study) increased levels of serum HBV DNA were found to be associated with an increased cumulative incidence of both cirrhosis and hepatocellular carcinoma. Significantly, serum HBV DNA was found to be an independent risk factor for both cirrhosis and hepatocellular carcinoma. The results from the REVEAL HBV study were true for HbeAg positive and negative participants, but most participants in this study were HbeAg negative. Additional analysis from the REVEAL-HBV study found that persistently elevated levels of serum HBV DNA increase the risk of hepatocellular carcinoma. This was the only example of an analysis which examined serum HBV DNA at more than one time point. Two other studies reported that serum HBV DNA levels were the strongest prognostic factor for hepatocellular carcinoma among patients with HBV-related cirrhosis.

Results from another study indicated that patient survival is increased when serum HBV DNA are low at diagnosis of hepatocellular carcinoma. Two studies provided evidence that increased levels of serum HBV DNA is the most significant prognostic factor for recurrence after hepatocellular carcinoma resection. One study reported that patients who had spontaneous HBV DNA seroconversion (sustained or not sustained) had an increased risk of hepatocellular carcinoma death. Lower levels of serum HBV DNA were also found to be associated with a reduced risk of hepatocellular carcinoma death.

Considered together, the data presented in these studies indicate that serum HBV DNA measurement, even at one time point, is an important prognostic factor of long term

chronic hepatitis B clinical outcomes. Further research is required to establish if changes in patient management in response to serum HBV DNA testing result in improved long term outcomes.

Cost-effectiveness

Listing the HBV DNA test on the MBS, would increase the costs of initial assessment of patients presenting with HBV (one test), as well as for the monitoring of patients on drug- or non-drug treatment (three or four test for patients on antivirals or interferon; one test per year for patients not on drug treatment). Each test would cost Medicare Australia about $130.

The overall budget impact is estimated to be at $2.5 million in the first year and is expected to increase slightly over time, reaching $2.7 million in the third year after listing. This increase is caused by an expected increase of patients to be monitored while on drug treatment. The presented calculations were based on conservative estimates of future interferon treatment uptake and there was potential for underestimation.

The HBV DNA test is also beneficial in detecting the development of drug resistance earlier than it would be possible with serological testing only. An earlier switch to other drug treatment options would result in costs incurred to the PBS earlier than without HBV DNA testing, which might have an increasing effect on annual PBS budget in the first year after funding the HBV DNA test under the MBS. On the other hand, the PBS could be positively affected as patients inadequately treated for HBV infection (tested negative for HBV DNA) could be taken off unnecessary drug treatment. In case HBV DNA testing picks up more patients with drug resistance, there would be an overall increase in the PBS budget.

Improvements in the detection of drug resistance can be expected to contribute to improved management of chronic hepatitis B patients, considering that disease progression might be influenced positively and complications could be avoided. Considering the advanced stages of chronic hepatitis B infection are shown to be more expensive to treat, avoiding disease progression or complications could potentially create substantial savings for Medicare Australia as well as other healthcare funders.

Recommendation

MSAC has considered the safety, effectiveness and cost effectiveness of the use of hepatitis B assays in the pre-treatment assessment and in the monitoring of patients with chronic hepatitis B.

MSAC finds there is sufficient evidence of the safety, effectiveness and cost effectiveness of hepatitis B assay in the pre-treatment and in the monitoring of patients with chronic hepatitis B.

MSAC recommends that public funding be provided for the use of hepatitis B assay in patients with chronic hepatitis B.

MSAC further recommends that the number of hepatitis B assays for pre-treatment assessment or for the monitoring of patients with chronic hepatitis B who are not on antiviral therapy be restricted to one assay in a twelve month period and for patients on antiviral therapy the number of assays be restricted to four assays in a twelve month period.

–The Minister for Health and Ageing accepted this recommendation on 4 June 2007–

Appendix A MSAC terms of reference and membership

MSAC's terms of reference are to:

- advise the Minister for Health and Ageing on the strength of evidence pertaining to new and emerging medical technologies and procedures in relation to their safety, effectiveness and cost-effectiveness and under what circumstances public funding should be supported;
- advise the Minister for Health and Ageing on which new medical technologies and procedures should be funded on an interim basis to allow data to be assembled to determine their safety, effectiveness and cost-effectiveness;
- advise the Minister for Health and Ageing on references related either to new and/or existing medical technologies and procedures; and
- undertake health technology assessment work referred by the Australian Health Ministers' Advisory Council (AHMAC) and report its findings to AHMAC.

The membership of MSAC comprises a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member	Expertise or Affiliation
Dr Stephen Blamey (Chair)	general surgery
Associate Professor John Atherton	cardiology
Professor Syd Bell	pathology
Associate Professor Michael Cleary	emergency medicine
Dr Paul Craft	clinical epidemiology and oncology
Dr Kwun Fong	thoracic medicine
Ms Catherine Farrell	Acting Assistant Secretary, Department of Health and Ageing
Dr David Gillespie	gastroenterology
Dr Debra Graves	medical administrator
Professor Jane Hall	health economics
Professor John Horvath	Chief Medical Officer, Department of Health and Ageing
Associate Professor Terri Jackson	health economics
Professor Brendon Kearney	health administration and planning
Professor Frederick Khafagi	nuclear medicine
Dr Ray Kirk	health research
Dr John McEwen	Senior Health Advisor, Department of Health and Ageing

Associate Professor Donald Perry-Keene	endocrinology
Dr Ewa Piejko	general practice
Mrs Sheila Rimmer	consumer health issues
Professor Ken Thomson	radiology
Dr Douglas Travis	urology
Dr Mary Turner	Australian Health Ministers' Advisory Council representative
Dr David Wood	orthopaedic sugery

Appendix B Advisory panel

Advisory panel for MSAC application 1096
Hepatitis B DNA testing for chronic hepatitis B

Professor Syd Bell (Chair) Area Director of Microbiology South East Sydney Area Health Service NSW	Chair and MSAC member
Professor Peter Angus Medical Director Liver Transplant Unit Austin and Repatriation Medical Centre Heidelberg, Victoria	Co-opted member
Mr Martyn Goddard Australian Hepatitis Council nominee Hobart, Tasmania	Co-opted member
Dr Debra Graves Chief Executive Officer Royal College of Pathologists of Australasia Surry Hills, NSW	MSAC member
Dr Geoff Higgins Chief Pathologist Infectious Diseases Laboratories Institute of Medical and Veterinary Science Adelaide, South Australia	Co-opted member
Ms Sharyn McGregor Director Hepatitis C Section Department of Health and Ageing	Hepatitis C Section Department of Health and Ageing
Dr Janney Wale Consumer representative Claremont, Western Australia	Consumers' Health Forum of Australia nominee
Dr Katrina Watson Gastroenterologist St Vincent's Hospital Melbourne, Victoria	Co-opted member

Evaluators for MSAC application 1096

Dr John Gillespie BSc (Hons) PhD	M-TAG Pty Ltd A unit of IMS Health
Ms Antje Smala BAgEng (Hons) BEng (Hons)	M-TAG Pty Ltd A unit of IMS Health
Dr Nathan Walters BSc (Hons) PhD	M-TAG Pty Ltd A unit of IMS Health
Dr Liesl Birinyi-Strachan BSc (Hons) PhD	M-TAG Pty Ltd A unit of IMS Health

Appendix C Studies included in the review

Table 41 presents characteristics and results extracted from studies that assessed the additional value of HBV DNA testing compared with current clinical practice.

Table 42 presents characteristics and results extracted from studies that assessed the value of serum HBV DNA levels in the initial assessment and monitoring of patients not undergoing antiviral therapy.

Table 43 presents characteristics and results extracted from studies that assessed the predictive value of HBV DNA testing of patients undergoing antiviral therapy.

The systematic review presented in Table 44 investigated the relationship between serum HBV DNA levels and clinical outcomes.

Table 45 presents the characteristics and results extracted from primary studies that evaluated the relationship between HBV DNA levels and clinical outcomes.

Table 41 Characteristics and results of studies assessing the additional value of HBV DNA testing on patient outcomes

Study (year) Country Study design	Population (N)	Other tests	Test characteristics	Treatment	Results	Study quality
Chan (2002) China Cohort study: 1983–2000 Prospective data collection from January 1996 Unclear patient enrolment	**Period I cohort (1983 to 1995):** HBsAg positive transplant patients (n = 52) **Period II cohort (1996 to 2000):** HBsAg positive *de novo* transplant patients (n = 15) **HBsAg negative cohort (1983 to 2000)[a]:** (n = 442) **Exclusions:** anti-hepatitis C positive (antibodies; significant alcohol consumption; cirrhosis on biopsy; decompensated liver disease	ALT; alkaline phosphatase; γ-glutamyl transpeptidase; and bilirubin[b] Prothrombin time; HBeAg/anti-HBeAg status; anti-hep D; liver biopsy (consenting patients only—not obligatory criterion for lamivudine treatment). Further tests NR	**Index test**: Digene Hybrid Capture II assay. Sensitivity limit of 1.4 x 10^5 copies/mL[b] **Comparator**: ALT (details NR)	Lamivudine (100 mg/day) begun if: – elevated HBV DNA levels (>2.83 x 10^8 copies/mL) or – elevated HBV DNA levels (>2.83 x 10^7 copies/mL) with abnormal ALT/liver biopsy specimen **Period I**: Treatment initiated for elevated HBV DNA (n = 7); treatment initiated for elevated HBV DNA with abnormal ALT/liver biopsy specimen (n = 8); patients with pre-emptive lamivudine treatment[c] (n = 15[d]) **Period II**: Treatment initiated on elevated HBV DNA (n = 5); treatment initiated for elevated HBV DNA with abnormal ALT/liver biopsy specimen (n = 6); patients with pre-emptive lamivudine treatment[c] (n = 11[e])	**Survival[f,g]**: *Period I* 100%; *Period II* 73% **RR of death (95% CI)[h]**: *Period I* 9.7 (4.7, 19.9); *Period II* NR **RR of liver related death (95% CI)[h]**: *Period I* 68.0 (8.7–533.2); *Period II* NR **Treatment duration (weeks) until undetectable HBV DNA**: *Period I* 6.4 (SD 2.1, range 3–10) *Period II* 6.4 (SD 4.0, range 3–14) **Treatment duration (weeks) until ALT normalisation**: *Period I* 14.5 (SD 11.5, range 1–39) *Period II* 15.6 (SD 12.2, range 5–38)	Possible selection bias: Cohort study, patient selection not randomised Possible non-consecutive recruitment Applicability: Applicability of HBV DNA testing regimen to Australian clinical practice unknown

Study (year) Country Study design	Population (N)	Other tests	Test characteristics	Treatment	Results	Study quality
Lampertico (2005) Italy Cohort study: 1997–2003 Prospective patient enrolment (consecutive NR)	HBeAg-negative during previous 12 months; ≥18 years; HBsAg carriers; with histological or clinical diagnosis of chronic hepatitis or cirrhosis. Unresponsive to or did not meet the criteria for interferon therapy Patients developing lamivudine resistance **Phenotypic resistant cohort** (n = 46) Genotype D 92% (of 39 tested); pre-Adefovir serum HBV DNA levels (log_{10} copies/mL), median 7.3, range 4.3–9.3; Pre-Adefovir serum ALT levels (IU/mL), median 145, range 42–2870 **Genotypic resistance cohort** (n = 28) Genotype D 94% (of 17 tested); Pre-Adefovir serum HBV DNA levels (log_{10} copies/mL), median 4.5, range 3.4–5.9; Pre-Adefovir serum ALT levels (IU/mL), median 38, range 20–70	HbeAg; anti-HBeAg; Histological or clinical assessment of chronic hepatitis B or cirrhosis	**Index test**: Versant 3.0 (bDNA), Bayer. Sensitivity limit of 2000 copies/mLor 3.3 log_{10} copies/mL. Serum HBV DNA assayed every 2 months **Comparator**: ALT (standard laboratory procedures, further details NR)	Lamivudine (100 mg/day); adefovir 10 mg (orally) added to lamivudine monotherapy **Phenotypic resistant cohort**: Adefovir added 33 months[i] (average) after lamivudine initiation **Genotypic resistance cohort**: Adefovir added 41 months[i] (average) after lamivudine initiation	**Response**[k]: *Pheno* 46%; *Geno* 100% **Serum ALT normalisation**[l]: *Pheno* 3 months 37%, 6 months 50%, 24 months 93%; *Geno* 100% **2 year clearance rate of serum HBV DNA**: *Pheno* 78%; *Geno* 100% **Onset of adefovir resistance**[m]: *Pheno* 0%; *Geno* 0%	Potential for bias: patients who were treated using adefovir at different stages of lamivudine resistance were not randomised Non-consecutive recruitment Comparator test not interpreted independently of HBV DNA test Reduced applicability: less frequent HBV DNA testing in Australian clinical practice

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid; NR, not reported; RR, relative risk; SD, standard deviation

[a] Acceptance criteria for renal transplant patients did not change between 1983–2000

[b] Testing schedule: (1) Every 2 to 4 wks (a) for the first 12 months after transplantation on: (b) for 4 months after pulse steroid and/or anti-lymphocyte therapy for acute rejection; (c) when serial HBV DNA levels show increasing trend; (d) during treatment with lamivudine. (2) Every 4 months in clinically stable long term renal allograft recipients on low-dose immunosuppressive medications

[c] Period 1 patients: Lamivudine treatment started 71 +/– 27 months (range 15 to 118) after transplantation; Period II patients: Lamivudine treatment started 71 +/– 27 months (range 1 to 18) after transplantation. Duration of lamivudine therapy was 36.3 +/– 11.4 months and 32.6 +/– 13.3 months for Period I and Period II patients respectively (p = 0.118)

[d] Of the 52 period I patients, 14 died before Januarary 1996. Of the remaining 38 patients, 15 satisfied the criteria for pre-emptive lamivudine treatment

[e] Of the 15 period II patients, 3 were late referrals who received lamivudine as salvage therapy after the development of liver decompensation. These patients were excluded from between-group comparisons

[f] Renal allograft survival was similar between each cohort and HBsAg negative patients, irrespective of lamivudine therapy

[g] For period 1 patients this value for survival refers to the period before January 1996. Two other patients (not treated with lamivudine) died from non-liver related causes after January 1996

[h] Relative risks compared with HBsAg negative renal transplant patients

[i] Phenotypic resistance defined as high levels of HBV DNA (>6 log10 copies/mL) and high ALT levels (not reported by study)

[j] Because genotypic resistance is identified before phenotypic resistance it is suspected that the timing of adefovir addition to therapy has not been reported correctly in this study and that adefovir treatment was begun at 33 months for the genotypic resistant cohort and at 41 months for the phenotypic resistant cohort

[k] Patients (%) with undetectable serum HBV DNA after 3 months

[l] Patients (%) with greater than 1 log rebound of serum HBV DNA compared with on-treatment (adefovir addition) nadir.

[m] None of 10 patients with detectable serum HBV DNA at the end of the study had adefovir-related mutations, rtN236T AND rtA181V

Table 42 Study characteristics and results: Evaluating the predictive value of serum HBV DNA testing in initial assessment and monitoring among patients not undergoing antiviral therapy

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
Chan (2003) China Cohort study: not reported Unclear patient enrolment	Two groups of treatment naïve patients: 1. HBeAg positive at study entry, then sustained seroconversion for at least 6 months with normal ALT (n = 33) Sustained seroconversion: n = 20: 11 M, 9 F, median age 29 (range 12–39);HBeAg reversion: n = 13, 11 M, 2 F, median age 39 (range 13–68) 2. HBeAg negative, anti-HBeAg positive at study entry(n = 40) Persistent HBeAg negative: n = 29, 12 M, 17 F, median age = 41 (range 21–65); HBeAg reversion: n = –11, M 8, F 3, median age = 49 (range 37–67)	Tests before study entry not reported	**Index test**: Realtime PCR (TaqMan standardised to EUROHEP gentotype D, HBV std), sensitivity range = 10^2 to 10^9 copies/mL **Reference standard**: Sustained seroconversion (HBeAg negative, anti-HBeAg positive) or reversion to HBeAg positive (details not reported)	(1) HBeAg positive at study entry[a] **Sustained seroconversion (proportion of patients)**: *Serum HBV DNA (<4 log_{10} copies/mL)* 55.0%; *serum HBV DNA (4–5 log_{10} copies/mL)* 40.0%; *serum HBV DNA (>5 log_{10} copies/mL)* 5.0% **HBeAg reversion (proportion of patients)**: *Serum HBV DNA (<4 log_{10} copies/mL)* 15.4% (LR 0.3); *serum HBV DNA (4–5 log_{10} copies/mL)* 38.5% (likelihood ratio 1.0); *serum HBV DNA (>5 log_{10} copies/mL)* 46.2% (LR 9.2) (2) HBeAg negative at study entry[b] **Sustained seroconversion (proportion of patients)**: *Serum HBV DNA (<4 log_{10} copies/mL)* 65.5%; *serum HBV DNA (4–5 log_{10} copies/mL)* 24.1%; *serum HBV DNA (>5 log_{10} copies/mL)* 10.3% **HBeAg reversion (proportion of patients)**: *Serum HBV DNA (<4 log_{10} copies/mL)* 18.2% (LR 0.3); *serum HBV DNA (4–5 log_{10} copies/mL)* 18.2% (likelihood ratio 0.8); *serum HBV DNA (>5 log_{10} copies/mL)* 53.8% (LR 5.2)	P1 Q3 *Applicability*: Unclear Criteria for patient inclusion not reported *Quality:* Poor Direction unclear Potential selection bias Inadequate reporting of reference standard
Lindh (2000) Sweden Cohort study: not reported Prospective, non-consecutive patient enrolment	Chronic hepatitis B patients attending regular check-up invited to take part (irrespective of clinical signs of disease) Excluded: HCV or HDV coinfected patients excluded (2 patients had previous interferon treatment 6 years before) n = 160 (105M, 55F) Mean age = 34 (range 16–66) HBeAg positive: n = 36 (mean age 30 years), HBeAg negative: n = 124 (mean age 40 years)	Tests before study entry not reported	**Index test**: Amplicor HBV monitor (Roche), detection range = 10^3 to 10^7 copies/mL (measured at time of liver biopsy) **Comparator**: ALT (details not reported, Indexed ALT[c], ALTi used for analysis) **Reference standard**: Liver biopsy (details not reported), blinded histological scoring using HAI (Knodell method) histological activity index inflammatory score and histological activity index fibrosis score	**Prediction of HAIinf >3 (PPV)**: *Serum HBV DNA (10^4 copies/mL)* 25%; *serum HBV DNA (2 x 10^5 copies/mL)* 68%; *serum HBV DNA (10^7 copies/mL)* 100%; *ALTi* (0.5) 28%; *ALTi* (1.0) 44%; *ALTi* (2.0) 67% **Prediction of HAIinf >3 (NPV)**: *Serum HBV DNA (10^4 copies/mL)* 84%; *serum HBV DNA (2 x 10^5 copies/mL)* 87%; *serum HBV DNA (10^7 copies/mL)* 81%; *ALTi* (0.5) 97%; *ALTi* (1.0) 88%; *ALTi* (2.0) 82% **Prediction of HAIinf >6 (PPV)**: *Serum HBV DNA (10^4 copies/mL)* 14%; *serum HBV DNA (2 x 10^5 copies/mL)* 36%; *serum HBV DNA (10^7 copies/mL)* 83%; *ALTi* (0.5) 14%; *ALTi* (1.0) 20%; *ALTi* (2.0) 42% **Prediction of HAIinf >6 (NPV)**: *Serum HBV DNA (10^4 copies/mL)* 97%; *serum HBV DNA (2 x 10^5 copies/mL)* 95%; *serum HBV DNA (10^7 copies/mL)* 93%; *ALTi* (0.5) 100%; *ALTi* (1.0) 94%; *ALTi* (2.0) 93%	P1 Q2 *Applicability*: Applicable *Quality*: Medium Valid reference standard, blinded to test results Potential for selection bias: non-consecutive recruitment

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
Manesis (2003) Greece Cohort Study: 1997–1999 Unclear patient enrolment	HBeAg negative chronic hepatitis B patients; HBsAg and HBeAg negative ≥ 6 months before study entry. Excluded: HDV, HCV or HIV co-infected and patients with decompensated liver disease and/or hepatocellular carcinoma (n = 196) Classified as HBeAg negative chronic hepatitis B if ALT [d] activity increased and liver histology compatible with chronic hepatitis B Classified as HBeAg negative inactive carriers if had persistent normal ALT[d] during follow-up	HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBeAg by enzyme immuno-assay HDV, HCV, HIV serology by enzyme immuno-assay Liver biopsies scored for histological grade and stage according to Ishak score	**Index test**: PCR; Amplicor HBV Monitor (Roche), LLD = 400 copies/mL **Comparator**: IgM anti-HBc index by semi quantitative ELISA (Abbot), ALT, AST (Abbot spectrum auto analyser) **Reference standard**: HBeAg carrier status; classified as *HBeAg negative* chronic hepatitis B if ALT activity increased and liver histology compatible with chronic hepatitis B; classified as *HBeAg negative inactive carriers* if had persistent normal ALT during follow-up	**ALT (> *vs* ≤ ULN)**: *chronic hepatitis B (n)* 109 vs 25; *inactive carriers (n)* 0 vs 62; Correct classification 87.2%; PPV 100%; NPV 73.1%; Sn 81.3%; Sp 100% **AST (> *vs* ≤ ULN)**: *chronic hepatitis B (n)* 92 vs 42; *inactive carriers (n)* 0 vs 62; Correct classification 78.6%; PPV 100%; NPV 59.6%; Sn 68.7%; Sp 100% **IgM anti-HBC (≥ vs <0.2000)**: *chronic hepatitis B (n)* 104 vs 30; *inactive carriers (n)* 5 vs 57; Correct classification 82.1%; PPV 95.4%; NPV 65.5%; Sn 77.6%; Sp 91.9% **HBV DNA (≥ vs <30 000 copies/mL)**: *chronic hepatitis B (n)* 120 vs 14; *inactive carriers (n)* 0 vs 62; Correct classification 92.9%; PPV 100.0%; NPV 81.6%; Sn 89.6%; Sp 100.0% **HBV DNA (≥ vs <100 000 copies/mL):** *chronic hepatitis B (n)* 117 vs 17; *inactive carriers (n)* 0 vs 62; Correct classification 91.3%; PPV 100.0%; NPV 78.5%; Sn 87.3%; Sp 100.0% **HBV DNA IgM anti-HBc index (positive[e] vs negative)**: *chronic hepatitis B (n)* 128 vs 6; *inactive carriers (n)* 5 vs 57; Correct classification 94.4%; PPV 96.2%; NPV 90.5%; Sn 95.5%; Sp 91.9% **IgM anti-HBC (≥ vs <0.2000), normal ALT & AST**: *chronic hepatitis B (n)* 14 vs 11; *inactive carriers (n)* 5 vs 57; Correct classification 81.6%; PPV 73.7%; NPV 83.8%; Sn 56.0%; Sp 91.6% **HBV DNA (≥ vs <30 000 copies/mL), normal ALT & AST**: *chronic hepatitis B (n)* 20 vs 5; *inactive carriers (n)* 0 vs 62; Correct classification 90.8%; PPV 100.0%; NPV 88.6%; Sn 68.0%; Sp 100.0% **HBV DNA (≥ vs <100 000 copies/mL), normal ALT & AST**: *chronic hepatitis B (n)* 17 vs 8; *inactive carriers (n)* 0 vs 62; Correct classification 90.8%; PPV 100.0%; NPV 88.6%; Sn 68.0%; Sp 100.0% **HBV DNA IgM anti-HBc index (positive[e] vs negative), normal ALT & AST**: *chronic hepatitis B (n)* 23 vs 2; *inactive carriers (n)* 5 vs 57; Correct classification 92.0%; PPV 82.1%; NPV 96.6%; Sn 92.0%; Sp 91.9%	P1 Q3 *Applicability*: Applicable HBV DNA test regimen considered applicable to Australian clinical practice *Quality*: Poor Index test interpreted with knowledge of reference standard Some potential for selection bias: direction unclear, consecutive enrolment

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
Peng (2003) China Cohort Study: 1998–2000 Prospective patient enrolment (non-consecutive test-based enrolment)	HBsAg positive; anti-HBs negative; no antiviral immunosuppressive therapy during period ≥6 mo before study. No immunocompromising diseases. Hepatitis A, E, C or D virus co-infected or other liver disease patients excluded (n = 743) HBeAg positive = 64.1%; HBeAg negative = 35.9%. HBeAg positive group (27.1+/- 8.3 years) vs HBeAg negative group (32.7+/– 15.3 years) (p<0.001). Gender ratio: HBeAg psoitive vs. HBeAg negative group: 413/63 vs 234/33 (M/F) (647 M, 96 F) Patients with liver biopsy specimens <1.0 cm not enrolled	HBsAg; anti-HBs	**Index test**: Dot-blot hybridisation-based, in-house assay, Semi-quantitative ranges: <20, 20–100, 100–200, 200–500, 500–1000 and >1000 pg/mL[f] **Reference standard**: Liver biopsy: Surecut needles (16G x 70–90 mm), specimens formalin fixed, paraffin embedded and stained with hematoxylin, eosin and reticulin. Slides read blindly. Histological scoring using HAI (Knodell method) HAIinf (inflammatory score) & HAIfib (fibrosis score)	1. HBeAg positive patients **Diagnostic performance compared to HAIinf**: *Serum HBV DNA (<20pg/mL)* LR+ 1.00, LR– 0.00[g]; *Serum HBV DNA (20–100pg/mL)* LR+ 0.75, LR– 4.84; *Serum HBV DNA (100–200pg/mL)* LR+ 0.44, LR– 2.86; *Serum HBV DNA (200–500pg/mL)* LR+ 0.24, LR– 1.61; *Serum HBV DNA (500–1000pg/mL)* LR+ 0.41, LR– 1.10; *Serum HBV DNA (>1000pg/mL)* LR+ 0.35, LR– 1.03 **Diagnostic performance compared to HAIfib**: *Serum HBV DNA (<20pg/mL)* LR+ 1.00, LR– 0.00[g]; *Serum HBV DNA (20–100pg/mL)* LR+ 0.70, LR– 3.83; *Serum HBV DNA (100–200pg/mL)* LR+ 0.39, LR– 2.36; *Serum HBV DNA (200–500pg/mL)* LR+ 0.27, LR– 1.41; *Serum HBV DNA (500–1000pg/mL)* LR+ 0.49, LR– 1.07; *Serum HBV DNA (>1000pg/mL)* LR+ 0.77, LR– 1.01 2. HBeAg negative patients **Diagnostic performance compared to HAIinf**: *Serum HBV DNA (<20pg/mL)* LR+ 1.00, LR– 0.00[g]; *Serum HBV DNA (20–100pg/mL)* LR+ 2.15, LR– 0.07; *Serum HBV DNA (100–200pg/mL)* LR+ 4.18, LR– 0.48; *Serum HBV DNA (200–500pg/mL)* LR+ 1.21, LR– 0.98; *Serum HBV DNA (500–1000pg/mL)* LR+ 0.26, LR– 1.06; *Serum HBV DNA (>1000pg/mL)* LR+ NR, LR– not reported **Diagnostic performance compared to HAIfib**: *Serum HBV DNA (<20pg/mL)* LR+ 1.00, LR– 0.00[g]; *Serum HBV DNA (20–100pg/mL)* LR+ 1.65, LR– 0.17; *Serum HBV DNA (100–200pg/mL)* LR+ 2.31, LR– 0.60; *Serum HBV DNA (200–500pg/mL)* LR+ 1.61, LR– 0.97; *Serum HBV DNA (500–1000pg/mL)* LR+ 0.00, LR– 1.02; *Serum HBV DNA (>1000pg/mL)* LR+ NR, LR– not reported	P2 Q2 *Applicability*: Limited In-house assay *Quality*: Medium Potential selection bias

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
Seo (2005) Japan Cohort study: 1989–2002 Retrospective patient enrolment (consecutive NR)	chronic hepatitis B patients : HBeAg negative inactive carriers (with persistently normal ALT levels) (n = 22) HBeAg negative chronic hepatitis B (persistent or intermittent ALT elevation) (n = 26) Patients followed up for mean 51.5 months (range 5–157 months) and see every 1–3 months	HBsAg; anti-HBs, HbeAg, anti-HBeAg enzyme immunoassay (Dianabot); ALT measurement NR	**Index test**: PCR; Amplicor HBV Monitor (Roche), detection range = 10^3 to 10^7 copies/mL **Reference standard**: HBeAg carrier status; classified as *HBeAg negative inactive* if HBeAg –ve and normal ALT; classified as *HBeAg negative active* if HBeAg negative and persistent on intermittent ALT elevation	**Serum HBV DNA (<4.5 log copies/mL)**: *One time point*[h] Sn 77%, Sp 50%; *Two time points*[i] Sn 80%, Sp 71.4% **Serum HBV DNA (<5 log copies/mL)**: *One time point*[h] Sn 82%, Sp 45%; *Two time points*[i] Sn 90%, Sp 71.4% **Serum HBV DNA (<5.5 log copies/mL)**: *One time point*[h] Sn 91%, Sp 32%; *Two time points*[i] Sn 100%, Sp 57.1% **Serum HBV DNA (<6 log copies/mL)**: *One time point*[h] Sn 100%, Sp 14%; *Two time points*[i] Sn 100%, Sp 35.7%	P1 Q3 *Applicability*: Applicable *Quality*: Poor No Blinding Potential selection bias: patient selection criteria unknown, retrospective analysis.

Abbreviations: ALT, alanine aminotransferase; ALTi, indexed ALT; AST, aspartate aminotransferase; chronic hepatitis B, chronic hepatitis B; EUROHEP, European concerted action on viral hepatitis; HAI, histological activity index; HAIinf, histological activity index inflammatory score; HAIfib, histological activity index fibrosis score; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus s antigen; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; LR, likelihood ratio; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RCT, randomised controlled trial; ROC, receiver operating characteristic; Sn, sensitivity; Sp, specificity; ULN, upper limit of normal

[a] Serum HBV DNA levels at time of seroconversion

[b] Serum HBV DNA levels at time of study entry

[c] ALTi = ALT divided by upper reference value (URV; 0.8 µkat–1 for males and 0.6 µkat–1 for females)

[d] Patients with abnormal ALT levels at baseline (>ULN) followed by monthly ALT determination., with liver biopsy if increased ALT at least twice. Patients with normal ALT followed for at least 24 months: ALT tested every three months for first two years and every six months thereafter

[e] Positive = serum HBV DNA $\geq$ 30 000 copies/mLor IgM anti-HBc $\geq$0.200. All other cases considered negative

[f] 1 pg/mL = 3 x 10^5 copies/mL

[g] When the value for true negative = 0, this has been adjusted to 0.1 to allow a meaningful LR– to be calculated

[h] Measured at the end of follow-up (patient monitoring). HBeAg negative inactive patients (n = 22); HBeAg negative active patients (n = 26)

[i] Mean time between 2 measurements was 4 +/– 2 months. HBeAg negative inactive patients (n = 10); HBeAg negative active patients (n = 14)

Table 43 Study characteristics and results: Evaluating the predictive value of serum HBV DNA testing among patients undergoing antiviral therapy

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
Buti (2001) Spain Cohort study: not reported Prospective patient enrolment (consecutive not reported)	HBV DNA positive chronic hepatitis B patients Lamivudine 100 mg/day >1 year (range 12–24 months) (N = 35) **Characteristics at baseline:** Mean age = 46.1 (range 24–62 years); Serum ALT (mean 133 U/L, range 49–364 U/L); HBeAg +ve = 15 (25 M, 10 F)	Pre-treatment liver biopsy in 31 patients (details NR)	**Index test:** *Study entry* chemiluminescent bDNA assay (Quantiplex, Chiron) LLD = 7.0 x 10^5; *month 3* real-time PCR assay, LLD = approx. 1 x 10^3 genome copies/mL **Comparator**: HBsAg (Abbot-Auszyme Mc); HBeAg and anti-HBe detected by radioimmunoassay (Diasorin) **Reference standard**: *Maintained treatment response* defined as undetectable serum HBV DNA throughout therapy period (≤1 year); *Non-response* persistence of serum HBV DNA or reappearance after initial clearance	**Predictive ability of HBV DNA for maintained therapy response**: Sn 73%, Sp 88%, Acc 74%, PPV 95%	P2 Q3 *Applicability*: Limited Patient selection criteria unknown HBV DNA test outdated, LLD poor *Quality*: Poor Index test is also reference standard Inadequate data reporting
Lindh (2001) Denmark, France, Spain, Sweden, UK Patients sampled from RCT: 1987–1990	HBeAg positive chronic hepatitis B patients: Placebo followed by interferon n = 47 Interferon after prednisolone n = 51 (2 wks prednisolone at 0.6 mg/kg/d , then 1 week at 0.45 mg/kg/d, then 1 week at 0.25 mg/kg/d, with 2 weeks rest before interferon) (Interferon at 10 μ for 1 week induction, then 10 μ trice week for 11 weeks)	NR	**Index test**: Amplicor HBV monitor (Roche), linear detection range = 10^3 to 10^7 copies/mL Serum samples taken before prednisolone treatment, before interferon, at end of treatment and on follow-up (mean 204 days after interferon cessation) **Comparator**: HBeAg, anti-HBeAg by radio immunoassay or enzyme immunoassay. Indexed aminotransferase value AST or ALT (if no AST) in relation to ULN (Test details not reported) **Reference standard**: *Sustained response* defined as HBV DNA level <10^6 copies/mL at follow-up with at least 1.5 log reduction in viral load compared with pre-interferon treatment. *Non responders* were patients who did not fulfil these criteria	**Predictive ability of HBV DNA (log 8.7 copies/mL) for sustained therapy response**: Sn 60%, Sp 75%, PPV 67%, NPV 67%	P2 Q3 *Applicability*: Applicable HBV DNA test regimen *Quality*: Poor Index test part of reference standard

Study (year) Country Study design	Population (N)	Prior tests	Test characteristics	Results	Study quality
van der Eijk(2006) Netherlands, Germany, Belgium, United Kingdom, Denmark, France, Spain, Italy, Greece Retrospective analysis of data from prospective RCT[a]: not reported	Patients: aged 18–70 years; HbsAg positive ≥6 months; HBeAg positive and HBV DNA positive (hybridisation methodology); AST or ALT elevation (3 x in 3 months before study entry); liver biopsy evidence of chronic hepatitis (during 6 month period before study enrolment). Completion of study regimen and sufficient serum samples required (n = 133) **Characteristics (N = 133 at baseline)** Age (median): 34 (range 16–70); M 97,:F 36; ethnicity: Caucasian 80%, Asian 16%, other 5%; cirrhosis: 16%; HBV DNA (log copies/mL) (median): 8.7 (range 4.1–10.0); AST (ULN) (median): 1.5 (range 0.55–16.7) Genotype: A 46%; B 5%; C 12%; D 32%; other 6%	HbsAg; HBeAg; HBV DNA (hybridisation methodology); AST; ALT; liver biopsy; anti-HDV, HCV, or HIV; serum levels of haemoglobin, platelets and leukocytes	**Index test**: Real-time PCR assay (TaqMan, calibrated according to EUROHEP HBV DNA standards[b]) **Comparator**: HBeAg, AxSYM HBe 2.0 (Abbott) **Reference standard**: *Sustained response* at week 52[c]: defined as loss of HBeAg, (<0.27 PEI U/mL), with a decrease in HBV DNA <10^5 copies/mLat week 52. *Non responders* were patients who did not fulfil these criteria	**Predction of treatment response by HBV DNA testing (Log HBV DNA at baseline >8 & <1.0 log decrease) at week 12[d]**: NPV 100%, PPV 46%, Sn 100%[e], Sp 61%[f], FN rate 0, FP rate 39 **Predction of treatment response by HBV DNA testing (Log HBV DNA at baseline >9 & <2.5 log decrease) at week 12[d]**: NPV 100%, PPV 38%, Sn 100%[e], Sp 46%[f], FN rate 0, FP rate 54 **Prediction of treatment response by HBV DNA testing (Log HBV DNA at baseline >8 & <1.0 log decrease) at week 8[d]**: NPV 96%, PPV 43%, Sn 93%[e], Sp 60%[f], FN rate 7, FP rate 40 **Prediction of treatment response by HBV DNA testing (Log HBV DNA at baseline >9 & <1.5 log decrease) at week 8[d]**: NPV 100%, PPV 38%, Sn 100%[e], Sp 46%[f], FN rate 0, FP rate 54	P2 Q3 *Applicability*: Limited HBV DNA testing frequency different from Australian practice *Quality*: Poor Retrospective analysis; index test also part of reference standard
Zollner (2001) Germany Cohort study: not reported Prospective patient enrolment; consecutive enrolment	Treatment naïve chronic hepatitis B patients, HCV negative Lamivudine 100 /day Median duration = 12 months (range 6–31 months); (n = 28); M 25, F:3) Mean age = 45 years (range = 17–69) **Characteristics at baseline:** Mean log HBV DNA viral load = 6.71/mL (range = 3.47–7.69); HBeAg positive = 24 (86%)	Test before study entry NR	**Index test**: Real time PCR (light cycler DNA master SYBER GreenI Roche) detection limit 10^2 genomes/mL, dynamic range. ≤10^9 genomes/mL. (EUROPHEP standard calibrated). HBV DNA measured every 3 months **Comparator**: HBsAg, HBeAg, anti HBeAg, anti-HBsAg, anti-HBC — all by EIA (axysm, Abbot) **Reference standard**: *Seroconversion* HBeAg, anti HBeAg (axysm, Abbot); *Lamivudine resistance*: Definition not clear, possibly serum HBV DNA above detection limit and contiuoulsy detectable HBeAg	**Predictive value of HBV DNA (< log 2 copies/mL) for seroconversion[g,h]**: *3 months* PPV 100%, NPV 76%, *p* value NS; *6 months* PPV 50%, NPV 85%, *p* value NS; *9 months* PPV 50%, NPV 91%, *p* value NS; *12 months* PPV 54%, NPV 100%; *p* value 0.017; *15 months* PPV 54%, NPV 100%, *p* value 0.017 **Predictive value of HBV DNA (< log 2 copies/mL) for lamivudine resistance[g,h]**: *3 months* PPV 66%, NPV 35%, *p* value NS; *6 months* PPV 82%, NPV 47%, *p* value NS; *9 months* PPV 82%, NPV 47%, *p* value NS; *12 months* PPV 83%, NPV 50%; *p* value NS; *15 months* PPV 100%, NPV 53%, *p* value 0.023	P1 Q3 *Applicability*: Applicable *Quality*:Poor Index test and reference standard not independently interpreted Inadequate reporting of reference standard

Abbreviations: ALT, alanine aminotransferase; Acc, accuracy; AST, aspartate aminotransferase; EUROHEP, European concerted action on viral hepatitis; FN, false negative; FP, false positive; HAI, histological activity index; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus s antigen; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; LLD, lower limit of detection; NPV, negative predictive value; NR, not reported; NS, not significant; PCR, polymerase chain reaction; PPV, positive predictive value; RCT, randomised controlled trial; Sn, sensitivity; Sp, specificity

[a] Janssen et al (1999) *Hepatology* 30(1): 238-243

[b] Heerman et al (1999) *J Clin Microbiol* 37(1):68–73

[c] Definition of virologic response recommended by NIH workshop on chronic hepatitis B: Lok et al (2001) *Gastroenterology* 120(7): 1828–1853

[d] Study authors defined these test criteria as “abnormal test”, which is understood to mean “test negative” ie test predicts non-responders

[e] Proportion of sustained responders correctly predicted by the test

[f] Proportion of non-responders correctly predicted by the test

[g] PPV provided for the probability for the loss of e-antigen and maintenance of lamivudine sensitive HBV with copy numbers below the limit of detection, respectively

[h] NPV provided for the probability of continuously detectable e-antigen and emergence of lamivudine resistance with copy numbers above the limit of detection, respectively

[i] Treatment discontinued if both resistant HBV (not defined by study authors) with rising ALT levels detected.

Table 44 Characteristics and results of a systematic review evaluating the relationship between serum HBV DNA levels and clinical outcomes

Systematic review	Objective	Search strategy Inclusion/exclusion criteria (included studies)	Methodology	Results	Study quality Comment[a]
Mommeja-Marin (2003)	To investigate the relationship between viral load level or suppression and treatment response	Medline was search from 1996–2002 with the following search terms: hepatitis B; HBV; and HBV DNA **Inclusion criteria**: Published in English; reported HBV DNA levels; specified other end-points (ALT, serology, & histology); reported medians, means or data for estimation of median; prospective patient enrolment; >25 chronically infected patients; non-immuno-depressed; viral loads reported at start and/or end of therapy (including placebo, if applicable). Studies in children and cirrhotic patients also included. **Exclusion criteria**: Post-liver transplantation studies; HIV and/or hepatitis C co-infection. (n = 26)	Data abstraction by two separate (blinded) reviewers Viral loads reported as pg/mLwere converted to copies/mL[b] Some study characteristics summarised. Results not summarised for individual studies An assessment of study quality was not undertaken Sources of heterogeneity were explored by subgroup analysis Results presented as correlation co-efficients: exponential and linear regressions compared to determine best data fit; statistical significance explored by *p* value calculation	**Coefficient of correlation between HBV DNA and baseline HAI score**: *Overall* 0.78, p = 0.0001; *HBeAg positive subgroup* 0.61, p = 0.05; *HBeAg negative subgroup* 0.95, p = 0.0004; *Cirrhosis subgroup* ND; *Nucleoside analogue treated subgroup* NA; *interferon treated subgroup* NA; *Liquid hybridisation assay LOD corrected* 0.65, p = 0.002 **Coefficient of correlation between HBV DNA and final HAI score**: *Overall* 0.71, p = 0.003; *HBeAg positive subgroup* 0.71, p = 0.01; *HBeAg negative subgroup* 0.66, p = 0.3; *Cirrhosis subgroup* ND; *Nucleoside analogue treated subgroup* 0.76, p = 0.006; *interferon treated subgroup* 0.65, p = 0.08; *Liquid hybridisation assay LOD corrected* 0.67, p = 0.01 **Coefficient of correlation between HBV DNA and proportion of patients with normal ALT**: *Overall* 0.62, p = 0.004; *HBeAg positive subgroup* 0.66, p = 0.008; *HBeAg negative subgroup* 0.71, $p < 0.05$; *Cirrhosis subgroup* 0.8, p = 0.003; *Nucleoside analogue treated subgroup* 0.82, p = 0.001; *interferon treated subgroup* 0.55, p = 0.02; *Liquid hybridisation assay LOD corrected* 0.61, p = 0.005 **Coefficient of correlation between HBV DNA and incidence of HBeAg seroconversion**: *Overall* 0.72, p = 0.002; *HBeAg positive subgroup* NA; *HBeAg negative subgroup* NA; *Cirrhosis subgroup* ND; *Nucleoside analogue treated subgroup* 0.92, p = 0.001; *interferon treated subgroup* ND; *Liquid hybridisation assay LOD corrected* 0.55, p = 0.3 **Coefficient of correlation between HBV DNA and median change in HAI score**: *Overall* 0.96, $p = 10^{-6}$; *HBeAg positive subgroup* 0.98, $p = 6 \times 10^{-6}$; *HBeAg negative subgroup* ND; *Cirrhosis subgroup* ND; *Nucleoside analogue treated subgroup* 0.96, p = 0.003; *interferon treated subgroup* ND; *Liquid hybridisation assay LOD corrected* NA **Coefficient of correlation between HBV DNA and median change in proportion of patients with normal ALT**: *Overall* 0.5, p = 0.06; *HBeAg positive subgroup* ND; *HBeAg negative subgroup* ND; *Cirrhosis subgroup* ND; *Nucleoside analogue treated subgroup* 0.76, p = 0.003; *interferon treated subgroup* ND; *Liquid hybridisation assay LOD corrected* NA **Coefficient of correlation between HBV DNA and median change in incidence of HBeAg seroconversion**: *Overall* 0.72, p = 0.0002; *HBeAg positive subgroup* NA; *HBeAg negative subgroup* NA; *Cirrhosis subgroup* 0.87, p = 0.00005; *Nucleoside analogue treated subgroup* 0.7, p = 0.05; *interferon treated subgroup* ND; *Liquid hybridisation assay LOD corrected* NA	Medium quality: Heterogeneity explored by subgroup analyses No quality assessment reported for included studies Medium validity: Subgroup analysis not performed for PCR HBV DNA assays

Abbreviations: ALT, Alanine aminotransferase; HBeAg, hepatitis e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; LOD, lower limit of detection; NA, not applicable; ND, not done

[a] Systematic review appraised by applying the quality criteria described in **Appendix D**

[b] Conversion: 1 pg/mL = 3×10^5 copies/mL

Table 45 Characteristics and results of studies evaluating the relationship between serum HBV DNA levels and clinical outcomes

Study (year) Country Study design	Population (N)	Test Characteristics	Results	Study quality/Comment
REVEAL-HBV study group (results reported by Chen et al (2006) & Iloeje et al (2006) Taiwan Cohort study: 1991–1992 Prospective, non-consecutive patient enrolment (population based)	89,293 residents (30–65 years) in 7 townships invited to participate: 23,820 enrolled—4155 were HBsAg +ve: of these 3851 had serum samples frozen at enrolment tested for DNA 198 excluded (195 Anti-HCV seropositive, 3 lacked adequate sample for anti-HCV test) **Chen et al (2006):** final cohort N = 3653[a]; Average follow-up = 11.4 years; Males = 62%; Age >39 years = 67%; Never drank alcohol = 87%; HbeAg +ve = 15%; ALT ≥45 IU = 6%; No liver cirrhosis = 98% **Iloeje et al (2006):** a further 71 excluded from analysis (2 deaths within 6 mo of cohort entry—non-hepatic causes—69 diagnosed with cirrhosis within 6 mo of enrolment): final cohort N = 3582; Median follow-up = 11 years (range NR); Median age = 45; Males = 61.3%; Alcohol drinkers = 12%; HbeAg +ve = 15.2%; Median HBV DNA = 10^3 copies/mL (range NR); ALT ≥45 IU = 5.7%; deaths during follow-up 8.7%	**Index test**: PCR (COBAS Amplicor; Roche), lower limit of detection of 300 copies/mL (tested at baseline and every 6–12 months during follow-up examination) **Other tests**: HBsAG, HBeAg byradioimmunoassay,(Abbot); anti-HepC by radioimmunoassay (Abbott); ALT by serum chemistry auto-analyser (model 736; Hitachi) (tested at baseline and every 6–12 months during follow-up examination)	**Cumulative hepatocellular carcinoma incidence stratified by baseline HBV DNA Level (copies/mL):** *Undetectable (<300)* = 1.30% ; *serum HBV DNA (300–9.9 x 10^3)* = 1.37%; *serum HBV DNA (1.0– 9.9 x 10^4)* = 3.57%; serum *HBV DNA (1.0–9.9 x 10^5)* = 12.17%; *serum HBV DNA (≥1.0 x 10^6)* = 14.89% **HR[b] of hepatocellular carcinoma (95% CI) stratified by baseline HBV DNA level (copies/mL):** Undetectable (<300) = 1.0 (reference); *serum HBV DNA (300–9.9 x 10^3)* = 1.1 (0.5–2.3, *p* = 0.86); *serum HBV DNA (1.0 – 9.9 x 10^4)* = 2.3 (1.1–4.9, *p* = 0.02); *serum HBV DNA (1.0–9.9 x 10^5)* = 6.6 (3.3–13.1, *p* <0.001); *serum HBV DNA (≥1.0 x 10^6)* = 6.1 (2.9–12.7, *p* <0.001) **HR[b] of hepatocellular carcinoma (95% CI) by other variables**: HbeAg –ve = 1.0 (reference); HbeAg +ve = 2.6 (1.6–4.2, p<0.001) ALT (IU) <45 = 1.0 (reference); ALT ≥45 = 1.5 (1.1–2.1, *p* = 0.64) Liver cirrhosis[c] = 9.1 (5.9–13.9, p<0.001) **Cumulative cirrhosis[d] incidence stratified by baseline HBV DNA level (copies/mL):** *Undetectable (<300)* = 4.5%; *serum HBV DNA (300–9.9 x 10^3)* = 5.9%; *serum HBV DNA (1.0–9.9 x 10^4)* = 9.8%; *serum HBV DNA (1.0–9.9 x 10^5)* = 23.5%; *serum HBV DNA (≥1.0 x 10^6)* = 36.2% **RR[b] of cirrhosis (95% CI) stratified by baseline HBV DNA level (copies/mL):** *Undetectable (<300)* = 1.0 (reference); *serum HBV DNA (300–9.9 x 10^3)* = 1.4 (0.9–2.2, *p* value NR); *serum HBV DNA (1.0 – 9.9 x 10^4)* = 2.5 (1.6–3.8, *p* < 0.01); *serum HBV DNA (1.0–9.9 x 10^5)* = 5.6 (3.7–8.5, *p* < 0.01); *serum HBV DNA (≥1.0 x 10^6)* = 6.5 (4.1–10.2, *p* < 0.01) **RR[b] of cirrhosis (95% CI) by other variables:** HbeAg –ve = 1.0 (reference); HbeAg +ve = 1.7 (1.2–2.3, *p* < 0.01) ALT (IU) <45 = 1.0 (reference); ALT ≥ 45 = 1.5 (1.1–2.1, *p* < 0.05) **HR of hepatocellular carcinoma (95% CI)[e]**: *Serum HBV DNA (baseline < 10 000)* lifestyle adjusted 1.0, liver damage adjusted 1.0; *Serum HBV DNA (baseline 10 000–99 999, final <10 000))* lifestyle adjusted 1.6 (0.7–3.9), liver damage adjusted 1.3 (0.5–3.1); *Serum HBV DNA (baseline 10 000–99 999, final 10 000–99 999)* lifestyle	Prospective Potential for selection bias: Majority of invited individuals did not participate Non-consecutive enrolment Population considered applicable to Australian clinical practice

Study (year) Country Study design	Population (N)	Test Characteristics	Results	Study quality/Comment
			adjusted 0.5 (0.1–3.6), liver damage adjusted 0.4 (0.1–3.2); *Serum HBV DNA (baseline 10 000–99 999, final >100 000)* lifestyle adjusted 3.5 (1.4–9.2), liver damage adjusted 2.9 (1.0–9.8); *Serum HBV DNA (baseline >100 000, final <10 000)* lifestyle adjusted 3.8 (1.7–8.4), liver damage adjusted 1.9 (0.8–4.4); *Serum HBV DNA (baseline >100 000, final 10 000–99 999)* lifestyle adjusted 7.3 (3.5–15.3), liver damage adjusted 4.3 (2.0–9.3); *Serum HBV DNA (baseline >100 000, final >100 000)* lifestyle adjusted 10.0 (6.3–16.2), liver damage adjusted 5.3 (2.9–9.7)	
Harris (2003) China Cohort study: 1992–2000 Prospective, non-consecutive patient enrolment	HbsAg +ve, HBV DNA +ve (dot blot hybridisation), with 3 or more serum samples available for analysis , hepatocellular carcinoma-free at study entry (n = 114) (subjects selected from α-fetoprotein intervention arm of an early detection screening study)	**Index test**: Dot blot hybridisation, quantified by bio-imaging analyser system (Fuji medical systems), sensitivity limit = 3 x 10^5 virions/mL (= copies/mL?) **Other tests**: AFP by enzyme immunoassay	**RR of hepatocellular carcinoma death among patients with stable HBV DNA seroconversion (95% CI)**: 2.2 (0.9, 5.3) **RR of hepatocellular carcinoma death among patients with unstable HBV DNA seroconversion (95% CI)**: 1.6 (1.1, 7.4) **Adjusted[f] RR of hepatocellular carcinoma death in patients with HBV seroconversion (95% CI)**: 2.8 (1.1, 7.4) **RR of hepatocellular carcinoma death among patients with HBV DNA load of <1.8 virions/mL(95% CI)**: 0.51 (0.19, 1.4)	Prospective Potential for selection bias: non-consecutive patient selection Potential for spectrum bias: patients selected from screening study Treatment not applicable to Australian clinical practice HBV DNA test outdated
Ishikawa (2001) Japan Cohort study: NR Unclear patient enrolment (non-consecutive)	HbsAg +ve cirrhotic patients, excluded patients with non-viral cirrhosis (n = 65)	**Index test**: TMA and hybridisation protection assay (details NR), serum samples taken at time of first diagnosis of cirrhosis **Other tests**: HbsAg by enzyme immunoassay (Dainabot) HbeAg, anti-HBeAg, by enzyme immunoassay; anti-HBc by reversecell Ab kit (Yamanouchi), serum samples taken at time of first diagnosis of cirrhosis	**RR[g] of heptacellular carcinoma (95% CI)**: *ALT levels (≥100 IU/L)* 4.525 (1.202–17.030); *LDH levels (≥480 IU/L)* 2.880 (0.881–9.412); HBV DNA (≥3.7 LGE/mL) 7.712 (1.511–39.365)	Direction unclear Potential for selection bias: non-consecutive patient selection Reduced applicability to Australian clinical practice: less sensitive HBVDNA test

Study (year) Country Study design	Population (N)	Test Characteristics	Results	Study quality/Comment
Kubo (2000) Japan Cohort study: 1990–1998 Unclear patient enrolment (non-consecutive)	HBV DNA positive patients, negative for anti-HCV, who had curative[h] liver resection for hepatocellular carcinoma. (n = 40)	**Index test**: Branched DNA assay (Chiron Amplex) (Serum samples for test obtained before surgery) **Other tests**: Anti-HCV by ELISA; serum HCV RNA by PCR, HBeAg, anti-HBeAg, HBsAg,and anti-HBsAg by enzyme immunoassay (International Reagents Corp) (Serum samples for test obtained before surgery)	**Tumour free survival rate among patients with high (≥0.7 mEq/mL) HBV DNA levels after three years**: 0% **Tumour free survival rate among patients with low (<0.7 mEq/mL) HBV DNA levels after nine years**: 64% **RR of hepatocellular carcinoma recurrence among patients with high (≥0.7 mEq/mL) HBV DNA levels (95% CI)**: 5.13 (1.57, 16.67) **RR of hepatocellular carcinoma recurrence among patients woth positive surgical margins (95% CI)**: 2.14 (1.10, 6.80)	Potential selection bias: direction unclear, non-consecutive enrolment Applicability to Australian clinical practice potentially limited: less sensitive HBV DNA test
Kubo (2003) Japan Cohort study: 1989–2001 Unclear patient enrolment (non-consecutive)	HBV DNA positive patients, negative for anti-HCV, who had curative[h] liver resection for hepatocellular carcinoma. (n = 52)	**Index test**: TMA-HPA (Chugai diagnostics): sensitivity range = 3.7 –8.7 LGE/mL[i] Branched DNA assay (Quantiplex); detection limit 0.7 mEq/mL(7 x 10^5 copies/mL) (Serum samples for test obtained before surgery) **Other tests**: Anti-HCV by ELISA ; HBeAg, HBsAg by enzyme immunoassay (International Reagents Corp) (Serum samples for test obtained before surgery)	**Hepatocellular carcinoma recurrence in proportion of patients with low serum HBV DNA (<3.7 LGE/mL) vs proportion of patients high serum HBV DNA (≥3.7 LGE/mL)**: 14.3% (n = 2) vs 63.2 (n = 24) **RR of hepatocellular carcinoma recurrence among patients with high (≥3.7 LGE/mL) HBV DNA levels (95% CI)**: 6.58 (1.52, 28.57)	Potential selection bias: direction unclear, non-consecutive enrolment Applicability to Australian clinical practice potentially limited: less sensitive HBV DNA test
Mahmood (2005) Japan Cohort study: 1996–2003 Unclear patient enrolment	Patients diagnosed with HBV related cirrhosis by liver biopsy and/or peritoneoscopy. HbsAg positive, anti-hep C negative at study commencement. Cirrhotic patients with possible hepatocellular carcinoma association at the time of hepatocellular carcinoma diagnosis were excluded from (n = 91; 23 patients were treated using interferon[j])	**Index test**: Amplicor Monitor (Roche) **Other tests**: HBsAg, anti-HBsAg by chemiluminescent enzyme immunoassay (Fuji); HBeAg, anti-HBeAg by enzyme-linked immunosorbent assay (Abbot) ALT (test details NR)	**Logistic regression analysis of HBV DNA levels to predict hepatocellular carcinoma occurrence**: $p = 0.029$ **Logistic regression analysis of ALT levels to predict hepatocellular carcinoma occurrence**: $p = 0.062$ **OR of hepatocellular carcinoma occurrence by serum HBV DNA level (95% CI)**: 2.33 (1.1, 5.6)	Potential selection bias: direction unclear and enrolment NR HBV DNA test applicable to Australian clinical practice

Study (year) Country Study design	Population (N)	Test Characteristics	Results	Study quality/Comment
Okhubo(2002) Japan Cohort study: 1983–1998 Unclear patient enrolment	Patients (74) with HBV-associated hepatocellular carcinoma, HBsAg +ve and anti-HCV –ve. (selected from 404 patients identified with hepatocellular carcinoma histopathologically or clinically) Excluded: HBsAg –ve, anti-HCV +ve patients Observation began at time of hepatocellular carcinoma diagnosis and ended at death or end of Oct 1999 Patient Characteristics: Males = 74.3%, Female = 25.7%; Age range = 32–76 years; Cirrhosis = 87.8% **Hepatocellular carcinoma diagnosis**: Histopathology n = 20 Ultrasonography, CT and hepatic arteriography n = 54 **hepatocellular carcinoma treatment**: Surgical resection n = 13 Non-surgical[k] treatment n = 43 No treatment n = 18	**Index test**: Transcription-mediated amplification method, details NR, detection limit[l] = 3.7 LGE/mL(Serum sample taken at time of hepatocellular carcinoma diagnosis) **Other tests**: HBsAg, HBeAg, and anti-HbeAg assayed by commercially available radio-immunoassay kits (Dainabot); Anti-HCV, enzyme-linked immunoabsorbent assay (Ortho Diagnostics) ALT assay methods NR (Serum sample taken at time of hepatocellular carcinoma diagnosis)	**Median survival time (years)**: *Serum HBV DNA (<3.7 LGE/mL vs ≥3.7 LGE/mL)* 5.8 vs 1.2 (p = 0.0002); *Serum ALT levels (<30 IU/L vs ≥30 IU/L)* 4.9 vs 0.9 (p = 0.0008); *Clinical stage (I vs II or III)* 3.2 vs 0.9 (p = 0.0007); *Liver tumour (solitary vs multiple)* 4.3 vs 0.7 (p = 0.0002); tumour size (< 2cm vs ≥ 2 cm) 4.9 vs 1.0 (p = 0.0008)	Potential for selection bias: direction unclear Did not investigate whether hepatocellular carcinoma treatment impacted on survival Limited Applicability: HBV DNA test not applicable to Australian clinical practice

Study (year) Country Study design	Population (N)	Test Characteristics	Results	Study quality/Comment
Yuen (2004) China Cohort study: 2001–2003 Unclear patient enrolment	chronic hepatitis B patients undergoing liver biopsy; no antiviral therapy; HbsAg +ve ≥6 months ; excluded patients with concomitant liver diseases including hepatitis C or D, alcoholic liver disease, autoimmune hepatitis, Wilson's disease, primary biliary cirrhosis and drug induced hepatitis. (n = 94) [m]HBeAg +ve = 43 (46.9%); [m]HBeAg –ve = 51 (54.3%); [m]Median ALT level, U/L = 91 (range = 21–602); [m]Median HBV DNA level, copies/mL = 1.9×10^7 (range = <200–2.0×10^{13}); Median age, yr = 43.3 (range = 20–51); M = 74, F = 20	**Index test**: Cobas Amplicor, HBV Monitor test (Roche), lower limit of detection = 200 copies/mL (HBV DNA tested in serum samples taken 2 weeks before liver biopsy) **Other tests**: HBsAg, HBeAg, anti- HBeAg by microparticle enzyme immunoassay (Abbot) ALT test details NR Liver histology graded by criteria of Knodell et al (1981). Histology assessed blind to liver biochemistry and HBV DNA test results	**Correlation[n] of HBV DNA (Anti-HBeAg positive) with liver histology**: *Total HAI score* 0.37 ($p = 0.008$); *HAI-NI score* 0.31 ($p = 0.014$); *HAI-F score* 0.33 ($p = 0.017$) **Correlation[n] of ALT levels (Anti-HBeAg positive) with liver histology**: *Total HAI score* 0.31 ($p = 0.028$); *HAI-NI score* 0.35 ($p = 0.011$) **Correlation[n] of ALT levels (HbeAg positive) with liver histology**: *Total HAI score* 0.40 ($p = 0.009$); *HAI-NI score 0.43* ($p = 0.004$) **Relation between serum HBV DNA and median necroinflammatory score (range)**: *Serum HBV DNA (<10^5 copies/mL)* 2 (0–5); *serum HBV DNA (<10^6 copies/mL)* 3 (0–10); *serum HBV DNA (<10^7 copies/mL)* 2 (0–18) **Relation between serum HBV DNA proportion of patients with necroinflammatory score ≤ 3**: *Serum HBV DNA (<10^5 copies/mL)* n = 6 (85.7%); *serum HBV DNA (<10^6 copies/mL)* n = 9 (81.8%); *serum HBV DNA (<10^7 copies/mL)* n = 15 (57.7%) **Relation between serum HBV DNA and median fibrosis score (range)**: *Serum HBV DNA (<10^5 copies/mL)* 0 (0–4); *serum HBV DNA (<10^6 copies/mL)* 0 (0–4); *serum HBV DNA (<10^7 copies/mL)* 1 (0–4) **Relation between serum HBV DNA proportion of patients with fibrosis score = 0**: *Serum HBV DNA (<10^5 copies/mL)* n = 6 (85.7%); *serum HBV DNA (<10^6 copies/mL)* n = 7 (63.6%); *serum HBV DNA (<10^7 copies/mL)* n = 11 (42.3%)	Direction unclear, possibly prospective Potential selection bias – only included patients with liver biopsies Blinding between liver histology and test results Patient population considered applicable

Abbreviations: AFP, α-fetoprotein tests; ALT, alanine aminotransferase; CHB, chronic hepatitis B, CI, confidence interval; ELISA, enzyme linked immunoassay; F, female; HAI, histologic activity index; HAI-F, histologic activity index fibrosis; HAI-NI, histologic activity index necroinflammation; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid;. HCV, hepatitis C virus; HDV, hepatitis D virus; HR, Hazard ratio; U, international units; LDH, lactate dehydrogenase; LGE, logarithm of genome equivalent; M, male; NR, not reported; PCR, polymerase chain reaction; REVEAL-HBV, The Risk Evaluation of Viral Load and Associated Liver Disease/Cancer-in Hepatitis B Virus; RNA, ribonucleic acid; RR, relative risk; TMA, transcription mediated assay

[a] Chen et al (2006) examined the impact of changing HBV DNA level on hepatocellular carcinoma risk in a subset of these participants (n = 1619) who had serum HBV DNA level of ≥ 10 000 copies/mLat study entry. Follow-up serum sampleswere taken at the last follow-up examination or at the follow-up examination preceding the diagnosis of hepatocellular carcinoma

[b] Multivariate-adjusted hazard ratio and relative risk determined by Cox proportional hazard regression analysis

[c] Diagnosed with ultrasonography within 6 months of study entry

[d] Cirrhosis diagnosed by high resolution real time ultrasound, with a quantative scoring system from liver surface appearance, liver parenchymal texture, intrahepatic blood vessel size, splenic size. All patients had baseline screening ultrasound; ultrasounds also performed every 6–12 months at follow-up. Ultrasound blinded to HBV DNA testing

[e] The hazard ratios were adjusted for lifestyle (sex, age, cigarette smoking & alcohol consumption) and liver damage (HBeAg positive, liver cirrhosis, ALT level)

[f] The relative risk was adjusted for factors such as family history of hepatocellular carcinoma, acute hepatitis history, alcohol consumption, cigarette smoking

[g] Relative risks calculated by Cox regression analysis

[h] Curative resection defined as complete resection of all macroscopically evident tumour. Absence of tumour cells along the parenchymal transection line confirmed histologically

[i] Kubo et al (2003) reported that this range is equivalent to 5 x 10^3 to 5 x 10^8 copies/mL of serum HBV DNA
[j] Patients received either i)interferon-α or interferon-β at 6 MU/day for 4 weeks: proportions receiving interferon-α or interferon-β not reported
[k] Transcatheter arterial embolisation, and/or percutaneous ethanol injection
[l] Okhubo et al (2002) reported that the detection limit of the branched DNA assay (0.7 millieqiuvalent per mL) corresponds to 5.8 LGE/mL by the TMA method
[m] At time of liver biopsy
[n] Correlation between continuous variables with skewed distribution was tested by Spearman's rank correlation. Two tailed *p* value <0.05 considered statistically significant

Appendix D Quality criteria

Study design	Quality checklist
Systematic review	Was the research question specified?
	Was the search strategy documented and adequate?
	Were the inclusion and exclusion criteria specified, appropriate and applied in an unbiased way?
	Was a quality assessment of included studies undertaken?
	Were the methods of the study appraisal reproducible?
	Were the characteristics and results of the individual studies summarised?
	Were the methods for pooling the data appropriate?
	Were sources of heterogeneity explored?
	Was a summary of the main results and precision estimates reported?
Studies evaluating effectiveness of an intervention on health outcomes	
Randomised controlled trial	Were the inclusion and exclusion criteria specified?
	Was the assignment to the treatment groups really random?
	Was the treatment allocation concealed from those responsible for recruiting subjects?
	Was there sufficient description about the distribution of prognostic factors for the treatment and control groups?
	Were the groups comparable at baseline for these factors?
	Were outcome assessors blinded to the treatment allocation?
	Were the care providers blinded?
	Were the subjects blinded?
	Were all randomised participants included in the analysis?
	Was a point estimates and measure of variability reported for the primary outcome?
Cohort study	Were subjects selected prospectively or retrospectively?
	Was the intervention reliably ascertained?
	Was there sufficient description about how the subjects were selected for the new intervention and comparison groups?
	Was there sufficient description about the distribution of prognostic factors for the new intervention and comparison groups? Were the groups comparable for these factors?
	Did the study adequately control for potential confounding factors in the design or analysis?
	Was the measurement of outcomes unbiased (ie blinded to
	treatment group and comparable across groups)?
	Was follow-up long enough for outcomes to occur?
	What proportion of the cohort was followed-up and were there exclusions from the analysis?
	Were drop-out rates and reasons for drop-out similar across intervention and unexposed groups?
Case-control study	Was there sufficient description about how subjects were defined and selected for the case and control groups?
	Was the disease state of the cases reliably assessed and validated?
	Were the controls randomly selected from the source of population of the cases?
	Was there sufficient description about the distribution of prognostic factors for the case and control groups? Were the groups comparable for these factors?
	Did the study adequately control for potential confounding factors in the design or analysis?

	Was the new intervention and other exposures assessed in the same way for cases and controls and kept blinded to case/control status?
	How was the response rate defined?
	Were the non-response rates and reasons for non-response the same in both groups?
	Was an appropriate statistical analysis used?
	If matching was used, is it possible that cases and controls were matched on factors related to the intervention that would compromise the analysis because of over-matching?
Case series	Was the study based on a representative sample selected from a relevant population?
	Were the criteria for inclusion and exclusion explicit?
	Did all subjects enter the survey at a similar point in their disease progression?
	Was follow-up long enough for important events to occur?
	Were the techniques used adequately described?
	Were outcomes assessed using objective criteria or was blinding used?
	If comparisons of sub-series were made, was there sufficient description of the series and the distribution of prognostic factors?
Study of diagnostic accuracy	Was the patient spectrum representative of those who would undergo the test in practice?
	Were selection criteria clearly described?
	Is the reference standard likely to correctly classify the target condition?
	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?
	Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?
	Did patients receive the same reference standard regardless of the index test result?
	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?
	Was the execution of the index test described in sufficient detail to permit replication of the test?
	Was the execution of the reference standard described in sufficient detail to permit its replication?
	Were the index test results interpreted without knowledge of the results of the reference standard?
	Were the reference standard results interpreted without knowledge of the results of the index test?
	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?
	Were uninterpretable/ intermediate test results reported?
	Were withdrawals from the study explained?

Appendix E Literature search strategies

Table 46 PreMedline and Medline search for HBV DNA testing, 1966 to April Week 1 2006

Search history		References retrieved
1	exp hepatitis b/	31281
2	hepatitis b.ti,ab.	34703
3	or/1-2	45243
4	dna, viral/	60324
5	(dna or deoxyribonucleic acid).ti,ab.	484140
6	or/4-5	504997
7	3 and 6	8143
8	hepatitis b virus/	11728
9	hepatitis b surface antigens/	12831
10	(hepatitis b virus or hbv).ti,ab.	21810
11	or/8-10	31040
12	7 and 11	7566
13	dna, viral/an,bl	21699
14	(hbv dna or HBV DNA or hbv deoxyribonucleic acid).ti,ab.	3739
15	(hepatitis b virus dna or hepatitis b virus deoxyribonucleic acid).ti,ab.	1091
16	or/14-15	4309
17	16 and (test$ or assay$).ti,ab.	1509
18	exp nucleic acid amplification techniques/ or gene amplification/	204572
19	nucleic acid hybridisation/	44353
20	polymerase chain reaction/	138175
21	20 and exp reagent kits, diagnostic/	690
22	20 and polymerase chain reaction/mt	29441
23	(versant or amplification).ti,ab.	49574
24	(hybrid capture or digene).ti,ab.	543
25	cobas.ti,ab.	822
26	roche pcr.ti,ab.	21
27	or/13,17-19,21-26	277696
28	12 and 27	4213
29	viral load/	7811
30	(viral adj (burden or load or dynamics or decline)).ti,ab.	6395
31	virolog$.ti,ab.	12307
32	or/29-31	21752
33	28 and 32	453
34	28 and comparative study/	531
35	or/33-34	899

Table 47 EMBASE search for HBV DNA testing, 1980 to 2006 Week 15

Search history		References retrieved
1	exp hepatitis b/	21336
2	hepatitis b.ti,ab.	28437
3	or/1-2	34684
4	virus dna/	20854
5	(dna or deoxyribonucleic acid).ti,ab.	389398
6	or/4-5	394343
7	3 and 6	7146
8	hepatitis b virus/	16625
9	(hepatitis b virus or hbv).ti,ab.	18830
10	or/8-9	24029
11	7 and 10	6428
12	virus dna/ec	8744
13	exp endogenous compound/	3217
14	(hbv dna or HBV DNA or hbv deoxyribonucleic acid).ti,ab.	3373
15	(hepatitis b virus dna or hepatitis b virus deoxyribonucleic acid).ti,ab.	1033
16	or/14-15	3949
17	16 and (test$ or assay$).ti,ab.	1398
18	exp assay/	295483
19	exp gene amplification/	196233
20	branched dna signal amplification assay/	52
21	cobas amplicor monitor test/	1
22	cobas amplicor hepatitis b virus monitor test/	1
23	digene hybrid capture test/	1
24	(versant or amplification).ti,ab,dv.	44123
25	(hybrid capture or digene).ti,ab,dv.	584
26	(cobas or amplicor).ti,ab,dv.	1866
27	roche pcr.ti,ab,dv.	20
28	or/12-13,17-27	497118
29	11 and 28	3371
30	exp virus examination/	45861
31	dna determination/	28154
32	(viral adj (burden or load or dynamics or decline or quantification)).ti,ab.	6168
33	virolog$.ti,ab.	9729
34	(dna adj (level$ or value$ or quantitation or concentration$)).ti,ab.	4348
35	or/30-34	84103
36	29 and 35	1387

Table 48 Cochrane Library search for HBV DNA testing (Issue 2, 5 May 2006)

Search history		References retrieved
1	MeSH descriptor Hepatitis B explode all trees in MeSH products	1102
2	"hepatitis b" in All Fields in all products	2719
3	(#1 OR #2)	2719
4	MeSH descriptor DNA, Viral explode all trees in MeSH products	467
5	dna or "deoxyribonucleic acid" in All Fields in all products	2892
6	(#4 OR #5	2892
7	(#3 AND #6)	570
8	MeSH descriptor Hepatitis B virus explode all trees in MeSH products	365
9	MeSH descriptor Hepatitis B Surface Antigens explode all trees in MeSH products	395
10	"hepatitis b virus" or hbv in All Fields in all products	1229
11	(#8 OR #9 OR #10)	1389
12	(#7 AND #11)	502
13	"hbv dna" or "HBV DNA" or "hbv deoxyribonucleic acid" in All Fields in all products	422
14	"hepatitis b virus dna" or "hepatitis b virus deoxyribonucleic acid" in All Fields in all products	74
15	(#13 OR #14)	471
16	test* or assay* in All Fields in all products	100406
17	(#15 AND #16)	180
18	versant or amplification in All Fields in all products	405
19	"hybrid capture" or digene in All Fields in all products	50
20	cobas or amplicor or "roche pcr" in All Fields in all products	104
21	viral near (burden, load, dynamics, decline, quantification) in All Fields in all products	1075
22	dna near (level*, value*, quantification, concentration*) in All Fields in all products	311
23	virolog* or compar* in All Fields in all products	244191
24	MeSH descriptor Viral Load explode all trees in MeSH products	593
25	comparative study in All Fields in all products	118940
26	(#17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25)	244672
27	(#12 AND #26)	423

Secondary databases

Searches of the following secondary databases/sites were also performed:

- Agencia de Evaluación de Tecnologías Sanitarias, España (Spain)
- Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS) (Quebec, Canada)
- Agence Nationale d'Accreditation et d'Evaluation en Santé (France)
- Agency for Healthcare Research and Quality (USA)
- Alberta Heritage Foundation for Medical Research (Canada)
- Austrian Institute of Technology Assessment
- British Columbia Office of Health Technology Assessment (Canada)
- Blue Cross Blue Shield Association Technology Evaluation Center (USA)
- Canadian Agency for Drugs and Technologies in Health (CADTH) [formerly Coordinating Office for Health Technology Assessment (CCOHTA)]
- Catalan Agency for Health Technology Assessment (CAHTA)
- Centre for Health Program Evaluation (Monash University, Australia), Monash University Evidence Centre Reports (Australia)
- Centers for Medicare and Medicaid Services (USA)
- Centre for Reviews and Dissemination (University of York, UK)
- Current Controlled Trials metaRegister and ISRTCN register
- Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)
- Department of Health Publications (UK)
- ECRI (formerly Emergency Care Research Institute) (USA)
- Finnish Office for Health Technology Assessment (FinOHTA)
- German Institute for Medical Documentation and Information (DIMDI)
- Harvard Centre for Risk Analysis: Program on the Economic Evaluation of Health Technology (USA)
- Health Council of the Netherlands
- Health Economics Research Group (Brunel University, UK)

- Health Information Research Unit (HIRU) internal database (McMaster University, Canada)
- Health Technology Advisory Committee (Minnesota Department of Health, USA)
- Health Technology Assessment International Conference Proceedings
- Health Technology Board for Scotland (UK)
- Institute for Clinical Evaluative Sciences (Canada)
- Institute for Medical Technology Assessment Erasmus MC (Netherlands)
- International Network of Agencies for Health Technology Assessment (INAHTA)(Sweden)
- International Society of Technology Assessment in Health Care (Montreal, Canada)
- Israel Centre for Technological Assessment of Health Care Services
- Medion Database (Netherlands)
- Monash University Evidence Centre Reports (Australia)
- National Guidelines Clearinghouse (USA)
- National Health and Medical Research Council Australia publication list
- National Health Service Health Technology Assessment Programme (UK)
- National Information Center on Health Services Research and Health Care Technology (HSTAT database) (USA), National Library of Medicine Health Services/Technology Assessment Text (HSTAT) (USA)
- New Zealand Health Technology Assessment
- Scottish Intercollegiate Guidelines Network (SIGN) (Scotland)
- Swedish Council on Technology Assessment in Health Care (SBU)
- Swiss Centre for Technology Assessment (TA-SWISS)
- Swiss Network for Health Technology Assessment (SNHTA)

Appendix F List of studies excluded after retrieval

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Excluded: Case referent:

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Excluded: Case control

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Appendix G Examples of treatment studies

Treatment effectiveness of Lamivudine has been illustrated in studies by Dienstag et al 1999 and Liaw et al 2004. Patients were given Lamivudine for 52 weeks (Dienstag et al 1999) or were planned to undergo lamivudine treatment for a maximum of five years (Liaw et al 2004). The characteristics and outcomes of these studies are presented in Table 49.

The study by Diensteg at el (1999) included HBeAg positive patients with liver biopsy confirmation of chronic hepatitis B. In this prospective double-blind RCT, 52 weeks of lamivudine treatment resulted in an improvement in HAI score in a significantly greater proportion of lamivudine patients than placebo patients (52% and 23% respectively, $p<0.001$). Histological deterioration occurred in a significantly greater proportion of the placebo group compared with the lamivudine group (24% and 11% respectively, $p<0.001$). Significantly different favourable outcomes in terms if necroinflammatory activity and fibrosis were also found in the lamivudine group compared with the placebo group (see Table 49). Lamivudine treatment resulted in a significantly greater cumulative proportion of patients with sustained suppression of serum HBV DNA levels throughout the 52 week treatment period (44% for lamivudine and 16% for placebo $p<0.001$). Suppression of HBV DNA levels was paralleled with HBeAg seroconversion: by week 52, 17 per cent of the lamivudine group had seroconverted compared with 6 per cent of the placebo group ($p<0.04$). Serum ALT levels returned to normal in a significantly greater proportion of patients in the lamivudine group than in the placebo group (41% and 7% respectively, $p<0.001$). To illustrate that the favourable outcomes reported for the lamivudine group did not result from differences in baseline variables, odds ratios for the likelihood of histologic response and HBeAg seroconversion were calculated after adjusting for several variables (see Table 49). The odds ratios remained significantly different after adjustment.

The study by Diensteg et al (1999) indicates that 52 weeks of lamivudine therapy results in improved health outcomes for HBeAg positive chronic hepatitis B patients.

The study by Liaw et al (2004) included patients who were HBeAg negative or positive and had histologically confirmed cirrhosis or advanced liver disease (see Table 49). Liaw et al (2004) planned to give patients lamivudine (100 mg daily) up to a maximum of five years. Treatment was stopped for patients who reached a clinically confirmed endpoint (disease progression) or had HBeAg seroconversion during the double-blind phase. Patients who reached an endpoint were offered open label lamivudine for one year and patients who had seroconversion were offered further lamivudine therapy if they suffered serologic relapse. If the trial was terminated according to predefined criteria patients were offered open label treatment for one year. Overall, the median duration of treatment was 32.4 months (range 0–42 months): at study termination 71 per cent of patients underwent medication for at least 30 months.

Overall disease progression was less in the lamivudine group compared withthe placebo group: 7.8 per cent versus 17.7 per cent; with a hazard ratio of 0.45 (95% CI: [0.28, 0.73]) ($p<0.001$). This finding was paralleled by changes in Child-Pugh score: an increase was reported in 3.4 per cent of the lamivudine group versus 7.4 per cent of the placebo group and the hazard ratio for the lamivudine group was 0.45 (95% CI: [0.22, 0.90]) ($p<0.02$).

Similarly, a smaller proportion of patients in the lamivudine group developed hepatocellular carcinoma compared withthe placebo group: 3.9 per cent versus 7.4 per cent respectively; hazard ratio = 0.49 (95% CI: [0.25, 0.99]) (p<0.047).

Covariate modelling of time to disease progression was used to investigate factors other than treatment that significantly affected outcome: the incidence of disease progression in relation to several baseline variables was investigated. Liaw et al (2004) found that baseline serum HBV DNA levels (see Table 49) were not significant factors that affected outcome. The investigators did not report if baseline HBV DNA levels would make a difference depending on whether a patient is HBeAg positive or negative. Although serum samples were taken for HBV DNA level measurements throughout the study (Liaw et al 2004) the authors did not report these data. It was not possible to determine whether the degree of change from baseline HBV DNA levels is correlated with time to disease progression and so forth.

The results of this study indicate that lamivudine therapy can produce favourable outcomes in chronic hepatitis B and advanced liver disease patients who are either HBeAg negative or positive.

Table 49 Chronic hepatitis B treatment studies: efficacy of lamivudine therapy

Study	Study design	Inclusion/Exclusion criteria	Patient characteristics	Treatment	Outcomes	Study quality
Dienstag et al 1999	Multicentre (34, USA) prospective double-blind RCT (1995–1997)	**Inclusion criteria:** ≥18years of age; no prior therapy; HBsAg +ve (≥6 mo); HBeAg +ve (≥1 mo); ALT = 1.3 to 10 x ULN (≥3 mo); liver biopsy evidence of chronic hepatitis; detectable serum HBV DNA (by hybridisation assay: Abbot, detection limit = 1.6pg, 10^6 genomes per mL) **Exclusion criteria:** previous antiviral therapy for chronic hepatitis B; treatment with antiviral agents, immunomodulatory drugs or corticosteroids within 6 mo before the study; bilirubin >2.5 mg/dl; prothrombin time >3secs longer than normal; albumin level of <3.5g/dl; history of ascites, variceal haemorrhage or hepatic encephalopathy, coinfection with hep C virus, hep D or HIV; nuclear antibody titer of >1:160; a creatine level of >1.5 mg per dl.; haemoglobin level <11 g/dl and white cell count <3000 per cubic mL; neutrophil count of <1500 per cubic mL; platelet count <100,000 per cubic mL; confounding illness or other types of liver disease.	**Lamivudine group (n = 66):** Median age 40 yr (range = 18–73); 86% male; ethnicity: 59% white, 24% Asian, 15% black, 2% other/unknown; cirrhosis 6%, HAI score median 10 (range 0–15); serum HBV DNA (pg/mL) median 102.2, (range 0.8–1753); serum ALT (U/l), median 125 (range 46–401) **Placebo group (n = 71):** Median age 38 years (range 20–67); 80% male; ethnicity: white 56%, 17% Asian, 18% black, 9% other/unknown; cirrhosis 14%; HAI score, median 11 (range 3–17); serum HBV DNA (pg/mL), median 56.5 (range 0.8–653); serum ALT, median 135 (range 33–592)	100 mg lamivudine or placebo daily for 52 wks (monitored for additional 16wks post therapy)	**Histologic response:** HAI score reduction (decrease ≥2pts): lamivudine group = 52%, placebo group = 23% (p<0.001). Median HAI score reduction: lamivudine group = 3, placebo group = 0. Histologic worsening (HAI score increase ≥2pts): lamivudine group = 11%, placebo group = 24% (p<0.001) Necroinflammatory activity decrease: lamivudine group = 64%, placebo group = 34% (p = 0.001) Increased fibrosis: lamivudine group = 5%, placebo group = 20% (p = 0.01) **Virological response:** Lamivudine group: serum HBV DNA suppression (undetectable levels in nearly all treated) with median of 4 wks. Cumulative % of patients with undetectable HBV DNA at least once during treatment: lamivudine group = 98%, placebo group = 33%. Cumulative % with sustained suppression of serum HBV DNA levels through wk 52 lamivudine group = 44%, placebo group = 16% (p<0.001). Median level of suppression of serum HBV DNA: lamivudine group = 95 to 99% thru 52 wks of therapy. Placebo group = approx 40%. HBeAg seroconversion (by wk 52): lamivudine group = 17%, placebo group = 6% (p = 0.04). **Biochemical response:** Serum ALT returned to normal (52 wk): lamivudine group = 41%, placebo group = 7%. (p<0.001)	Randomised controlled trial Spectrum of disease in patients considered comparable to accuracy study populations Appropriate blinding: liver biopsy slides read blind of treatment group

Study	Study design	Inclusion/Exclusion criteria	Patient characteristics	Treatment	Outcomes	Study quality
					Effect of baseline variables: Likelihood of histologic response: lamivudine group, OR = 7.5 (95% CI, 2.7–20.9, *p*<0.001) HbeAg seroconversion; lamivudine group OR = 9.7 (95% CI, 1.7 – 56.1, *p* = 0.01) (Significantly higher than placebo group, after adjustment for baseline covariates of serum ALT, HBV DNA levels, HAI score, race, age, sex, weight, presence or absence of cirrhosis)	
Liaw et al 2004	Multicentre (9), double-blind RCT.	**Inclusion criteria:** >16years of age; HBsAg positive (≥6 mo); HBeAg –ve or +ve and detectable serum HBV DNA at screening; liver biopsy with Ishak fibrosis score ≥4 (0 = no fibrosis, 6 = cirrhosis) at screening/during previous 2 years. **Exclusion criteria:** hepatocellular carcinoma evidence; ALT >10x ULN; hepatic decompensation; autoimmune hepatitis; hepatitis C, D or HIV co-infection; serious concurrent illness; pancreatic amylase or lipase levels >2 xULN; elevated serum creatinine kinase; hemoglobin <8g/dl; treatment with immunomodulatory or chronic antiviral therapy within 6 mo before screening, treatment with any investigational drug within the 6 mo before screening; previous lamivudine treatment; pregnant women	**Lamivudine group (n = 436):** 85% male; 98% Asian; median age 43years (range 17–74); Child-Pugh score 5 = 78%, 6 = 17%, ≥ 7 = 5%. Ishak fibrosis score 4 = 40%, 5 = 29%, 6 = 31%. HBV DNA mEq/mLMedian 11.7, range <0.7–109,800. HBV DNA ≥ 0.7 mEq/mL– 79% HbeAg +ve = 58% ALT U/l – median = 70, range = 14–959. ALT >1 ULN, 78%. **Placebo group (n = 215):** 85% male; 98% Asian; median age 44 years (range 22–71), Child-Pugh score 5 = 73%, 6 = 19%, ≥ 7 = 8%; Ishak fibrosis score 4 = 35%, 5 = 26%, 6 = 39%; median HBV DNA (mEq/mL) 21.5, (range <0.7–4234). HBV DNA ≥ 0.7 mEq/mL = 81%; HbeAg +ve = 58%; ALT U/l, median = 68 (range = 7–821) ALT >1 ULN = 80%.	Lamivudine 100 mg daily. Double blind phase terminated at 2nd interim analysis (24mo) as results reached predefined boundary for efficacy Median duration of treatment was 32.4 mo (range, 0–42 mo), 71% patients had received study medication for at least 30mo when study terminated	**Overall disease progression:** Lamivudine group = 7.8% Placebo group = 17.7% Lamivudine vs placebo group: hazard ratio = 0.45 (95% CI, 0.28–0.73) (*p*<0.001) **Increase in Child-Pugh score:** Lamivudine group = 3.4% Placebo group = 8.8%, Lamivudine vs placebo group: hazard ratio = 0.45 (95% CI, 0.22–0.90) (*p*<0.02). **Hepatocellular carcinoma:** Lamivudine group = 3.9% Placebo group = 7.4% Lamivudine vs placebo group: hazard ratio = 0.49 (95% CI, 0.25–0.99) (*p*<0.047) (Hazard ratios derived from Cox Model adjusted for country, sex, baseline ALT, CP score and Ishak fibrosis score.) **Baseline HBV DNA & Incidence of disease progression** Lamivudine Group: Undetectable: 4% 0.7–10 meq/mL: 11% >10–100 meq/mL:6% >100 meq/mL: 8% Placebo group	Randomised controlled trial Spectrum of disease in patients considered comparable to accuracy study populations Appropriate blinding: liver biopsy slides read blind of treatment group

Study	Study design	Inclusion/Exclusion criteria	Patient characteristics	Treatment	Outcomes	Study quality
					Undetectable: 22% 0.7–10 meq/mL: 18% >10–100 meq/mL: 16% >100 meq/mL: 16%	

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid;. IU, international units; NR, not reported; PCR, polymerase chain reaction

Appendix H Major capital equipment cost

There is probably no need for the pathology labs to finance major capital equipment in addition to already their existing equipment or outside a reagent rental agreement with the HBV DNA test manufacturer(s).

For illustration purposes, the costs presented here describe the situation that a pathology lab would need to purchase major equipment to process a HBV DNA sample:

To calculate major capital cost, a linear depreciation over an estimated equipment lifespan of four years, with a residual value of zero, has been applied. Maintenance costs of 10 per cent were considered for three years as well as a one-year warranty period. Opportunity costs were considered as interest to be paid for the undepreciated investment costs and maintenance costs (8.2%, based on data provided by Medfin Finance, Sydney, November 2006). A discount rate of 5 per cent per annum was applied to the undepreciated investment costs, maintenance costs, opportunity costs, as well as a returned benefit in terms of tests performed.

Based on the described calculation, capital costs amount to $20 per HBV DNA test, if 20 tests are performed per week (see Table 50).[10]

[10] The capital costs would be $13 per test where 30 tests were performed each week and $10 per test where 40 tests were performed per week.

Table 50 Calculation of capital costs per HBV DNA test

	Life cycle year			
	1	**2**	**3**	**4**
Investment				
Value of investment	$55,000	$41,250	$27,500	$13,750
Depreciation, per year[a]	$13,750	$13,750	$13,750	$13,750
Maintenance costs, per year[b]	$0	$5000	$5000	$5000
Interest costs of investment and maintenance[c]	$4510	$3793	$2665	$1538
Total costs per year	$18,260	$22,543	$21,415	$20,288
Present value of costs[d]	$17,384	$20,446	$18,503	$16,697
Total present value of costs	$73,029			
Return on investment				
Number of procedures	1040	1040	1040	1040
Present value of procedures[e]	990	943	899	856
Total present value of procedures	3,688			
Present capital costs per procedure	**$20**			

[a] Linear depreciation, no residual value, lifetime four years
[b] First year: warranty period, no maintenance costs
[c] Calculated by considering an interest rate of 8.2% for purchase and maintenance costs
[d] Discounted at 8.2% per annum to reflect current value of investment
[e] Discounted at 5% to reflect current value of procedures

Appendix I Decision tree

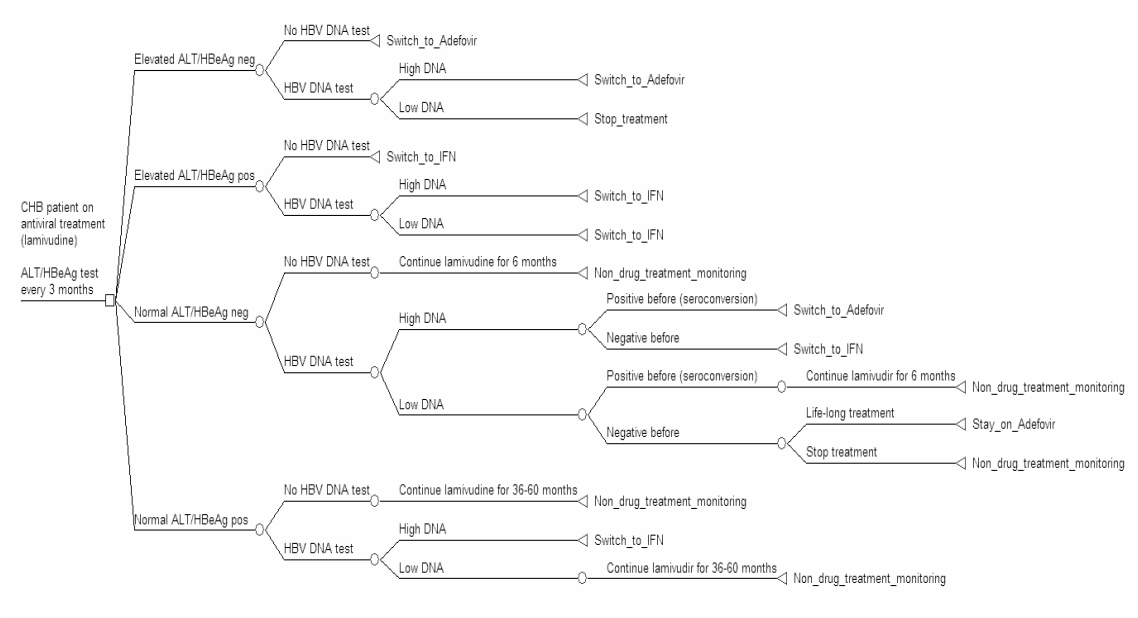

Figure 14 Decision-tree model for monitoring of patients on lamivudine treatment

Appendix J Cost for other healthcare funders

The following table presents the costs for other health care funders (laboratories) in case the HBV DNA test would be listed on the MBS. The costs were calculated by considering the number of tests and the capital costs for each test, and shows that the overall impact is expected to be close to $400,000 per year.

Table 51 Aggregated financial impact of HBV DNA test funding to other healthcare funders

		Year 1	Year 2	Year 3	Reference
Initial testing					
A	Number of incident cases	6475	6475	6475	Figure 11
B	Number of HBV DNA tests per year (initial assessments)	6475	6475	6475	One test during initial assessment
Monitoring antiviral treatment					
C	Number of patients on antiviral treatment under PBS and RPBS	1107	1265	1423	Figure 12
D	Number of HBV DNA tests/year for patients on antiviral treatment	4428	5060	5692	Four tests a year D = C * 4
E	Number of patients receiving interferon treatment under PBS and RPBS	74	65	58	Figure 13
F	Number of HBV DNA tests/year for patients receiving interferon treatment	221	196	174	Three tests during 12 months treatment F = E *3
Monitoring patients not on antiviral treatment					
G	Number of patients on non-drug treatment and showing elevated ALT = Number of tests	8214	8214	8214	Table 39 All patients showing elevated ALT
Totals					
H	Total number of HBV DNA tests	19,338	19,945	20,555	H = B + D + F + G
I	Cost per HBV DNA test	$20	$20	$20	Table 50
J	**Annual costs HBV DNA test**	**$382,952**	**$394,969**	**$407,041**	**J = H * I**

Abbreviations

AASLD	American Association for the Study of Liver Disease
ADV	adefovir dipivoxil
AFP	α-fetoprotein tests
AHMAC	Australian Health Ministers' Advisory Council
ALT	alanine aminotransferase
ALTi	indexed alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
AUD	Australian dollar
CHB	chronic hepatitis B
CI	confidence interval
CT	computed tomography
DNA	deoxyribonucleic acid
DOR	diagnostic odds ratio
ELISA	enzyme-linked immunosorbent assay
EUROHEP	European Concerted Action on Viral Hepatitis
FN	false negative
FP	false positive
HAI	histological activity index
HAI-fib	histological activity index fibrosis score
HAI-Infl	histolofical activity index (necro)inflammatory score
HBeAg	hepatitis B envelope antigen
HBsAg	hepatitis B surface antigen
HCC	hepatocellular carcinoma
HBV	hepatitis B virus

HCV	hepatitis C virus
HDV	hepatitis D virus
HIV	human immunodefiency virus
HR	hazard ratio
IgM	immunoglobulin
INF	interferon
INTREPED	interferon treatment with or without prednisolone priming
IU	international units
LAM	lamivudine
LDH	lactate dehydrogenase
LGE	logarithm of gene equivalent
LLD	lower limit of detection
LR	likelihood ratio
MBS	Medicare Benefits Schedule
MSAC	Medical Services Advisory Committee
NHMRC	National Health and Medical Research Council
NPV	negative predictive value
NA	not applicable
ND	not done
NR	not reported
NS	not significant
OR	odds ratio
PBS	Pharmaceutical Benefits Scheme
PCR	polymerase chain reaction
PPICO	population, prior tests, intervention, comparator, outcomes
PPV	positive predictive value
QUOROM	quality of reporting of meta-analyses

RCT	randomised controlled trial
REVEAL-HBV	The risk evaluation of viral load and associated liver disease/cancer in hepatitis B virus
RNA	ribonucleic acid
ROC	reciever operating characteristic
RPBS	Repatriation Pharmaceutical Benefits Scheme
RR	relative risk
SD	standard deviation
Sn	sensitivity
Sp	specificity
TGA	Therapeutic Goods Administration
TMA-HPA	Transcription-mediated amplification and hybridisation protection assay
ULN	upper limit of normal
US	ultrasound
WHO	World Health Organization

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