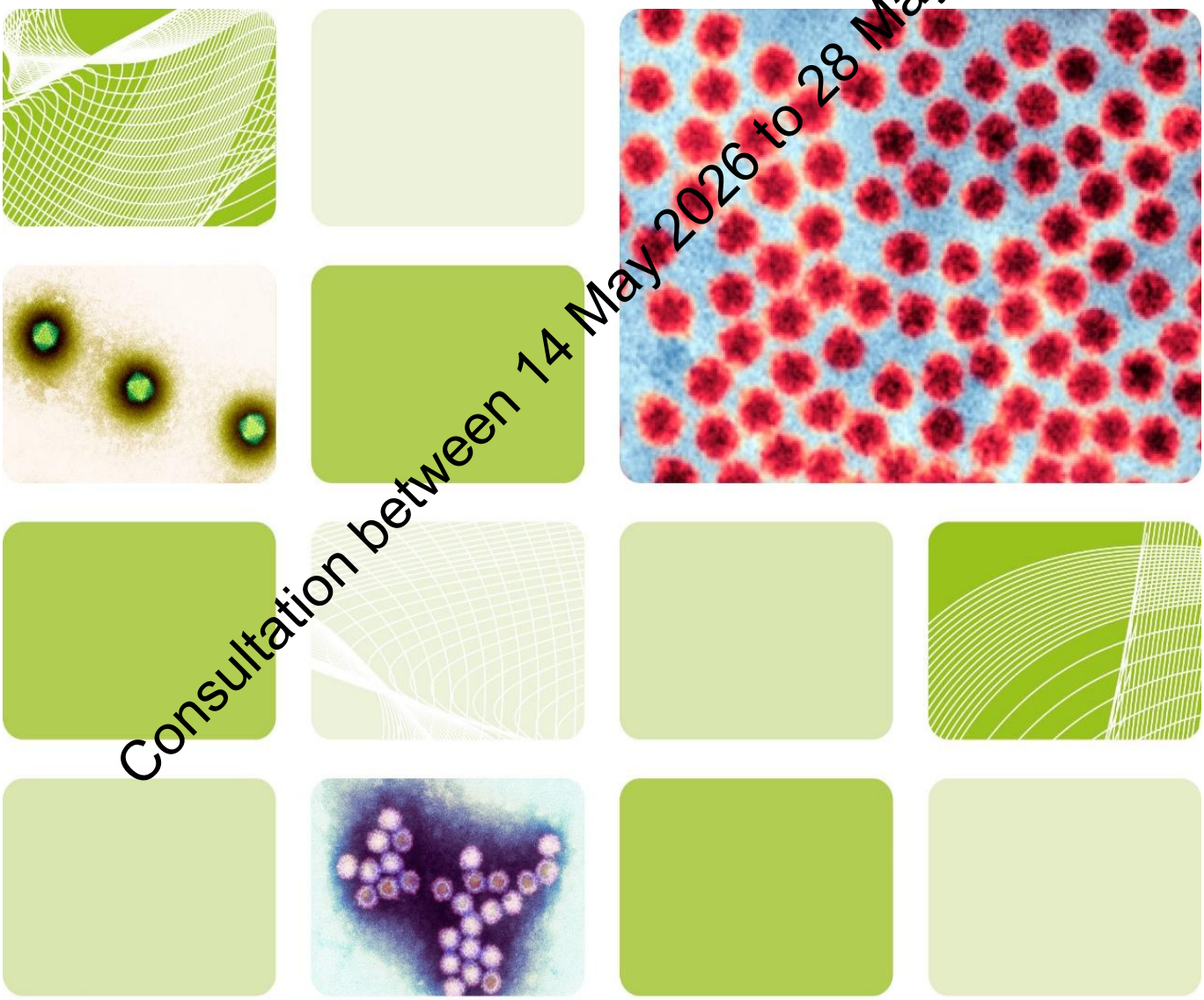
UK Health Security Agency

UK Standards for Microbiology Investigations

Laboratory diagnosis of hepatitis B virus and hepatitis D virus



Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by the UK SMI steering committee.

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UK SMIs are produced in association with:

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Contents

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Amendment table

The amendments since the previous version of this UK SMI document are listed in the amendments table below.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	x/dd.mm.yy
Issue number discarded	
Insert issue number	
Anticipated next review date*	dd.mm.yy
Section(s) involved	Amendment
Title	Title changed from 'Investigation of hepatitis B infection' to 'Laboratory diagnosis of hepatitis B virus [HBV] and hepatitis D virus [HDV]'

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document outlines laboratory-based serological testing for diagnosis of acute and chronic hepatitis B virus (HBV) and hepatitis D virus (HDV) infection, including in pregnancy. Refer to UK SMI S 1 - Acute infective hepatitis for further information regarding clinical presentations of acute infective hepatitis, and associated tests.

This document covers whole blood, plasma and serum samples. It includes the various methods of collection including venous and capillary blood sampling and dried blood spot (DBS) collection and addresses testing in specific patient groups. In addition, the algorithms aim to assist clinicians and laboratories in their decision making, by providing a framework for testing and the interpretation of results. It should be noted that the flowcharts included in this UK SMI may not be applicable in laboratories where testing for hepatitis B markers are carried out simultaneously once hepatitis B surface antigen (HBsAg) is found to be reactive.

All assays should be verified prior to use. If assays are to be used outside the scope for which the manufacturer has designated for its use, these should be validated and shown to be fit for purpose by the laboratory to suit its needs. For more information, refer to UK SMI Quality-related guidance.

This UK SMI is intended for use within healthcare settings. It is not intended to cover:

- testing methods or strategies commonly used in community testing such as POCT testing, self-sampling, or self-testing.
- testing of specimens other than blood, plasma and serum obtained by venepuncture, such as oral fluid and saliva.
- monitoring of HBV RNA viral load and sustained viral clearance (SVR) for those undergoing treatment.

Refer to the Green Book for interpretation of vaccine status. For the management and treatment of hepatitis B virus, refer to the EASL Clinical Practice Guidelines on the management of hepatitis B virus infection (1)

This UK SMI should be used in conjunction with other relevant UK SMIs.

Abbreviations

Abbreviation	Definition
HBV	hepatitis B virus
HDV	hepatitis D virus
BBV	Blood-borne virus
DBS	Dried blood spot
POCT	Point of care testing
PWID	People who inject drugs
NAAT	Nucleic acid amplification test
HBsAg	hepatitis B surface antigen
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBc IgM	IgM class antibody to the hepatitis B core antigen
HBsAb	Antibody to hepatitis B surface antigen
HBeAb	Antibody to hepatitis B e antigen

Definitions

For all antibody and NAAT testing, the following definitions apply:

Reporting stage for serology

These terms are used for final or preliminary reports.

Positive – Report-stage for reactive result.

Negative – Report-stage for a non-reactive result.

Indeterminate – Reactive result, that is just above the cut-off or one that cannot be confirmed.

Reporting stage for molecular assays

These terms are used for final or preliminary reports.

Scenarios	Qualitative result	Quantitative actual value	Quantitative Log_{10} of actual value
1	Detected	Actual value	Calculated log from actual value of the assay
2	Detected	Below the lower limit of quantitation of the assay	Calculated to the lower limit of the assay value
3	Detected	Above the upper limit of quantitation of the assay	Calculated to the upper limit of the assay value
4	Not detected	Not applicable	Not applicable
5	Inhibitory	Not applicable	Not applicable
6	Insufficient	Not applicable	Not applicable
7	Invalid	Not applicable	Not applicable

For reporting terminologies please refer to section 6 Post-laboratory processes.

4 Introduction

Hepatitis B virus (HBV) is a partially double stranded DNA virus from the *Hepadnaviridae* family virus for which humans are the only host and is transmitted via exposure to infectious bodily fluids, including transmission between mother and child (2).

Following the widespread successful implementation of childhood hepatitis B vaccinations, prevalence of chronic hepatitis B has reduced significantly (3). In the UK, hepatitis B is administered as part of the routine childhood immunisation programme. For further information on hepatitis B vaccination in the UK, please refer to chapter 18 of the Green book (4).

The UK government has adopted the Global Health Sector Strategy target set by the World Health Organization (WHO), which aims to eradicate hepatitis B virus as a public health threat by 2030 (5). Currently, the UK is on track to achieve these targets, however emphasis has been placed on increasing diagnosis, improving care pathways clinical management of people living with HBV infection (6).

4.1 Types of HBV infection

HBV causes both acute and chronic infection. Testing for HBV needs to differentiate between these two phases and may also identify past infections.

4.1.1 Acute infection

Acute infection may be asymptomatic or cause non-specific symptoms including fever, abdominal pain, nausea or jaundice. Symptoms are usually self-limiting and resolve in a matter of weeks to months (7).

HBV markers at the onset of jaundice are characterised by the presence of high plasma levels of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), IgM class antibody to the viral core (HBc IgM) and HBV DNA. Commonly, especially in adults, the viral antigens and HBV DNA fall rapidly over weeks and there is seroconversion of antibody to HBeAg (HBeAb). Seroconversion of antibody to HBsAg (HBsAb) occurs late after HBsAg clearance (2).

4.1.2 Chronic infection

Chronic HBV infection is defined by HBsAg being detectable for more than 6 months following acute infection and can be either asymptomatic or show similar symptoms to acute HBV infection (8). Chronic infection usually persists for decades and follows a typical pathway consisting of several discrete phases characterised by the serological markers present (9). Chronic HBV is more likely in those exposed to HBV perinatally, with the risk of developing chronic HBV increasing with age (4). Chronic HBV can lead to progressive liver disease and increased risk of developing hepatocellular carcinoma (10). For more information, please refer to The European Association for the Study of the Liver (EASL) guidelines (1).

4.2 HBV diagnostic approaches

In areas with high HBsAg prevalence (≥2% or ≥5% HBsAg seroprevalence), WHO recommends all adults be offered HBsAg testing. Additionally, WHO recommend blood donor screening and routine testing in pregnant women (11). As part of the infectious diseases in pregnancy screening (IDPS) programme, all pregnant women in the UK should be offered hepatitis B testing as early as possible in pregnancy (12). Anyone with confirmed Hepatitis B virus infection requires HDV antibody testing.

Targeted testing of high-risk groups is recommended. This includes migrants from endemic regions, partners or family members of infected persons, healthcare workers, people who inject drugs (PWID), people in prisons and custody , men who have sex with men (MSM), sex workers, and HIV-infected persons (13,14).

Screening is recommended in individuals with chronic liver disease, cirrhosis/fibrosis of the liver or liver cancer; individuals with end-stage kidney disease undergoing haemodialysis, HCV infected persons, individuals undergoing immunosuppressive or immunomodulatory therapy; individuals with congenital immunodeficiency; those considered for stem cell, bone or organ transplants (1). HBV screening should also be performed in blood, tissue, semen and organ donors to prevent transmission (1).

Following clinical suspicion of HBV infection, routine laboratory diagnosis consisting of a series of qualitative or quantitative serologic assays are required to detect biological markers that develop following infection. HBV DNA quantification using NAAT are required to stage the infection. Markers (antigens or antibodies) are detected by Enzyme Linked Immunosorbent Assays (ELISA), Enzyme immunoassays (EIA) or chemiluminescence immunoassays (CLIA) and used for diagnosis and monitoring of infection (11).

Following a positive HBsAg test, other serological markers can be tested to confirm between acute and chronic HBV infection and to stage infection.

In general, positive HBsAg and positive HBc IgM support the diagnosis of acute infection, whereas chronic HBV infection is indicated by positive HBsAg, positive HBcAb total and negative HBc IgM.

4.2.1 Markers of hepatitis B virus infection

- **HBsAg -** HBsAg is a marker of initial identification of current infection. It represents excess outer components of the virus envelope and is commonly detectable in plasma 6 to 12 weeks after infection, a few weeks later than HBV DNA. There is general acceptance that diagnostic assays should be capable of detecting 0.05 IU/mL HBsAg or less (15).
- **HB core total antibody (HBcAb)** is a marker of current or previous HBV infection. HBcAb is likely to persist for life and is a reliable marker of past infection following clearance of HBsAg. In this scenario, concurrent detection of HBsAb is taken to confirm the specificity of HBcAb (total). However, as a

significant number of recovered persons will not have detectable HBsAb, detection of isolated HBcAb is not uncommon (16,17).

- Some immunocompromised patients who are HBcAb positive and HBsAg negative are at risk of reactivation and will require protection against this through close monitoring and/or antiviral therapy. In such patients it may be important to confirm the specificity of isolated HBcAb reactivity. Reactivity in an alternative HBcAb assay (especially a combination of an indirect and a competitive ELISA) or the detection of HBeAb will serve to confirm specificity of the original HBcAb detection.
- **HB core IgM (HBc IgM)** is a marker of recent HBV infection and high levels will usually be present in acute icteric hepatitis B (18-20). It is also seen in HBV reactivation episodes. Detection of HBcAb IgM remains important in differentiating between acute and chronic hepatitis B infection. It is frequently detectable for at least 3 months after jaundice but can remain detectable at low levels in chronic infection for many years and therefore detection should be interpreted with caution (18,21). HBcAb avidity may be used to identify that acute illness is not a ‘flare’ during chronic infection.
- **HB surface antibody (HBsAb)** is directed against a range of epitopes on the ‘a’ determinant of the surface protein and is considered as a neutralizing antibody. HBsAb may be used to monitor recovery of HBV infection and post-immunisation vaccine responses. Levels of HBsAb of 10 mIU/mL are generally accepted to confer protection against HBV following a primary course of vaccination, however, the Department of Health and Social Care advises that it is preferable to achieve levels above 100 mIU/mL (4). HBsAb is used as a marker of resolution of infection, when found together with HBcAb after loss of HBsAg (22). Note that HBsAg and HBsAb can coexist, therefore the presence of detectable HBsAb alone cannot exclude active hepatitis B infection (23,24).
- **HBeAg and HBe antibody (HBeAb) -** HBeAg is a post-translationally modified derivative of the pre-core protein (spans the pre-core/core ORF) cleaved at both the c and n’ termini (25). It is exported from the liver when the virus is actively replicating in hepatocytes and, during pregnancy, can cross the placenta to act as a tolerogen to the foetus (17,26). HBeAg is a marker for high potential infectivity. In both acute infection and chronic infection an antibody response to this protein develops, termed e seroconversion. This HBeAb declines after clearance of HBsAg and may not persist as long as HBcAb after resolution of the infection. In practice, re-sampling of a patient a few weeks after jaundice will usually show ‘e’ seroconversion and significant falls of both HBsAg and HBV DNA, thus confirming an acute infection. Follow-up testing at 6 months, to demonstrate loss of HBsAg, should also occur, unless chronic infection develops.

- In HBeAg-negative patients, mutations in the precore and basal core promoter (BCP) regions can lead to reduced or absent HBeAg production while still allowing for ongoing HBV replication and HBsAg production from the integrated DNA (27).

4.2.2 HBV DNA

Hepatitis B viral DNA, carried as a single circular gene within the core of the 42nm virus particle, is now routinely quantified by nucleic acid amplification tests (NAATs) and expressed in international units per mL (IU/mL). HBV DNA quantification can be used to stage a patient's infection, to evaluate the risk of cirrhosis and to monitor response to antiviral therapy. It is also used to define the need for therapy in some patients. The best endpoint for anti-viral management is to reach a level of HBV DNA which is undetectable by current methods with a sensitivity of 10 to 15 IU/mL (1).

HBV DNA quantification can also be used to infer infectivity of a person infected with HBV. This is important for assessing HBV infected healthcare workers and antenatal mothers or birthing parents (refer to the green book) (22).

4.2.3 HBsAg quantification

HBsAg can be quantified using specific immunoassays. HBsAg quantification is used to characterize disease phase, define prognosis and guide treatment. HBsAg quantification is important for managing pegylated interferon-alfa (PEG-IFNa) treatment and stratification of patients eligible for stopping therapy with nucleos(t)ide analogues (NAs) (1).

4.3 Hepatitis B in pregnancy

Testing for HBsAg should be offered in pregnancy. The general testing, reporting and notification strategies for hepatitis B infected pregnant people are identical to those for other individuals (12). Additional arrangements, for reporting to specialist midwives or similar healthcare workers responsible for the care of pregnant people and their babies, should be in place locally. Perinatal transmission of hepatitis B to the neonate is a substantial risk and, wherever possible, prophylaxis for the neonate should be arranged in good time before delivery. Refer to Infectious diseases in pregnancy screening (NDPS): programme and the green book for more information (4,12). Local arrangements may vary.

4.4 Hepatitis D virus

Hepatitis D virus (HDV) is a defective RNA virus that requires HBV for infection. HDV can cause acute or chronic infection, with chronic HDV infection arguably being the most aggressive form of viral hepatitis (28). HDV can either coinfect with HBV, where both HDV and HBV are infected simultaneously, or by superinfection in patients with established chronic HBV (29). Acute HDV infection originating from coinfection with acute hepatitis B, is usually mild and self-limiting, however superinfection of HDV often leads to chronicity in a majority of cases (30). Screening for HDV should be performed

with a validated assay in all HBsAg positive patients (28). In contrast, any HDV assay must not be offered whenever HBsAg is negative; clinical order communication should reflect this.

4.4.1 Diagnosis of HDV

The diagnosis of HDV requires the serological testing to determine the presence of Hepatitis D specific markers. All HBsAg positive patients should be screened for HDV total antibodies and periodically retested where clinically indicated or annually in those remaining at risk of infection (28). Following this, positive samples are tested for HDV RNA using NAAT to indicate ongoing infection. Quantitative reverse transcription PCR can be used to identify viral load to monitor treatment response (31). HDV RNA assays are pan-genotypic and so they should perform well for all HDV genotypes (32).

5 Safety considerations

The section covers specific safety considerations related to this UK SMI, and should be read in conjunction with the general safety considerations (33-52).

Hepatitis B virus is a hazard group 3 organism but in most cases, it has a derogation for handling at containment level 2.

The guidance should be supplemented with local COSHH and risk assessments.

5.1 Specimen collection, transport and storage

Use aseptic technique to collect blood.

Collect adequate and appropriate specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

This guidance should be supplemented with local COSHH and risk assessments.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

5.2 Specimen handling and processing

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

6 Pre-laboratory processes

6.1 Specimen type

Whole blood, plasma, serum or dried blood spots

Note: Use of specific sample types in individual assays is subject to local verification and validation requirements, alongside the manufacturer's instructions. It is good practice to keep an aliquot for re-testing if required.

6.2 Specimen collection and handling

Collect specimens as soon as possible after onset of symptoms.

For safety considerations refer to Section 5.

Specimens collected in EDTA, or serum separator tubes are generally acceptable for testing. Please refer to manufacturer's instructions for specimen acceptability.

Refer to local laboratory protocols or the manufacturer's instructions for specific requirements on serum or plasma separation for NAAT.

Refer to current guidance on the safe handling of all organisms documented in the UK SMI general safety document.

6.3 Specimen transport and storage

This section covers specimen transport and storage consideration related to this UK SMI, and should be read in conjunction with the scientific information on our webpages.

Specimens should be transported and processed as soon as possible (53).

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens' (49).

6.4 Technical limitations

6.4.1 Sample types and collection methods:

The standard sample types are whole blood, serum and plasma.

Dried Blood Spots (DBS) and capillary blood samples are increasingly employed in hard to access populations, in people who inject drugs, as a public health tool in prison services and have been adapted for use in HBV serology and HBV quantification for HBV diagnosis (54,55) . Local validation and/or verification is required prior to their use to ensure accuracy and effectiveness in both diagnostic and monitoring context.

For information on managing inhibition and contamination in molecular methods, refer to UK SMI Q 4: Good practices when performing molecular amplification assays.

6.4.2 Immunoassays:

HBc antibody tests

HBc antibodies may not be detectable in early infection due to the time required for the development of the humoral immune response and may present as isolated HBcAb positivity in the absence of HBs antibody or antigen positivity. This may reflect previous HBV infection, false positivity, occult HBV infection, HBsAg mutations or testing during the window period. All results should be interpreted carefully and follow up testing may be required.

HBsAg assays

It is recommended that only HBsAg assays which are able to detect immune/vaccine escape variants should be used. Assays with a sensitivity level of 0.05 IU/mL or less for HBsAg should be used.

All results should be assessed in conjunction with relevant clinical information and patient history.

HBsAg Negative and HBV DNA Positive: this can occur in the following four situations:

- Mutations in the HBV '*s*' gene can lead to reduced sensitivity or failure to detect HBsAg in diagnostic tests. Clinically these could be because of surface mutants. Within the laboratory, this is also seen if monoclonal HB surface antibody is used for both capture and probe in the immunoassay (15,56,57).
- Fulminant HBV infection, in very early infection when HBV DNA is high and HBsAg can be negative because HBsAg producing hepatocytes are destroyed and remnant HBsAg is neutralised by HB surface antibody. HBc IgM and HBc IgG should be tested to guide acute diagnosis. HBc IgM will usually be high in these patients and forms the only means of serology diagnostics.
- Occult HBV infection is defined as HBsAg negative in plasma with very low and fluctuating levels of HBV DNA (58).

HBsAg false negative results in a qualitative assay may occur due to antigen levels being below the level of assay detection capability. HBsAg Positive and HBV DNA Negative:

- Vaccine-associated HBsAg: for 2-3 weeks following HBV vaccination, individuals may give transient positive results. HBsAg is usually low in these instances and neutralizable (59).
- HBsAg and HBV DNA dissociation occurs in chronic hepatitis B virus infection when the correlation between their levels breaks down, often due to HBV DNA integration into the host's genome or specific mutations, such as those affecting the precore region. This dissociation signifies a change in the disease's natural history, as HBsAg expression can persist from the integrated DNA even without

active viral replication [i.e. absence of HBV DNA in blood], influencing treatment strategies and the established criteria for HBV resolution (60).

- Individuals may be undergoing antiviral treatment, which may suppress HBV DNA production

Core/pre-core mutations:

Some patients may have mutations in the HBV core/pre-core region of the genome, resulting in reduced production and seroconversion of HBeAg (61). As a result of this, HBeAg may not be detectable.

6.4.3 Molecular Methods

Low level HBV DNA results should be interpreted with caution as there may be a variety of different causes. Careful evaluation, repeat testing and clinical correlation may be required. Laboratories should provide detailed quantification to ensure accurate result interpretation.

In addition, if dilution is performed it is recommended to report the dilution factor with the HBV DNA results to ensure accurate interpretation of viral load and support clinical decision-making. Refer to kit provider for assay threshold and recommended dilutions.

For examples on how to report on the dilution factor, please see below with a result comment and a dilution factor comment.

- For a “not detected” result after dilution, a suitable combined comment is: “HCV RNA was not detected following a 1:x dilution. After applying the dilution factor, the adjusted threshold is [value] IU/mL. Results should be interpreted within the clinical context.”
- For a “detected” result after dilution, a suitable combined comment is: “HCV RNA detected. After applying the 1:x dilution factor, the final viral load is [value] IU/mL. Results should be interpreted within the clinical context.”
- Example calculation (if needed): an analyzer readout of 200,000 IU/mL with a 1:2 dilution produces a final reported value of 400,000 IU/mL.

7 Laboratory processes

7.1 Laboratory diagnosis of HBV

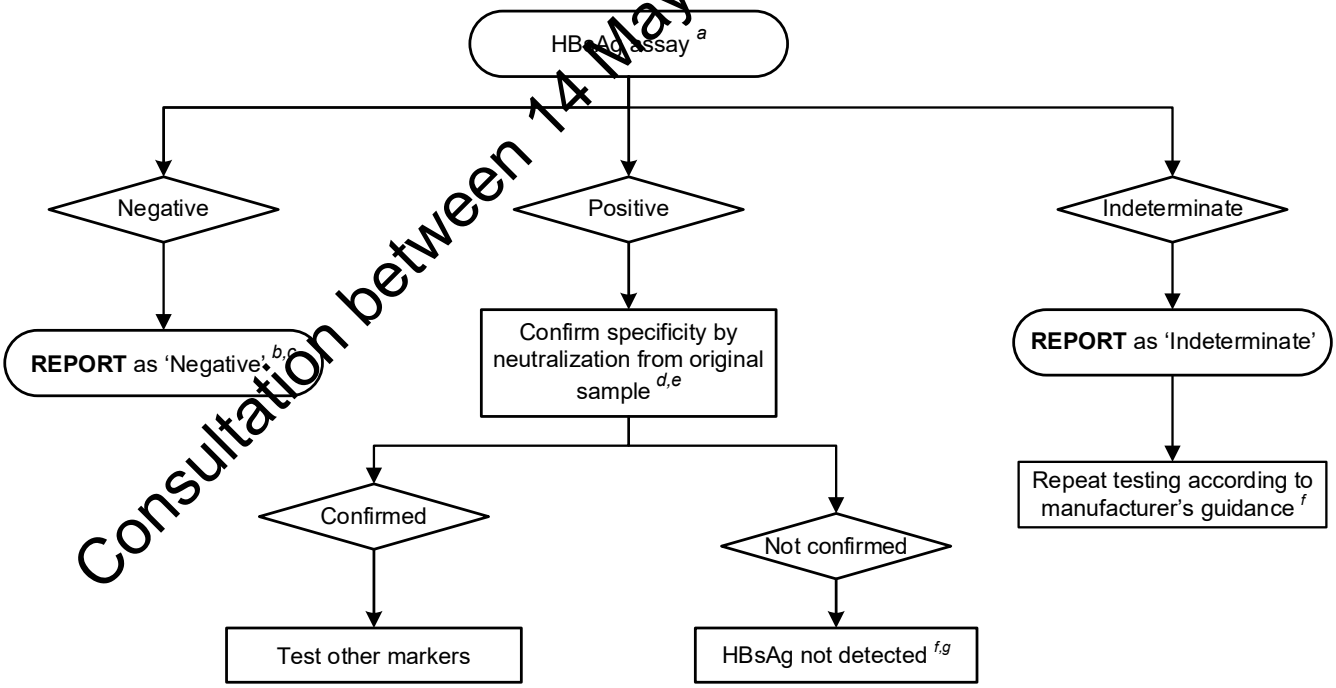
Routine laboratory diagnosis of HBV is based on the detection of HBV associated markers using serological methods. There must always be consideration of the clinical picture in addition to all HBV markers.

Initial tests must include HBsAg, confirmed either through neutralisation or by performing a second confirmatory test on the same sample.Low level positive results may require an alternative confirmatory assay, as there may be insufficient sample to perform neutralisation. High level positive results may require dilution prior to neutralisation or an alternative test for confirmation.

Following a positive test, other HBV markers are tested to stage the infection. These may include HBeAg, HBeAb, HBcAb, HBc IgM, HBsAb and HBV DNA.

Note: All patients found to be HBV markers in keeping with HBV infection should be tested for hepatitis D virus (28). If HDV testing is not available in house, laboratories should refer samples for testing.

7.1.1 Algorithm: Hepatitis B surface antigen confirmation by neutralization



Footnotes

a) It is recommended that only those assays which are able to detect immune / vaccine escape variants should be used. Assays with a sensitivity level of 0.05 IU/mL or less for HBsAg should be used.

b) Patients considered to be at an increased risk of HBV exposure or reactivation, and those with chronic liver disease, should be tested for HBcAb when found to be HBsAg negative.

c) In HBsAg negative patients, patient history and country of origin should be considered to rule out Occult HBV infection or gene mutations

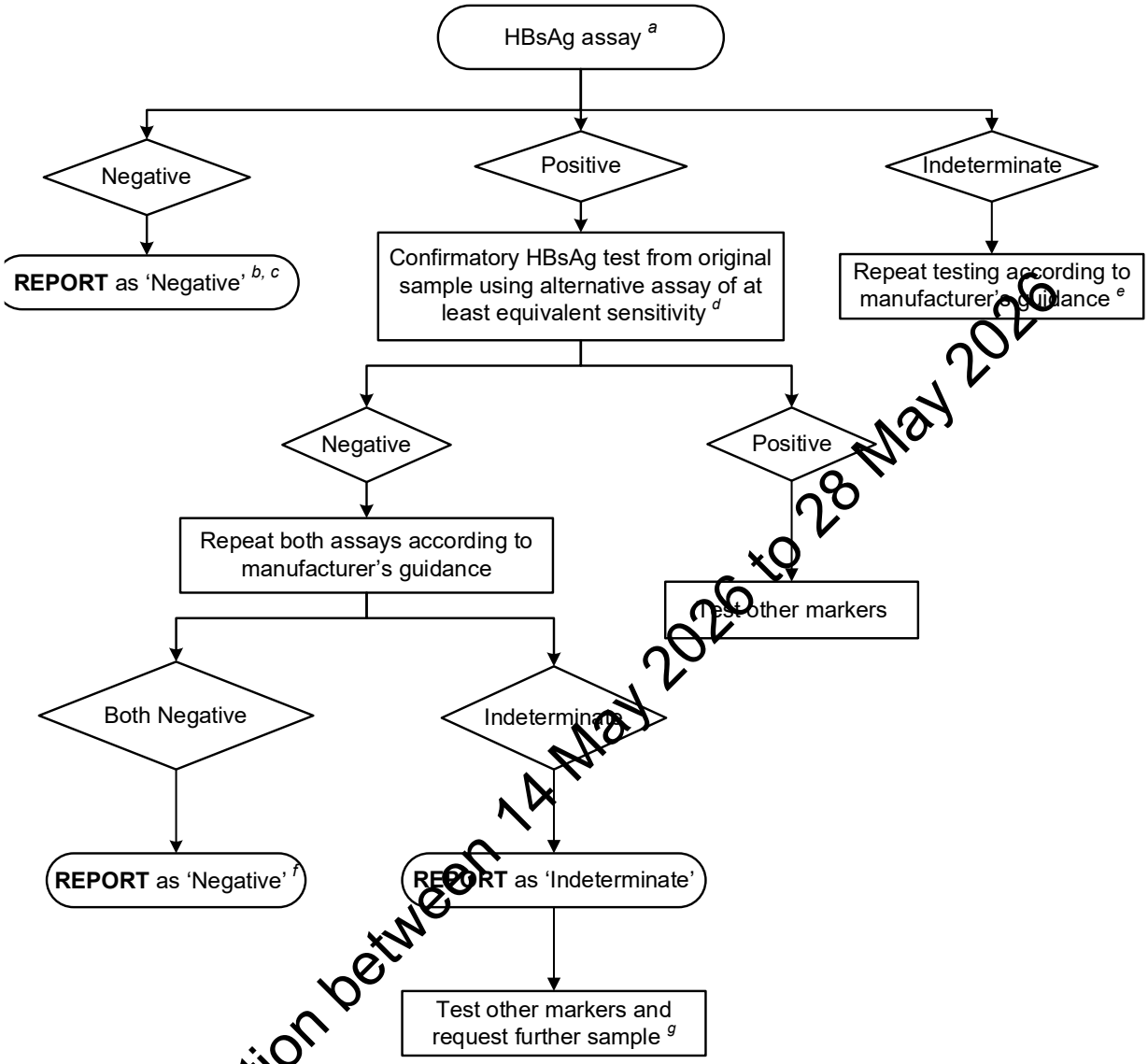
d) Haemolysed samples (for example, cadaver samples) may give non-neutralizable false reactive results.

e) Low level HBsAg may be of an insufficient level to perform neutralization by manufacturer's instructions. Very high HBsAg also might not neutralize unless diluted. Therefore, further testing using an alternative surface antigen test of equivalent sensitivity or HBV DNA is mandatory.

f) If results are persistently indeterminate refer to local protocol which may involve further serology including HBcAb (total), other HBV markers, and/or HBV DNA. Another sample may be required.

g) Consider patient history and country of origin.

7.1.2 Algorithm: Hepatitis B surface antigen confirmation by an alternative assay



Footnotes

a It is recommended that only those assays which are able to detect immune / vaccine escape variants should be used. Assays with a sensitivity level of 0.05 IU/mL or less for HBsAg should be used.

b Patients considered to be at an increased risk of HBV exposure or reactivation, and those with have chronic liver disease, should be tested for HBcAb when found to be HBsAg negative.

c In HBsAg negative patients, patient history and country of origin should be considered to rule out occult HBV infection or gene mutations.

d HBc IgG should be tested alongside an alternative assay to assist in the interpretation of negative and indeterminate HBsAg results

e If results are persistently indeterminate refer to local protocol which may involve further serology including HBcAb (total), other HBV markers, and/or HBV DNA. Another sample may be required.

f Consider patient history and country of origin

g Consider testing pre-track/pre-test aliquot for second HBsAg or HBV markers or HBsAg neutralisation to exclude contamination of the sample.

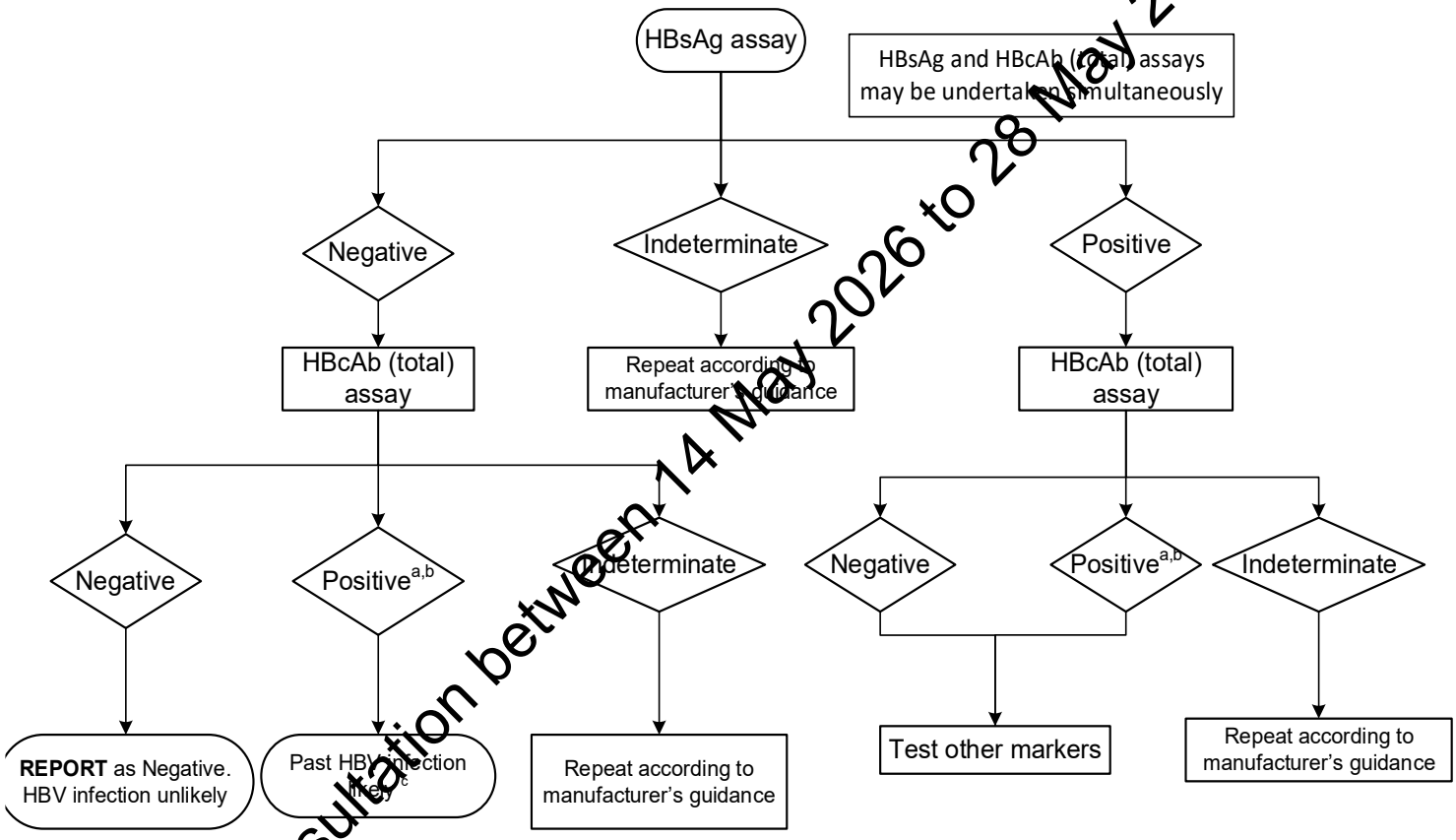
7.1.3 HBV testing in pre-immunosuppressive or donor screening

HBV testing in immunosuppressive and organ donor screening requires testing for HBsAg and HBcAb (total), regardless of HBsAg result. When results suggest current or past HBV infection, HBV DNA and HBsAb testing is recommended.

Laboratories may screen using HBsAg and reflex with HBcAb (total) or, alternatively, screen using HBcAb (total) and reflex using HBsAg.

All assays should be subject to local validation and/or verification prior to their use.

For information about deceased tissue or organ donor screening refer to the SaBTO microbiological safety guidelines (62).



Footnotes

a HBcAb (total) positives may be confirmed with a second assay.

b Patients that have recently been administered blood products or IVIG may demonstrate positive HBcAb (total) results.

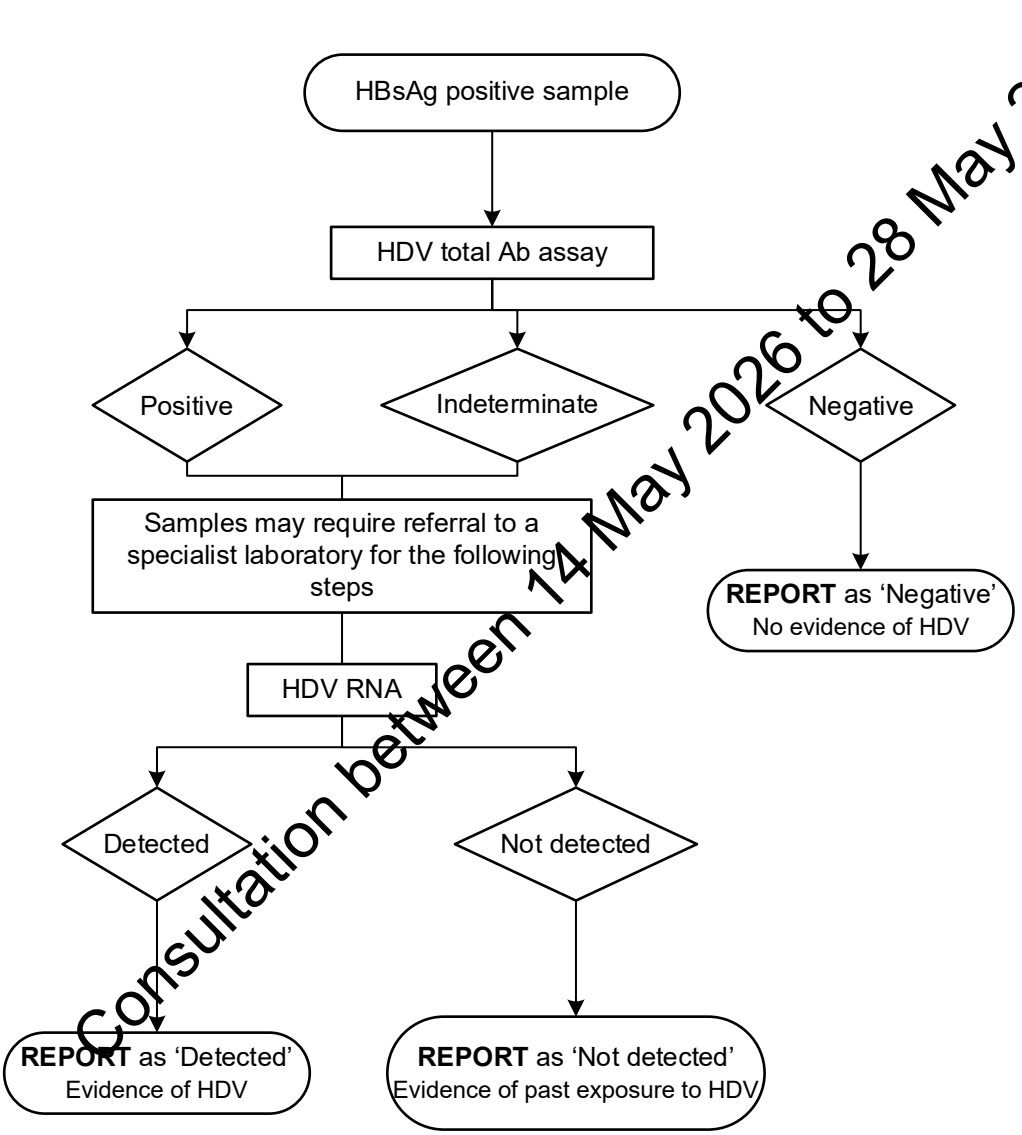
c Testing for all HBV markers may be considered depending on local protocol.

7.2 Laboratory diagnosis of HDV

HDV total antibody should be the first line of testing in all new HBsAg positive samples.

Following a positive or indeterminate HDV total antibody result HDV RNA should be tested. Some laboratories may also test HDV IgM. In known HDV RNA Positive patients, please monitor HDV RNA only.

Samples may be referred to a specialist laboratory for HDV testing.



8 Post-laboratory processes

8.1 Interpretation and reporting of results

Reporting terminology should be determined through local laboratory clinical governance, ensuring results are communicated in a way that optimises patient safety and supports accurate clinical interpretation. Laboratories should use clear, consistent terminology appropriate to their users, reflecting local clinical pathways and risk management processes. While UK SMIs provide guiding principles, the final choice of wording remains a matter of local professional judgement, informed by local disease population and accountability.

The tables below summarise the combinations of results that may occur. Suggested report comments are indicated but many results will require individualised comments based upon the result profile and clinical scenario.

The wording suggested here assumes this is the first sample received from this patient. Later samples may require modified report comments. For assistance in the interpretation of results in pregnant people refer to NHS Infectious Diseases in Pregnancy Screening Programme Laboratory Handbook (12).

Table 1: Reporting and interpretation of hepatitis B virus results

NT = Not tested

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
1	Negative	NT	NT	NT	NT	NT	NT	No evidence of current hepatitis B infection.	
2	Negative	Negative	NT	NT	NT	< 10	NT	No evidence of current or past hepatitis B infection. Interpret in light of immunisation history. For those with ongoing risk refer to the Green book.	
3	Negative	Negative	NT	NT	NT	≥ 10	NT	No evidence of current or past hepatitis B infection.	Indicates hepatitis B immunisation
4	Negative	Positive	NT	NT	NT	≥10	NT	Consistent with past exposure to hepatitis B and at risk of reactivation if immunocompromised. If this individual has received blood products, this result could be due to the presence of passively acquired antibody.	
5	Negative	Positive	NT	NT	NT	< 10	NT	Consistent with past exposure to hepatitis B and at risk of reactivation if immunocompromised. If this individual has received blood products, this result could be due to the presence of passively acquired antibody. Recommend HBV immunisation.	It is advisable to confirm isolated HBc total antibody positive results with a second assay, as isolated core total antibody sometimes represents false reactivity. HBeAb positive can also confirm an HBc total antibody positive status.

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
6	Negative	Positive	Positive	Negative	Negative or Positive	< 10	NT	Suggests relatively recent, resolving infection with hepatitis B virus. Please send a repeat sample to confirm. Notify Public Health team urgently.	In clinical scenario of recent acute liver failure (fulminant hepatitis) HBsAg may be negative due to the pronounced immune response [HBs antibody] and rapid viral clearance of HBV; HBcAb total and HBc IgM may then be the only positive serological markers. Consider whether there is there a history of infection or recent jaundice.
7	Positive [high level]	Negative	Negative	Negative	Negative	< 10	Detected	Consistent with early acute infection with hepatitis B virus. Please send a repeat sample to confirm and notify Public Health team urgently. Other causes of liver disease should be systematically excluded, including co-infections with HCV, HIV and HDV. Household and sexual contacts of people with acute or chronic hepatitis B infection should be tested and/or immunised as soon as possible to prevent acquisition. Refer to specialist in liver disease.	Notify Health Protection team urgently. The detection of HBsAg without evidence of HBc total and HBc IgM can be associated with early acute infection before antibody production. HBV DNA testing is essential to confirm this. Request repeat sample to confirm identity of patient and to confirm acute Hepatitis B virus infection by development of other markers. These can take many weeks to evolve and may not be accompanied by symptoms of acute hepatitis. If patient is pregnant, ensure appropriate treatment of baby or babies.

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
8	Positive [low level]	Negative	Negative	Negative	Negative	< 10	NT	HBsAg positive at low level. Please note this HBsAg low level result together with HB core total antibody negative and HB core IgM negative results. No evidence of past HBV infection. HBsAg result may be due to recent immunisation. Send further sample in one week, and EDTA blood for HBV DNA, if no history of vaccination.	If possible, laboratories should contact the clinician to find out if the patient has received HBV vaccination recently. This HBsAg is likely to represent antigenic component within recent HBV immunisation. The HBsAg in vaccine can be detectable for about one week after vaccination (63). Please send samples for HBsAg testing before immunising the patient so that there is no interference in the HBsAg assay. See comments above regarding early infection and seek HBV DNA testing.
9	Indeterminate	Negative	Negative	Negative	Negative	< 10	NT	Please note this HBsAg indeterminate result together with HB core total antibody negative and HB core IgM negative results. No evidence of past HBV infection. HBsAg indeterminate could be a non-specific reaction or could indicate recent hepatitis B immunisation, non-specific cross reactivity or very early infection. Send further sample in one week, and EDTA blood for HBV DNA, if no history of vaccination.	If possible, laboratories should contact the clinician to find out if the patient has received HBV vaccination recently. This HBsAg may represent antigenic component within recent HBV immunisation. The HBsAg in vaccine can be detectable for about one week after vaccination (63). Please send samples for HBsAg testing before immunising the patient so that there is no interference in the HBsAg assay. See also comments above regarding early infection and seek HBV DNA testing.

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
									Isolated HBsAg indeterminate is more likely to be non-specific reaction.
10	Indeterminate	Indeterminate	Negative	Negative	Negative	< 10	NT	Please note the HBsAg indeterminate result together with HB core total antibody indeterminate and HB core IgM negative results. This HBV profile is difficult to interpret. Please send another sample for HBsAg and HB core total antibody. Send further sample in one week, and EDTA blood for HBV DNA, if no history of vaccination.	If possible, laboratories should contact the clinician to find out if the patient has received HBV vaccination recently. The HBsAg in vaccine can be detectable for about one week after vaccination (63) See also comments above regarding early infection and seek HBV DNA testing.
11	Negative	Indeterminate	Indeterminate	Negative	Negative or Positive	< 10	NT	This HB core IgM indeterminate result could be due to non-specific reaction or cross-reaction to some other IgM or acute HBV. Send further serum sample for HBsAg and an EDTA blood for HBV DNA	HB core IgM can cross react with other assays. Very rarely, this can be a clinical scenario of recent acute liver failure (fulminant hepatitis) HBsAg may be negative due to the pronounced immune response [HBs antibody] and rapid viral clearance of HBV; HBc total and HBc IgM may then be the only positive serological markers. Consider whether there is there a history of infection or recent jaundice.

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
12	Positive	Negative	Negative	Positive	Negative	< 10	Detected at high levels	Consistent with early acute infection with hepatitis B virus. Send an immediate repeat to confirm and send another sample in 6 months to determine whether chronic infection has developed or resolution has occurred. Please repeat testing to confirm and notify public health team. Household and sexual contacts of people with acute or chronic Hepatitis B virus should be tested and/or immunised as soon as possible to prevent acquisition. Other causes of liver disease should be systematically excluded, including co-infections with HCV, HIV and HDV. Refer to specialist in liver disease.	Notify Health Protection team urgently. If pregnant, ensure appropriate treatment of baby or babies. HB core mutants HBV causing chronic infection can also lead to this profile, but this is rare.
13	Negative	Indeterminate	Negative	Not tested	Not tested	< 10	NT	The hepatitis B core antibody result is difficult to interpret. Unable to discriminate between non-specific reactivity and low level of hepatitis B core antibodies. HB core total antibody indeterminate could due to passively transferred IgG. No evidence of a current hepatitis B virus infection [HBsAg is Negative]. Hepatitis B vaccination recommended if at risk.	

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
								Please send another sample for HBsAg and HB core total antibody. Send further sample in one week, and EDTA blood for HBV DNA, if no history of vaccination.	
14	Negative	Indeterminate	Negative	Not tested	Not tested	≥10	NT	The hepatitis B core antibody result is difficult to interpret. Unable to discriminate between non-specific reactivity and low level of hepatitis B core antibodies. HB core total antibody indeterminate could due to passively transferred IgG. No evidence of a current hepatitis B virus infection [HBsAg is Negative]. Hepatitis B vaccination recommended is not recommended. Please send another sample for HBsAg and HB core total antibody.	
15	Positive	Positive	Positive	Positive	Negative	< 10	NT/ Detected	Consistent with recent infection with hepatitis B virus. Please send immediate repeat sample to confirm and notify Public health team urgently. Immediate repeat and send another sample in 3-6 months to check for resolution.	Notify Health Protection team urgently. Interpretation depends on HBc IgM level. Review clinical history and consider HBc IgG avidity testing (at reference laboratory).

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
								Household and sexual contacts of people with acute or chronic Hepatitis B should be tested and/or immunised as soon as possible to prevent acquisition Other causes of liver disease should be systematically excluded, including co-infections with HCV, HIV and HDV. Refer to specialist in liver disease.	A flare in chronic hepatitis B cannot be excluded. If pregnant, ensure appropriate treatment of baby or babies.
16	Positive	Positive	Negative	Positive/Negative	Positive/Negative	< 10 or not tested	NT or Not Detected/ Detected	Consistent with current HBV infection – most likely chronic HBV infection. Please review with clinical features and risk factors for acquisition. Please send further sample now and again in 6 months' time to confirm chronic infection. Household and sexual contacts of people with acute or chronic Hepatitis B should be tested and/or immunised as soon as possible to prevent acquisition. Other causes of liver disease should be systematically excluded, including co-infections with HCV, HIV and HDV. Refer to specialist in liver disease.	Notify Health Protection teams. If pregnant, ensure appropriate treatment of baby or babies.
17	Positive	Positive	Positive	Negative	Positive	Not tested	NT	Consistent with recent infection with hepatitis B virus. Please send immediate repeat sample to confirm and notify Public health team urgently Immediate repeat and send	Notify Health Protection teams depending on clinical history. Request another sample now to confirm result.

	HBsAg	HBc Ab (total)	HBc Ab IgM	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretive comment	Notes
								another sample in 3-6 months to check for resolution. Household and sexual contacts of people with acute or chronic Hepatitis B should be tested and/or immunised as soon as possible to prevent acquisition Other causes of liver disease should be systematically excluded, including co-infections with HCV, HIV and HDV. Refer to specialist in liver disease	Resolving acute infection cannot be excluded. A flare in chronic hepatitis B cannot be excluded.

Second sample where HBsAg is persistent for > 6 months can be interpreted as follows

- HBsAg high: Known chronic HBV infection
- HBsAg low: Known chronic HBV infection; albeit with low level of HBsAg as before

Table 2: Reporting and interpretation of hepatitis D virus results

	HDV total antibody	HDV RNA	Interpretive comment
1	Negative	No need to test	No past exposure to HDV
2	Indeterminate	Not detected	This HDV total antibody result is difficult to interpret. No evidence of HDV viremia.
3	Indeterminate	Detected	Evidence of HDV viremia. Please continue to monitor.
4	Positive	Not detected	Past exposure to HDV. No evidence of viremia
5	Positive	Detected	Evidence of HDV viremia. Please continue to monitor.

Table 3: HDV RNA expressed as qualitative and quantitative results

	Qualitative	Quantitative in IU/mL
1	Not detected	0
2	Detected below the limit of quantitation	< lower limit of quantitation
3	Detected	Actual value and log
4	Detected above the limit of quantitation	> Upper limit of quantitation

8.2 Public health management

HB core IgM positive results at levels consistent with new acute HBV infection should be reported urgently (for example, by telephone) to the local public health team, to facilitate timely public health interventions. All other new HBV infections are reported the next working day.

As part of its routine public health function, UKHSA undertakes surveillance of all cases of presumed acute hepatitis B and all cases of potential HBV transmission from mother or birthing parent to infant. Sequencing across the surface gene may be undertaken to define the genotype of acute infections, to confirm mother or birthing parent to child transmissions, and to identify vaccine escape mutants. In cases of reactivation and severe flares, sequencing of the core/pre-core genes may identify e-null viruses which have a greater propensity to lead to chronic liver disease. In the investigation and control of outbreaks of HBV transmission, phylogenetic analysis is used to confirm clusters and transmission pathways. Finally, phylogenetic analysis may be used to predict treatment response in some scenarios (1). For more

information regarding HBV reporting to UKHSA please refer to 'Reporting to UKHSA: a guide for diagnostic laboratories'.

In the UK, guidance for hepatitis B infected health care workers (HCW) is available (22). Refer to the UK advisory panel for healthcare workers living with bloodborne viruses page on GOV.UK.

For information regarding screening for HBV infection in pregnancy refer to the Infectious diseases in pregnancy screening programme: laboratory handbook (12).

For further information on public health management refer to UKHSA guidance on Hepatitis B: clinical and public health management.

National surveillance programmes for specific organisms should be taken into consideration when using the UK SMI. Refer to the data collection section of the Hepatitis B: guidance, data and analysis page on GOV.UK.

9 Referral to reference or specialist testing laboratories

When sending away isolates to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

England

Wales

Scotland

Northern Ireland

10 Public Health Responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g., routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

For suggested citation of UK SMIs, refer to UK SMI Development.

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