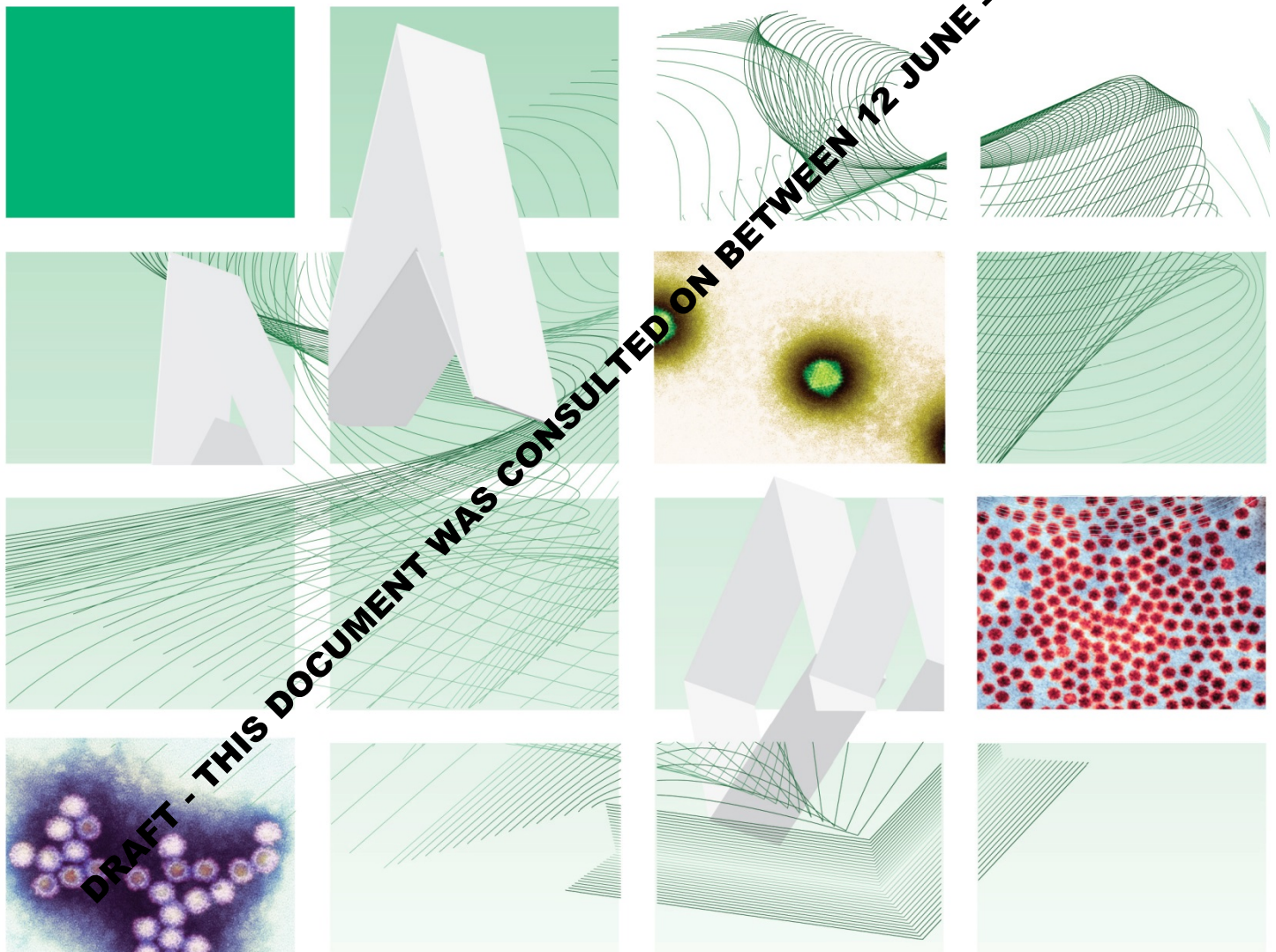




UK Standards for Microbiology Investigations

Investigation of hepatitis B infection



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365¹, 2016**. The original accreditation term began in **July 2011**."

Issued by the Standards Unit, Microbiology Services, PHE

Virology | V 4 | Issue no: dzz+ | Issue date: dd.mm.yy <tab+enter> | Page: 1 of 30

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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PHE Publications gateway number: 2015262

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	10/dd.mm.yy <tab+enter>
Issue number discarded	5.3
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Anticipated next review date*	
Section(s) involved	Amendment

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UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (YYYY <tab+enter>). Hepatitis B Diagnostic Serology in the Immunocompetent (including Hepatitis B in Pregnancy). UK Standards for

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Scope of document

Type of specimen

Whole blood, plasma, serum, dried blood spots

This UK SMI outlines laboratory testing for hepatitis B virus (HBV) infection, for diagnosis of acute infection and chronic infection, including in pregnant women.

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. Refer to the Green Book for interpretation of vaccine status.

Dried Blood Spot (DBS) samples are increasingly employed in hard to access populations and as a public health tool in prison services and in people who inject drugs (PWID). DBS can be used to collect whole blood specimens which are tested using standard CE marked Anti-HBV EIA and NAAT assays after verification and validation by accredited testing laboratories¹.

Refer to [S 1 - Acute infective hepatitis](#) for further information regarding clinical presentations of acute infective hepatitis, and associated tests.

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

Nomenclature of hepatitis B

Abbreviation	Definition
HBV	hepatitis B virus (complete infectious virion)
HBsAg	hepatitis B surface antigen (also called envelope antigen)
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
Anti-HBs, anti-HBc, and anti-HBe	Antibody to HBsAg, HBcAg, and HBeAg

Definitions

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

During testing process

Reactive – Initial internal-stage positive result pending confirmation.

Not reactive – Initial internal-stage negative result.

Equivocal – Result is not clearly positive or negative. Further testing is required.

The term 'equivocal' may be different for various platforms for example, 'indeterminate'.

Inhibitory – The term 'inhibitory' may be different for various platforms for example, 'invalid'.

Reporting stage

These terms are used for final or preliminary reports.

Detected – Report-stage confirmed reactive result.

Not detected – Report-stage not reactive result.

Indeterminate – Reactive result that cannot be confirmed.

Inhibitory – The term ‘inhibitory’ may be different for various platforms for example, ‘invalid’.

Introduction

Hepatitis B virus (HBV), a human representative of the family *Hepadnaviridae*, causes both acute infection, which may be associated with acute hepatitis and jaundice, and long term chronic infection associated with progression to severe liver disease and primary liver cell cancer. Testing for hepatitis B infection has to differentiate between these two conditions.

Acute infection

Acute infection may be asymptomatic; if it causes icteric illness (jaundice), hepatitis B, this is usually self-limiting and resolves in a matter of weeks to months².

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 (anti-HBc IgM) and HBV DNA and progresses to rapidly falling levels over weeks of the viral antigens and HBV DNA, coupled with seroconversion for antibody to HBeAg (anti-HBe). Seroconversion for antibody to HBsAg (anti-HBs) occurs late after HBsAg clearance.

Chronic infection

Chronic infection is defined by HBsAg being detectable for over 6 months³. The time period for chronic infection covers decades rather than weeks or months and normally follows a set pathway comprising a number of discrete phases over decades. This passage can however be reversed by immunosuppression and then the term “reactivation” is used⁴. In contrast the pathway may be speeded up by anti-viral interventions. A selection of HBV markers including HBV DNA quantification are used to characterise the infection in a patient and broadly place the individual on the “pathway”. The phases of a chronic HBV infection include immune tolerance, immune activation and inactive infection- the latter leading to either undetectable HBsAg antigenaemia or escape^{5,6}. Asymptomatic infections are more likely to become chronic than acute icteric infections. Infections in children are more likely to become chronic than those in adults.

Natural progression of HBV infection

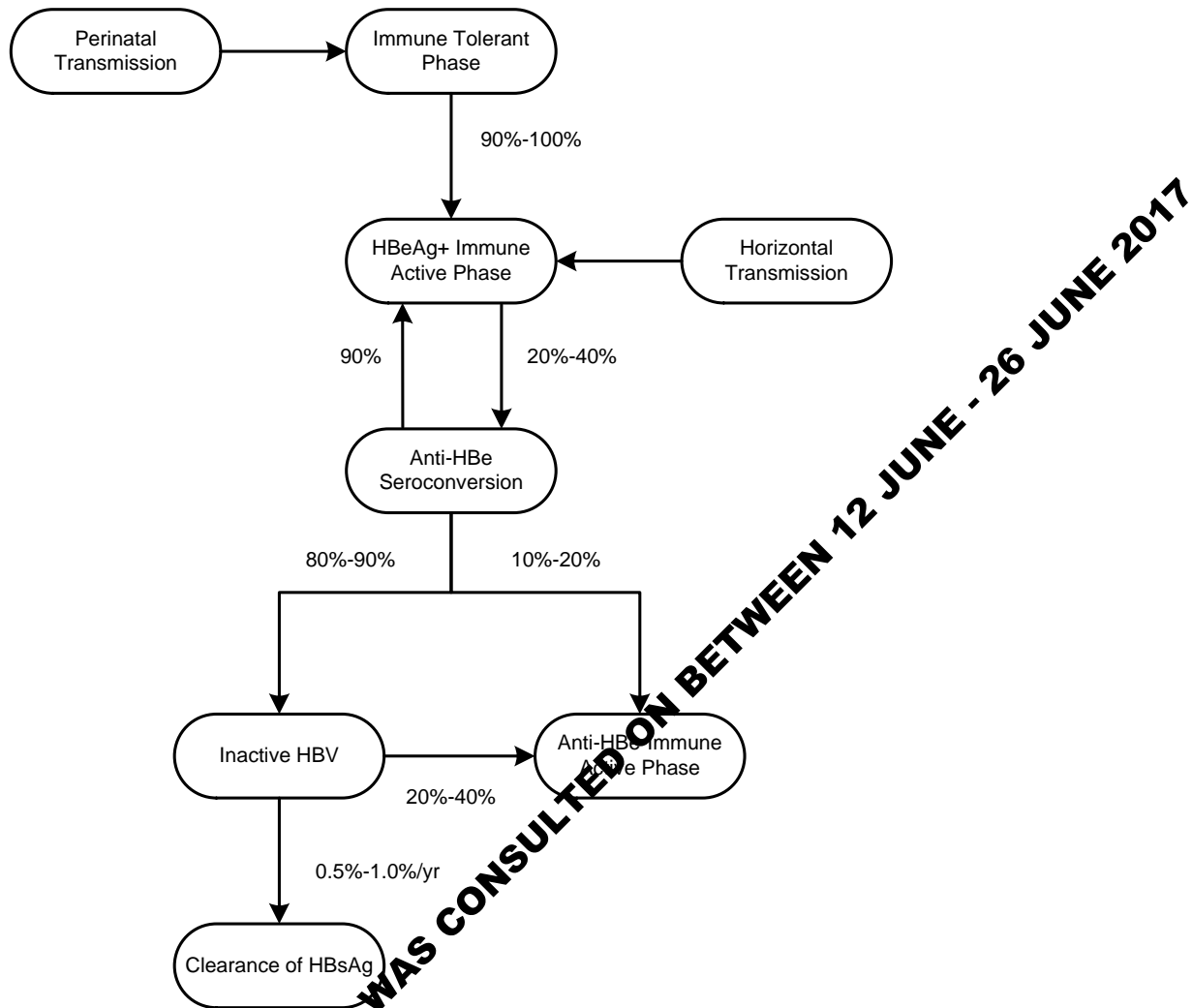


Fig. 1. Algorithm to display the natural history of chronic hepatitis B virus infection. (Adapted from Hepatology 2009; 49:S45-S55)

Phases of Chronic Hepatitis B Infection

Immune Tolerant Phase

- Occurs primarily after perinatal infection from HBsAg/HBeAg-positive mother
- ALT levels are normal
- HBV DNA $\geq 200,000$ IU/mL (≥ 1 million copies), often above 10^7 - 10^8 IU/mL
- Liver biopsy is normal or shows only minimal inflammation with no or minimal fibrosis
- Occurs most frequently in HBV genotype C infection

Immune Active (Clearance) Phase

- HBeAg-positive chronic hepatitis B

- Elevated ALT levels
- HBV DNA _ 20,000 IU/mL
- Anti-HBe–positive chronic hepatitis B
 - Elevated ALT
 - HBV DNA _ 2000 IU/mL
- Hepatic inflammation with or without fibrosis on biopsy often present in both HBeAg-positive and HBeAg-negative immune active phase

Inactive Phase

- Anti-HBe
- ALT levels normal
- HBV DNA _ 2000 IU/mL
- Hepatic inflammation minimal or absent
- Hepatic fibrosis may improve over time
- HBsAg clearance may eventually occur

In a proportion of patients, characterised by low levels of HBsAg, antigenaemia may slowly decline over years to undetectable levels with or without incident or subsequent loss of detectable HBV DNA (viraemia clearance). Anti-HBe and usually, but not invariably, anti-HBs will then persist for life. Covalently closed circular DNA (cccDNA) copies of the genome may persist at low density in the liver. Such patients may occasionally have persistence of plasma DNA and can be termed as “occult HBV” infections⁷⁻⁹. Immunosuppression of a patient with detectable anti-HBc with or without anti-HBs is likely to lead to reactivation, with loss of anti-HBs followed by a return of HBV DNA, HBsAg, HBeAg to tolerant phase levels^{10,11}. Similarly immunosuppression of a suppressed HBsAg seropositive patient can also lead to the same reactivation.

Escape occurs when virus variants which do not express HBeAg, “e null” viruses, appear in the plasma followed by HBV DNA and HBsAg returning to the levels seen in the immune tolerance phase. Such “e null” viruses are easily identified by sequencing as they are constitutively unable to translate the pre-core protein due to selection of premature stop codons or loss of start codons. These escapes may be periodic and associated with a loss of effective immune suppression, a burst of liver inflammation and even clinically apparent hepatitis¹². Such an escape/capture cycle is called hepatic flare, and is associated with continuing liver damage, a high level of anti-HBc IgM and may be difficult to differentiate from acute hepatitis¹³. Re-sampling within 3-4 weeks for HBsAg quantification could identify patients in whom HBsAg is being cleared; if the HBsAg level remains unchanged this may indicate those who are chronically infected and have undergone a flare^{12,14}.

Laboratory diagnosis

Serological investigations are usually conducted on plasma or serum and all markers can be preserved in plasma, preferably EDTA, or serum stored at -20°C and will be unaffected by freezing. In order to facilitate outreach testing sampling of capillary blood by dried blood spots is being widely used.

HBsAg detection and quantification

Identification of current infection in the first instance is based on detection of HBsAg, excess outer components of the virus envelope shed into the plasma. HBsAg can be detected in plasma shortly after infection, about 6-8 weeks before the onset of jaundice; HBV DNA can be detected a few weeks earlier than HBsAg¹⁵. There is general acceptance that assays should detect 0.05 mIU/mL HBsAg or less¹⁶. Mutations in the 's' gene can lead to reduced sensitivity or failure to detect HBsAg, especially if monoclonal anti-HBs is used for both capture and probe in the immunoassay¹⁶⁻¹⁸. HBsAg quantification is used by some laboratories since it can help to establish the nature of an infection; the reduction of HBsAg level can be a measure of anti-viral efficacy and if rapid falls over a short period are demonstrated (without the use of an anti-viral), then this may improve the accuracy of diagnosing acute icteric hepatitis B infection.

Failure to detect plasma HBsAg in acute hepatitis B can happen if the infection resolves quickly and sampling is delayed from the onset of jaundice. IgM anti-HBc will be detectable at high level in such situations often together with an early antibody response to HBsAg (anti-HBs).

Detection alone of HBsAg by immunoassay at a single time point does not give information on the nature or duration of infection. However, its continued presence in samples taken six months or more apart that defines chronic hepatitis B infection.

Differentiation between acute and chronic infections

This requires consideration of the clinical picture in addition to all HBV markers. During the course of an acute HBV infection an antibody to the core of the virus, anti-HBc, develops. Anti-HBc IgM detection needs to be interpreted with caution and detection of anti-HBc IgM remains important in differentiating between acute and chronic hepatitis B infection. Anti-HBc IgM detection may be consistent with recent HBV infection and in acute icteric hepatitis B high levels will be present, usually near the top of the range in most assays; values of sample/cut off ratio >8 will be achieved offering a sensitivity of 96.2% and a specificity of 89.7%¹⁹⁻²¹. It is usually detectable for at least three months after jaundice but can remain detectable at low levels in chronic infection for many years.

Anti-HBc avidity may be used to differentiate between acute and chronic infection - to identify that the acute illness is not a 'flare' during chronic infection. However, in both pregnancy and immunosuppression, patients may have low avidity antibody and interpretation in these situations is difficult. This test is available in only one or two centres in the UK. In practice short term re-sampling of a patient with jaundice a few weeks later will show 'e' seroconversion and significant falls of both HBsAg and HBV DNA thus confirming an acute infection. Follow up testing at six months to demonstrate loss of HBsAg should also occur.

Other markers

Anti-HBc is likely to persist for life following clearance of HBsAg and is considered as a reliable marker of past infection. Detection of anti-HBs in the same sample is taken to confirm the specificity of each antibody. However, as a significant number of recovered persons will not have detectable anti-HBs, detection of isolated anti-HBc is not uncommon^{22,23}. Some immunosuppressive regimens require identification of anti-HBc sero-positive patients in order to protect against reactivation and it may be necessary to confirm the specificity of isolated anti-HBc. A combination of an indirect

and a competitive ELISA will, if concordantly reactive, serve to confirm specificity of this antibody or the detection of anti-HBe.

HBeAg is a post-translationally modified soluble derivative of the precore peptide cleaved at both the c' and n' termini. It acts as a tolerogen, crosses the placenta inducing tolerance in the conceptus, and is exported from the liver when the virus is actively replicating in hepatocytes^{23,24}. It is associated with high levels of HBV DNA in the blood and is a marker for high potential infectivity. Both in the acute infection and in the long term persistent infection an antibody response to this protein develops termed e seroconversion, Anti-HBe declines after resolution of the infection and clearance of HBsAg and may not persist as long as anti-HBc.

Anti-HBs is directed against a range of epitopes on the 'a' determinant of the surface protein and is considered as a neutralising antibody¹⁵. Anti-HBs can be quantified and expressed as IU/mL and may be used to monitor post-immunisation vaccine responses. An initial level of 10 IU/mL is considered to confer protection against HBV²⁵. Anti-HBs is also used as a marker of resolution of infection (loss of HBsAg, detectable anti-HBc) when found together with anti-HBc. Note that HBsAg and anti-HBs can coexist, therefore the presence of detectable anti-HBs alone cannot exclude active hepatitis B infection^{26,27}. All healthcare workers exposed to bodily fluids are expected to be immunised against HBV infection.

Inferring infectivity

Hepatitis B DNA, carried as a single circular gene within the core of the 42nm Dane particle, is now routinely quantified by PCR and expressed in international units per ml (IU/mL). HBV DNA quantification can be used to stage a patient's infection (see above) and, when compared against a pre-treatment value, to monitor 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 10-15 IU/mL^{18,28}. HBV DNA level is also a significant prognostic marker for cirrhosis.

HBV DNA quantification can also be used to infer infectivity of a person chronically infected with HBV. This can be applied both to HBV infected healthcare workers and antenatal mothers (www.dh.gov.uk/greenbook)²⁹⁻³³.

Hepatitis B in pregnancy³⁴

Testing for HBsAg should be offered in pregnancy³⁴. The general testing strategies and reporting and notification patterns for hepatitis B infected pregnant women are identical to those for other individuals. Additional reporting to specialist midwives or similar healthcare workers responsible for the care of pregnant women and their babies should be in place locally. Vertical transmission of hepatitis B to the neonate is a substantial risk and prophylaxis for the neonate should be arranged well before delivery wherever possible. Local arrangements may vary. The guidance promulgated by the DH in Chapter 18 of '[Immunisation against infectious disease](#)' should be followed taking particular note of online Chapter updates. Reference should also be made to DH Guidance '[Screening for infectious diseases in pregnancy: Standards to support the UK antenatal screening programme](#)'. The following should also be referred to (www.dh.gov.uk/greenbook).

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Safety considerations

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

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

1 Specimen transport, storage and retention^{35,36}

1.1 Optimal transport and storage conditions

Specimens should be transported and processed as soon as possible³⁷.

Samples should be retained in accordance with The Royal College of Pathologists guidelines ‘The retention and storage of pathological records and specimens’³⁸.

Public health management

Positive anti-HBc IgM results at levels consistent with recent 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 PHE undertakes surveillance of all cases of presumed acute hepatitis B and all cases of potential HBV transmission from mother to infant. In addition, as part of this activity, sequencing may be undertaken to define the genotype of acute infections and is usually conducted across the surface gene. Sequencing is also carried out in mother-child infections, both to confirm that the mother was the source of the infection and to determine if the infant is carrying a vaccine escape mutant. In cases of reactivation and severe flares, sequencing of the

core/pre-core genes may identify e-null viruses which have a propensity to lead to chronic liver disease. Finally in the investigation and control of outbreaks of HBV transmission, phylogenetic analysis is used to confirm clusters and transmission pathways.

For information regarding notification to PHE (or equivalent in the devolved administrations) refer to the section on 'Notification to PHE or equivalent in the devolved administrations' at the end of this document. In the UK, guidance for hepatitis B infected health care workers (HCW) is available³⁰⁻³².

<https://www.gov.uk/government/groups/uk-advisory-panel-for-healthcare-workers-infected-with-bloodborne-viruses>

For information regarding screening for HBV infection in pregnancy refer to³⁴:

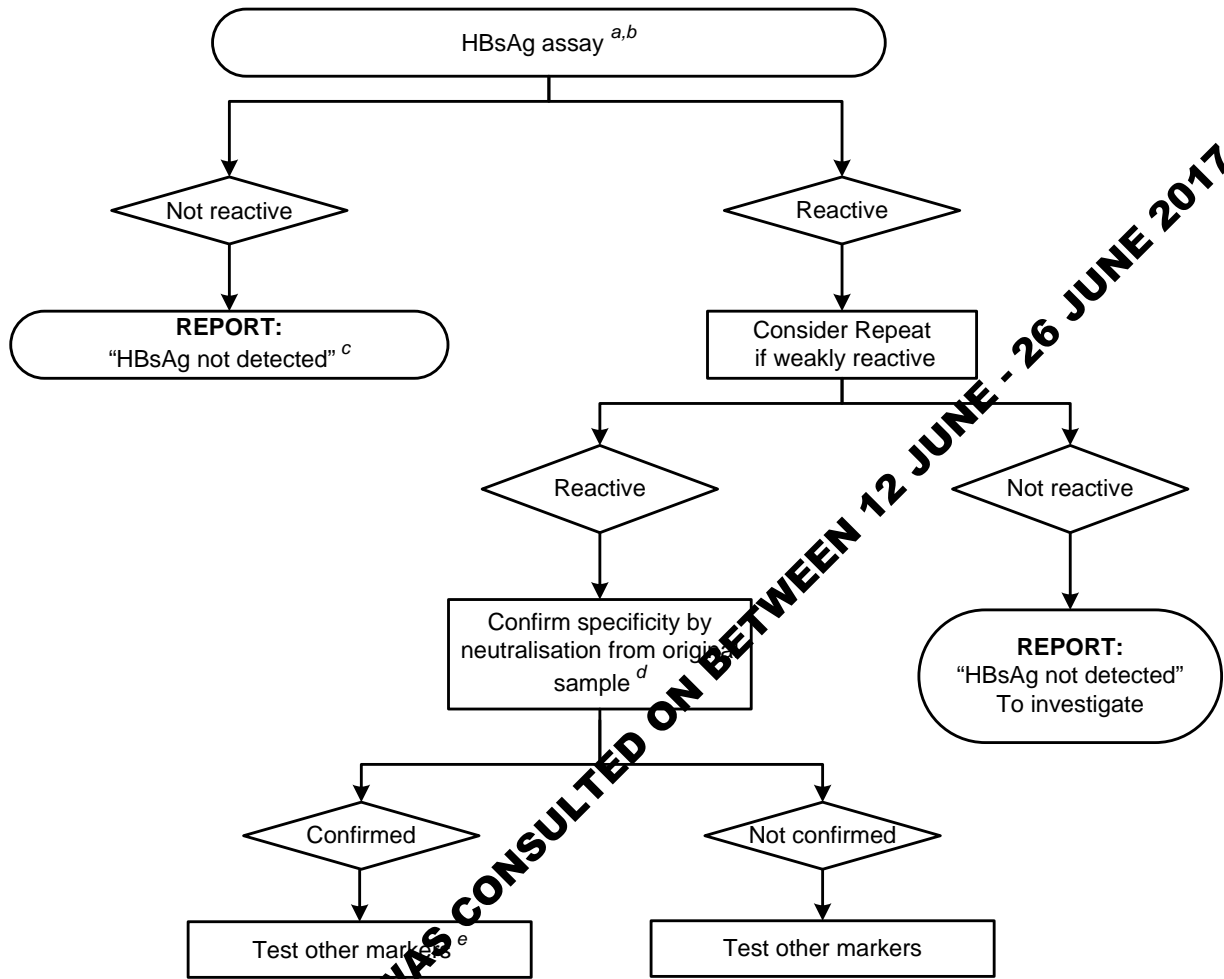
<https://www.gov.uk/government/publications/infectious-diseases-in-pregnancy-screening-programme-laboratory-handbook>

For further information on public health management refer to PHE guidance: <https://www.gov.uk/hepatitis-b-clinical-and-public-health-management> and <https://www.gov.uk/government/publications/hepatitis-b-and-c-local-surveillance-standards>.

National surveillance programmes for specific organisms should be taken into consideration when using the UK SMI. Add relevant information/link to surveillance.

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Hepatitis B surface antigen confirmed by neutralisation



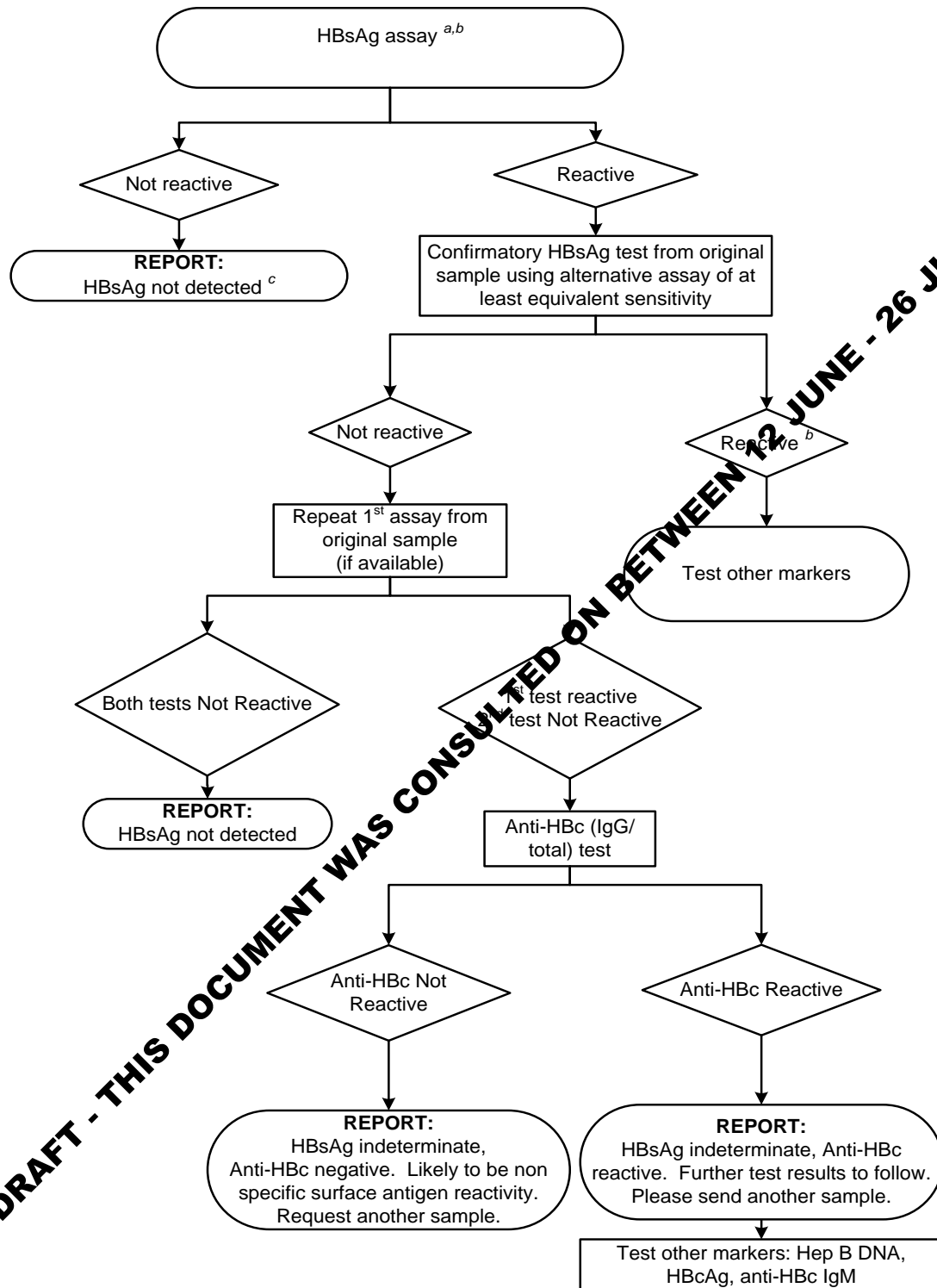
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Footnotes relating to investigation of hepatitis B surface antigen confirmed by neutralisation

- a) It is recommended that only those assays which are able to detect immune / vaccine escape variants should be used. Assays that have a HBsAg minimum target sensitivity level of 0.05 IU/mL should be used.
- b) Haemolysed samples (for example, cadaver samples) may give non-neutralisable false reactive results.
- c) Patients considered to be at an increased risk of HBV exposure or to have chronic liver disease can be tested for anti-HBc when found to be HBsAg negative.
- d) Low level HBsAg may be of an insufficient level to perform neutralisation by manufacturer's instructions. Very high HBsAg also might not neutralise unless diluted. An alternative surface antigen test of equivalent sensitivity is recommended.
- e) Clinical discussion could occur if required.

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Hepatitis B surface antigen confirmed by an alternative assay



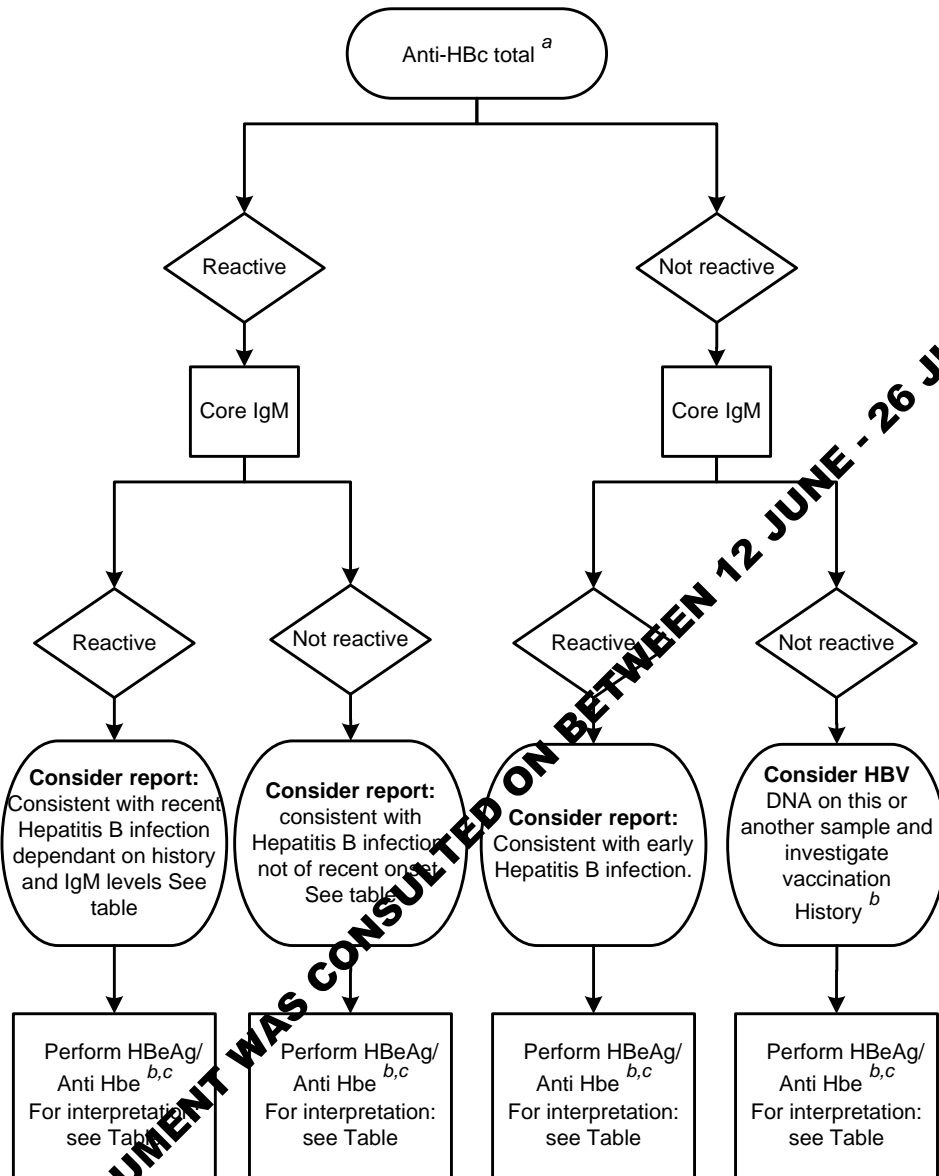
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Footnotes relating to investigation of hepatitis B surface antigen confirmed by an alternative assay

- a) It is recommended that only those assays which are able to detect immune/vaccine escape variants should be used. HBsAg minimum target sensitivity level of 0.05 IU/mL.
- b) Haemolysed samples (for example, cadaver samples) may give false reactive results.
- c) Patients considered to be at an increased risk of HBV exposure or to have chronic liver disease should be tested for anti-HBc when found to be HBsAg negative.

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Hepatitis B antibody testing (confirmed reactive)



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Footnotes relating to investigation of hepatitis B antibody testing (confirmed reactive)

- a) When interpreting anti-HBc reactivity consider the possibility of false reactivity, especially if low level reactivity is observed³⁹.
- b) It should be noted that hepatitis B surface antigen may be detectable following vaccination.
- c) Hepatitis D virus (HDV) testing should be done in all HBsAg positives at presentation of chronic HBV infection and during any clinical flares or during acute infection, especially if complicated by acute liver failure^{18,40}.
- d) HIV and hepatitis C testing should be carried out if hepatitis testing is positive⁴⁰. It is good practice to test for HIV⁴⁰.

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Hepatitis B reporting

There are other combinations of results (equivocal HBsAg reactive HBeAg is one) which have not been tabled but which do occur and require individual comments based upon profile and clinical setting, along with a further sample. For assistance in the interpretation of results in pregnant women refer to³⁴:

NB NT = Not tested

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment (see footnotes for further information and actions)	Notes to aid report comments
1	Not reactive	Not reactive	NT	NT	NT	Not reactive or NT	NT	No evidence of current or past hepatitis B infection.	
2	Not reactive	Reactive	NT	NT	NT	Reactive	NT	Consistent with past hepatitis B infection.	
3	Not reactive	Reactive	NT	NT	NT	Not reactive	NT	Consistent with past hepatitis B infection.	It is advisable to confirm isolated anti-HBc positive results with a second assay, as isolated anti-HBc sometimes represents false reactivity.
5	Not reactive	Reactive	Reactive	Not reactive	Not reactive or Reactive	Not reactive or Low Reactive	NT	Suggests relatively recent resolving infection with hepatitis B. Please send a repeat sample to confirm.	In clinical scenario of recent acute liver failure (fulminant hepatitis) HBsAg may be negative due to the pronounced immune response and rapid viral clearance of HBV; total anti-HBc and anti-HBc IgM may then be the only positive serological

Hepatitis B Diagnostic Serology in the Immunocompetent (including Hepatitis B in Pregnancy)

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment (see footnotes for further information and actions)	Notes to aid report comments
									markers. Is there a history of infection or recent jaundice?
6	Reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not reactive	Detected	Consistent with early acute infection with hepatitis B. Please repeat testing to confirm and notify public health team urgently.	Notify Public health team urgently. The detection of HBsAg without evidence of anti-HBc and anti-HBc IgM is 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 check for confirmation of 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.
7	Reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not tested	HBsAg detected. Send further sample in one week, or EDTA blood for DNA, if no history of vaccination.	Has this patient been recently immunised? The HBsAg in vaccine can be detectable for about one week after vaccination ⁴¹ .
8	Reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not detected	HBsAg detected. No evidence of viral replication	Has this patient been recently immunised? The HBsAg in

Hepatitis B Diagnostic Serology in the Immunocompetent (including Hepatitis B in Pregnancy)

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment (see footnotes for further information and actions)	Notes to aid report comments
									vaccine can be detectable for about one week after vaccination ⁴¹ .
9	Reactive	Not reactive	Not reactive	Reactive	Not reactive	Not reactive	Detected	<p>Consistent with early acute infection with hepatitis B.</p> <p>Send an immediate repeat to confirm and send another sample in 6 months to determine whether the chronic state has been reached or resolution has occurred. Please repeat testing to confirm and notify public health team.</p>	Notify Public Health team urgently.
10	Reactive	Reactive	Reactive	Reactive	Not reactive	Not reactive	Not tested NT	<p>Consistent with recent infection with hepatitis B.</p> <p>Please send immediate sample to confirm and notify Public health team urgently Immediate repeat and send another sample in 3-6 months to check for resolution.</p>	<p>Notify Public health team urgently.</p> <p>Interpretation depends on anti-HBc IgM level. Consider anti-HBc IgG avidity testing.(at reference laboratory)</p> <p>A flare in chronic hepatitis B cannot be excluded.</p>
11	Reactive	Reactive	Not reactive	+/-	+/-	Not reactive or not tested	Not tested or not detected or detected	<p>Consistent with current HBV infection – most likely chronic HBV infection.</p> <p>Please review with clinical features and risk factors for acquisition. Please send further sample now and again in 6 months' time to confirm the chronic infection</p>	

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	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment (see footnotes for further information and actions)	Notes to aid report comments
12	Reactive	Reactive	Reactive	Not reactive	Reactive	NT		Please interpret with clinical history and IgM levels. HBV DNA should be tested.	

Note: The testing algorithm wording of reports assumes this is the first sample received from this patient. Later samples will require modified report comments.

Note: Interpretative comments should be provided on reports: refer to ISO 15189:2012 .

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Notification to PHE^{42,43}, or equivalent in the devolved administrations⁴⁴⁻⁴⁷

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt-Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{44,45}, [Wales](#)⁴⁶ and [Northern Ireland](#)⁴⁷.

References

Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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