

Hepatitis B (*Hepatitis B virus*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *hepatitis B virus*.

Authorisation: PHLN

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Hepatitis B

1.1.1 Definitive Criteria – Recent hepatitis B

1. Detection of *hepatitis B* surface antigen (HBsAg) or hepatitis B virus (HBV) DNA in blood from a patient < 6 months of age
OR
2. Detection of HBsAg in blood from a patient shown to be HBsAg negative within the last 6 months
OR
3. Detection of HBV DNA in blood from a patient shown to be HBsAg and HBV DNA negative within the last 6 months
OR
4. Detection of HBsAg and IgM antibody to hepatitis B core antigen (IgM antiHBc) in blood in the absence of prior evidence of HBV infection

1.1.2 Definitive Criteria – Chronic hepatitis B

1. Detection of HBsAg or HBV DNA in a patient shown to be HBsAg or HBV DNA positive in blood at least 6 months previously
OR
2. Detection of HBsAg or HBV DNA in a patient shown to be IgM antiHBc negative

1.1.3 Definitive Criteria – Unspecified hepatitis B

1. Detection of HBsAg in blood
OR
2. Detection of HBV DNA in blood
AND
3. Does not meet the criteria for recent or chronic hepatitis B

2 Introduction

Hepatitis B virus attacks the liver and can cause life-threatening acute and chronic disease. The average incubation period is 90 days but HBV may be detected 30 to 60 days after infection and persist for a variable period of time. Worldwide, approximately two billion people have been infected and about 350 million have chronic infections. The majority of these people reside in the Asian region where infection in early childhood is commonplace and 8-10% of the adult population has chronic HBV infection. Approximately 25% of those with chronic HBV infection will later die from the chronic liver disease complications of cirrhosis and liver cancer.¹

The virus is transmitted predominantly through contact with the blood, semen or vaginal fluid of an infected person and is more infectious than hepatitis C virus and HIV. The common modes of transmission include perinatal, sexual contact or exposure to infected blood by transfusion or unsafe injection practices. HBV infection, however, is preventable due to the availability of a safe and effective (up to 95% protective) vaccine since 1982 and can be used with hyperimmune globulin for post-exposure prophylaxis. The likelihood that an HBV infection will become chronic depends upon the age at which a person becomes infected, with young children being the most likely to develop chronic infections. About 90% of infants infected during the first year of life develop chronic infections compared to 30% to 50% of children infected between one to four years of age, and 5 to 10% of healthy adults. All infants should receive the hepatitis B vaccine, as should higher risk groups such as injecting drug users, those at occupational risk of HBV infection and household contacts of HBV infected persons. The World Health Assembly passed a resolution in 1992 to recommend global vaccination against hepatitis B.

There is no specific treatment for acute hepatitis B. Chronic hepatitis B can be treated with drugs, including interferon and directly acting anti-viral agents, which can reduce the progression of chronic liver disease in some patients. HBV infection and hepatocellular carcinoma is a leading cause of liver transplantation in developed countries.

Hepatitis B virus is a partially double-stranded circular DNA virus. The virus consists of a core capsid which contains viral DNA surrounded by an envelope containing HBsAg. Both whole, intact virions and incomplete virus particles, consisting entirely of HBsAg, are produced during replication of HBV. The HBsAg particles vary greatly in morphology and are found in high concentrations in blood during early acute infection and continue to be produced in chronic disease. Other detectable blood markers of active HBV infection include HBV DNA and hepatitis B 'e' antigen (HBeAg), but not hepatitis B core antigen which is not released in the free form from the infected hepatocyte. An individual positive for HBsAg or HBV DNA is considered to be infected with HBV and the presence of HBeAg indicates the individual is of higher infectivity. Other HBV markers which can be used diagnostically to monitor an HBV infection include IgM antibody to hepatitis B core antigen (IgM anti-HBc), total antibody to hepatitis B core antigen (anti-HBc), antibody to HBeAg (anti-HBe), and antibody to HBsAg (anti-HBs). Seroconversion from HBeAg to anti-HBe correlates with reduced infectivity.

3 Tests

3.1 Detection of HBsAg

3.1.1 Qualitative HBsAg detection

HBsAg is the most commonly used marker of infection for diagnostic HBV screening and indicates active infection, acute or chronic. In newly infected persons, HBsAg is the only serologic marker detected during the first 3 to 5 weeks after infection. The average time from exposure to detection of HBsAg is 30 days (range: 6 to 60 days) and it is detectable before the onset of hepatitis. Resolution of acute infection is marked by loss of HBsAg and the appearance of anti-HBs. If the infection becomes chronic the HBsAg will persist usually for life if untreated. Spontaneous loss of HBsAg occurs in only a minority of HBV carriers, after HBeAg seroconversion. HBsAg does not cross the placenta and detection in the neonate usually indicates vertical transmission.

Several simple screening tests have been developed including agglutination, immunofiltration and immunochromatographic membrane tests. In general, these simple tests are most suitable for use in laboratories that have limited facilities or process low numbers of specimens daily.² The most widely used HBsAg screening tests worldwide are EIAs as they are the most appropriate for screening large numbers of specimens on a daily basis.³

The EIA system for HBsAg detection is similar to EIA formats for antibody detection but in this case an HBsAg-specific monoclonal antibody is immobilised onto the solid phase. This antibody is directed against the main “a” antigenic determinant of the HBsAg. The serum or plasma to be analysed is added to the well and any HBsAg present is captured to form an antibody-antigen complex. After washing another HBsAg-specific antibody labelled with an enzyme, or in some cases a co-enzyme, is added which binds to form an antibody-HBsAg-antibody/enzyme conjugate complex. A substrate solution is added which produces a colour change proportional to the amount of bound enzyme. Thus samples which do not contain HBsAg will not form a complex and therefore no colour reaction will take place. Sample wells that do contain HBsAg will show a colour change corresponding to the number of individual complexes formed. The colour produced is measured on a spectrophotometer to give a signal/cut off (S/CO) ratio by comparison with assay controls.

To confirm positive results in the HBsAg EIA, a neutralization test is usually carried out. A reagent containing anti-HBs is first added to an aliquot of the positive specimen. Two EIA tests, as described above, are then carried out contemporaneously, one with the specimen only and one with the combined specimen/anti-HBs. The results obtained are then compared to determine if a reduction in reading is observed in the neutralized sample and thus confirmation of the initial positive result. Some laboratories employ one of the simple HBsAg tests for confirmation if the screening HBsAg EIA S/CO ratio is high.

The sensitivity of the HBsAg EIA is reported to be < 1ng/mL and specificity is very high (>99%).⁴ False positivity can occur if blood sampling occurs soon after HBV vaccination⁵ and false negative results have been reported for HBsAg mutant viruses⁶ as the immunodominant “a” determinant is under anti-HBs selection pressure.

3.1.2 Quantitative HBsAg detection

Quantitative EIA detection of HBsAg has recently been introduced. A fall in the HBsAg load will occur before HBsAg seroconversion. There is an emerging role for quantitative measurement of HBsAg to monitor the virological response to HBV antiviral therapy.⁷

3.2 Detection of IgM antibody to hepatitis B core antigen

IgM anti-HBc is detected at the onset of acute hepatitis B and persists for up to 6 months if the infection resolves. In patients with chronic HBV infection, IgM anti-HBc can persist during viral replication at low levels that typically are not detectable by the EIA assays. However, persons with exacerbations of chronic infection can test positive for IgM anti-HBc. Because the positive predictive value of this test is low in asymptomatic persons, IgM anti-HBc testing for diagnosis of acute hepatitis B is most reliable in persons with clinical evidence of acute hepatitis or an epidemiologic link to a person with HBV infection.⁸ The commercial EIA tests use an antibody capture method where anti-human IgM is fixed to the solid phase. After adding the patient sample and washing an hepatitis B core antigen conjugate is added which binds to the captured IgM anti-HBc. The optical density ratio of the conjugate reaction is proportional to the amount of IgM anti-HBc in the blood sample.

3.3 Other hepatitis B serological markers

Other HBV markers which can be used diagnostically to monitor an HBV infection include HBeAg, total anti-HBc, anti-HBe and anti-HBs which can all be detected by EIA commercial tests. Serologic assays are not available for HBcAg, because no free HBcAg circulates in blood.

HBeAg can be detected in the serum of persons with acute or chronic HBV infection. The presence of HBeAg indicates active disease and that the individual is of higher infectivity due to high HBV DNA levels (10^6 to 10^{10} IU/mL). In an acute infection detection of anti-HBe suggests that the infected person is progressing towards resolving their infection. In HBV carriers seroconversion to anti-HBe usually correlates with reduced infectivity due to a reduction in the HBV DNA level, but may be due to the presence of HBV mutants in the pre-core or basal core promoter gene regions. With these mutants, despite the lack of HBeAg and the presence of anti-HBe, the HBV infection may be active with high HBV DNA levels.

In persons who recover from HBV infection, HBsAg and HBV DNA usually are eliminated from the blood, and anti-HBs appears. Individuals who have seroconverted from HBsAg to anti-HBs are usually immune to further HBV infection.

Total anti-HBc is usually detected for life after HBV infection. In certain persons, total anti-HBc is the only detectable HBV serologic marker. Isolated anti-HBc positivity can represent resolved HBV infection where the anti-HBs levels have waned, chronic infection in which circulating HBsAg is not detectable by commercial serology, or false-positive reaction.

3.4 Detection of hepatitis B DNA

HBV DNA can be quantified by means of target amplification techniques (e.g. PCR) or signal amplification techniques (e.g. branched DNA assay) which may also be coupled with an automated extraction platform. The PCR-based assays involve extraction of the viral DNA, then amplification and quantification. Signal amplification assays quantify the level of HBV DNA and require no purification step. The PCR-based assays for HBV DNA detection have the largest dynamic range of quantification (up to 8-9 log₁₀) and are the most sensitive tests (sensitivity from 5-10 IU/mL). Originally, the HBV DNA quantitative units of copies/mL used in the various assays did not represent the same amount of

HBV DNA in a clinical sample until the World Health Organization established an international standard of HBV DNA quantification called international units (IU). The WHO International Standard for HBV DNA consists of a dilution of the Eurohep standard reference 1, genotype A, HBsAg subtype adw assigned a potency of 10^6 IU/mL.⁹ The conversion factor is approximately 5–6 copies/mL = 1 IU/mL, depending on the assay. This allows recommendations and guidelines from clinical trials to be derived and applied in clinical practice with any of the HBV DNA assays.

HBV DNA can be detected in the serum of an infected person 10 to 20 days before detection of HBsAg and has recently been introduced into routine blood donation screening in Australia to improve detection of HBV during the serological window period. It is important to note that not all HBsAg positive carriers will have detectable HBV DNA and, rarely, HBV DNA may be detected in HBsAg-negative individuals with chronic HBV infection (so called occult HBV infection). The HBV DNA level is highly correlated with infectivity. Previously HBeAg was used to signify a high replicative state with higher infectivity but now HBV DNA load assays can measure this directly. Following HBeAg seroconversion the HBV DNA level usually falls and may become undetectable. A minority of these HBeAg seroconverted carriers will subsequently spontaneously lose detectable HBsAg (0.5% per year). The HBV genome still persists in the form of covalently closed circular DNA in the infected liver cells in a proportion of these people, as evidenced by the reappearance of HBsAg and HBV DNA in some of those who later undergo immunosuppressing therapies.

HBV viral load monitoring has been shown to correlate with the risk of HBV carriers developing cirrhosis and hepatocellular cancer. Those with viral loads $> 10^4$ copies/mL show a significantly increased risk of both cirrhosis and liver cancer.^{10,11} It is also useful for the pre-treatment evaluation and assessment of virological response to therapy.

Transient DNA detection from neonatal blood may occur soon after birth from an infected mother which does not always indicate infection of the neonate.

3.5 Hepatitis B mutations

3.5.1 HBeAg expression mutants

HBeAg is HBsAg with an extra 29 amino acids that is important for the HBV life cycle. Two major groups of mutations reduce or block HBeAg expression; a single base mutation at the end of the precore gene produces a stop codon blocking HBeAg production, and a second group of mutations affect the basal core promoter resulting in decreased HBeAg mRNA production and hence decreased HBeAg levels. These mutations may be associated with enhanced HBV replication, are often genotype-specific and can be detected by molecular means in reference laboratories.

3.5.2 Envelope gene mutants

Point mutations leading to amino acid substitutions in the antigenic determinant domain of HBsAg may result in immune escape from anti-HBs which can lead to breakthrough HBV infection following vaccination and/or HBV immune globulin prophylaxis. These mutations can be detected by molecular means in reference laboratories.

3.5.3 Antiviral resistance mutants

The main treatment modalities for HBV are pegylated interferon- α , as used to treat hepatitis C, and nucleoside and nucleotide analogue HBV polymerase inhibitors, many of which were developed for management of HIV infection. Response to interferon therapy is dependent on the HBV viral load,

the alanine aminotransferase (ALT) level, and the HBV genotype but there is no available genotypic interferon resistance test.

A major problem encountered with the use of antiviral drug therapy is genotypic resistance. The earliest indication for the emergence of drug-resistant HBV is the detection of resistance mutations in the HBV polymerase gene. Subsequently a rise of ≥ 1 logIU/mL in the HBV DNA level occurs which is then followed by biochemical breakthrough as evidenced by the development or worsening of hepatitis, indicated by a rising ALT level. Lamivudine was the first nucleoside analogue used to treat HBV but development of resistance was a major problem, becoming more likely the longer the drug was used. Entecavir and tenofovir have increased potency and a higher genetic barrier to mutational resistance and are now the first-line agents of choice. The specific mutations resulting in resistance to the DNA polymerase inhibitors have been defined¹² and can be detected by DNA polymerase sequencing and subsequent amino acid mapping. This molecular antiviral drug resistance testing is usually only available from reference laboratories.

3.6 HBV genotype detection

The eight genotypes of HBV have a distinct geographic distribution. Genotyping can be performed by genetic sequencing of the whole HBV genome or the S gene, by PCR-based methods and by commercial probe hybridisation methods.¹³ There is some HBV genotype variation in the propensity for more severe liver disease or liver cancer (genotype A and C respectively) and in the differential response to interferon therapy (genotype A, B more responsive than C, D),¹⁴ however, HBV genotyping is not routinely available.

3.7 Quality Assurance

Suitable and Unsuitable specimens

HBV EIA antigen and antibody testing can be performed on individual patient serum and plasma and HBV DNA testing can be performed on plasma. Samples for EIA may be stored on or off the clot or RBCs for up to 7 days or more at 2-8°C but samples for HBV DNA need to be separated from the clot within hours. The tests are not validated for cadaveric samples or for pooled specimens.

Internal QC

As the HBV EIA and HBV DNA tests are available commercially the manufacturer's instructions should be followed. Positive and negative controls are run every 24 hr on automated EIA machines and with each batch for plate EIAs and HBV DNA tests

External QC Programmes

RCPA QAP – HBV antibody, HBV DNA qualitative and quantitative
QCMD – hepatitis B genotyping

4 Nomenclature

- The international nomenclature for the organism is Hepadnaviridae, hepatitis B virus.

4.1 Laboratory Nomenclature for National Database Dictionary

Finding	SNOMED ConceptID
Type B viral hepatitis	66071002
Acute type B viral hepatitis	76795007
Chronic type B viral hepatitis	61977001

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