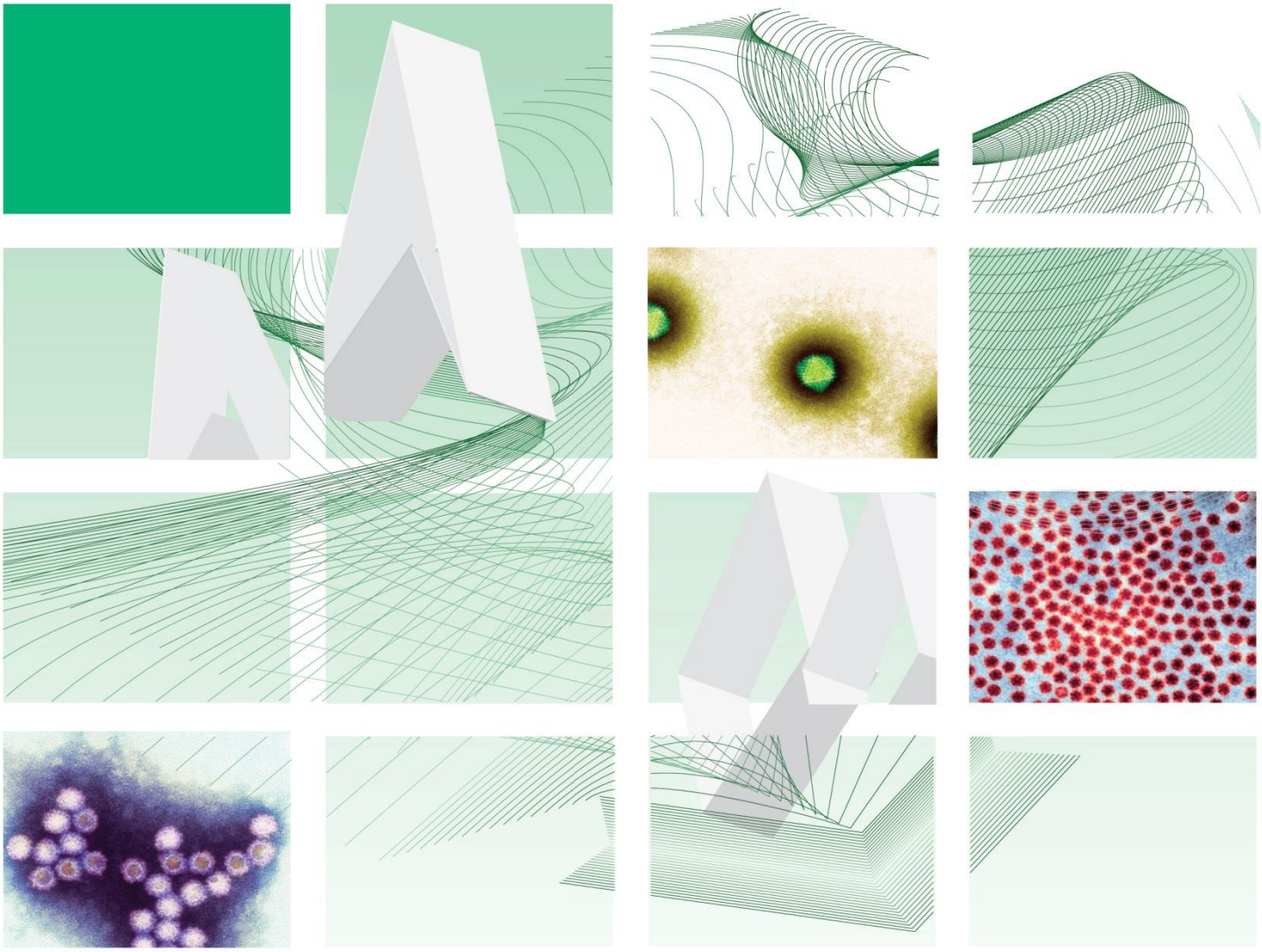
UK Standards for Microbiology Investigations

Investigation of hepatitis B infection



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Contents

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	11/20.07.21
Issue number discarded	6
Insert issue number	6.1
Anticipated next review date*	07.06.21
Section(s) involved	**Amendment**
Introduction	Under “HBsAg detection and quantification”, “0.05 mIU/mL HBsAg” has been corrected to “0.05 IU/mL HBsAg”
References	Reference 14 was incorrectly given as the October 2005 version of the JPAC guidelines (7th edition). This has been corrected to the March 2013 version of the JPAC guidelines (8th edition)
Whole document	Document template updated to latest version

Amendment number/date	10/07.06.18
Issue number discarded	5.3
Insert issue number	6
Anticipated next review date*	07.06.21
Section(s) involved	**Amendment**
Title.	The title has been amended.
Scope.	Scope of the document has been added.
Definitions.	Definitions have been added.
Introduction.	Introduction has been added to this document.
Technical information/Limitations.	The section has been added to the document.
Neutralization algorithm.	The algorithm has been updated and an additional footnote d added.

Hepatitis B surface antigen confirmation by an alternative assay algorithm.	Algorithm has been reviewed with minor amendments and a couple of additional footnotes added.
Hepatitis B surface antigen confirmed reactives algorithm.	Algorithm has been deleted as not adding value.
Hepatitis B reporting table.	Contents reviewed with an additional column added “Notes to aid reporting”.
Notes relating to the table.	The section has been deleted and the relevant information embedded in the table.

*Reviews can be extended up to 5 years subject to resources available.

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

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.

Dried blood spot (DBS) samples are increasingly employed in hard to access populations, in people who inject drugs and as a public health tool in prison services. DBS can be used to collect whole blood specimens which are tested using standard CE marked HBV antigen, antibody or NAAT assays after verification and validation by accredited testing laboratories as appropriate.

Refer to S 1 - Acute infective hepatitis for further information regarding clinical presentations of acute infective hepatitis, and associated tests. Refer to the Green Book for interpretation of vaccine status.

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

3.1 Nomenclature of hepatitis B

Abbreviation	Definition
HBV	hepatitis B virus (complete infectious virion)
HBsAg	hepatitis B surface antigen
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
anti-HBs, anti-HBc (total), and anti-HBe	antibody to HBsAg, HBcAg, and HBeAg

3.2 Hepatitis B infection

Hepatitis B virus (HBV), is a *Hepadnaviridae* family virus for which humans are the only host. It causes both acute infection, which may be associated with acute hepatitis and jaundice, and chronic infection, which can be associated with the development of severe liver disease and primary liver cell cancer. Testing for hepatitis B infection needs to differentiate between these two phases and may also identify past infections.

Acute infection

Acute infection may be asymptomatic; if it causes icterus (jaundice) this is usually self-limiting and resolves in a matter of weeks to months[1].

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. Commonly, especially in adults, the viral antigens and HBV DNA fall rapidly over weeks and there is seroconversion for antibody to HBeAg (anti-HBe). Seroconversion for antibody to HBsAg (anti-HBs) occurs late after HBsAg clearance.

Chronic infection

Chronic HBV infection is defined by HBsAg being detectable for more than 6 months following acute infection[2]. Chronic infection usually persists for decades, rather than weeks or months, and normally follows a typical pathway with a number of discrete phases[3,4,5]. Please refer to The European Association for the Study of the Liver (EASL) guidelines[6].

3.3 Laboratory diagnosis[6-13]

Serological investigations are usually conducted on plasma or serum depending on individual diagnostic kit specification.

HBsAg detection and quantification

Initial identification of current infection is based on detection of HBsAg. HBsAg 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[14].

Mutations in the HBV '*s*' gene can lead to reduced sensitivity or failure to detect HBsAg in diagnostic tests, especially if monoclonal anti-HBs is used for both capture and probe in the immunoassay[14-16]. HBsAg quantification is used by some laboratories as a stopping rule to interferon therapy; to assess the likelihood of disease progression; to stage the natural course of infection; or for pregnancy management[6].

Markers to differentiate between acute and chronic HBV infection

There must always be consideration of the clinical picture in addition to all HBV markers. Antibody to the core of the virus, (anti-HBc) develops during the course of an acute HBV infection 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 high levels will usually be present in acute icteric hepatitis B[17-19]. It is frequently detectable for at least 3 months after jaundice but can remain detectable at low levels in chronic infection for many years. Anti-HBc (IgM) detection needs to be interpreted with caution as it may also be detectable following a flare of chronic infection[17,20].

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, immunosuppressed patients may have persistent low avidity antibody, so interpretation in these situations is difficult. Anti-HBc avidity testing is available in very few centres in the UK. 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.

Other markers

Anti-HBc (total) is likely to persist for life and is considered to be a reliable marker of past infection following clearance of HBsAg. In this scenario, concurrent detection of anti-HBs is taken to confirm the specificity of anti-HBc (total). However, as a significant number of recovered persons will not have detectable anti-HBs, detection of isolated anti-HBc is not uncommon[21,22]. Some immunocompromised patients who are anti-HBc positive, 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 anti-HBc reactivity. Reactivity in an alternative anti-HBc assay (especially a combination of an indirect and a competitive ELISA) or the detection of anti-HBe will serve to confirm specificity of the original anti-HBc detection.

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[23]. 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[22,24]. HBeAg is associated with high levels of HBV DNA in the blood and is therefore a marker for high potential infectivity. Both in acute infection and in chronic infection an antibody response to this protein develops, termed e seroconversion. This anti-HBe declines after clearance of HBsAg and may not persist as long as anti-HBc after resolution of the infection.

Anti-HBs is directed against a range of epitopes on the 'a' determinant of the surface protein and is considered as a neutralizing antibody. Anti-HBs can be quantified in IU/mL and may be used to monitor post-immunisation vaccine responses. The World Health Organization considers that a minimum level of 10 IU/mL confers protection against HBV[25]. The Department of Health and Social Care advises that it is preferable to achieve levels above 100 mIU/mL[26]. Anti-HBs is also used as a marker of resolution of infection, when found together with anti-HBc after loss of HBsAg[27-29]. Note that HBsAg and anti-HBs can coexist, therefore the presence of detectable anti-HBs alone cannot exclude active hepatitis B infection[30,31].

3.4 Inferring infectivity

Hepatitis B DNA, carried as a single circular gene within the core of the 42nm virus 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), 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[6,16].

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)[27-29].

3.5 Hepatitis B in pregnancy[32]

Testing for HBsAg should be offered in pregnancy[32]. The general testing, reporting and notification strategies for hepatitis B infected pregnant people are identical to those for other individuals. 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. Local arrangements may vary. The guidance presented in chapter 18 of 'Immunisation against infectious disease' should be followed, taking particular note of online chapter updates. Reference should also be made to DHSC Guidance 'Screening for infectious diseases in pregnancy: Standards to support the UK antenatal screening programme'.

3.6 Public health management

Positive anti-HBc IgM 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. For more information regarding public health notification, refer to the section on 'Notification to PHE or equivalent in the devolved administrations', in section 2: scientific information.

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 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[6].

In the UK, guidance for hepatitis B infected health care workers (HCW) is available[27,29]. 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[32] the infectious diseases in pregnancy screening programme: laboratory handbook

For further information on public health management refer to PHE guidance on Hepatitis B: clinical and public health management and Hepatitis B and C: local surveillance standards.

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.

4. 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.

5. Specimen processing and procedure

5.1 Specimen type

Whole blood, plasma, serum, dried blood spots

5.2 Specimen transport and storage conditions[33,34]

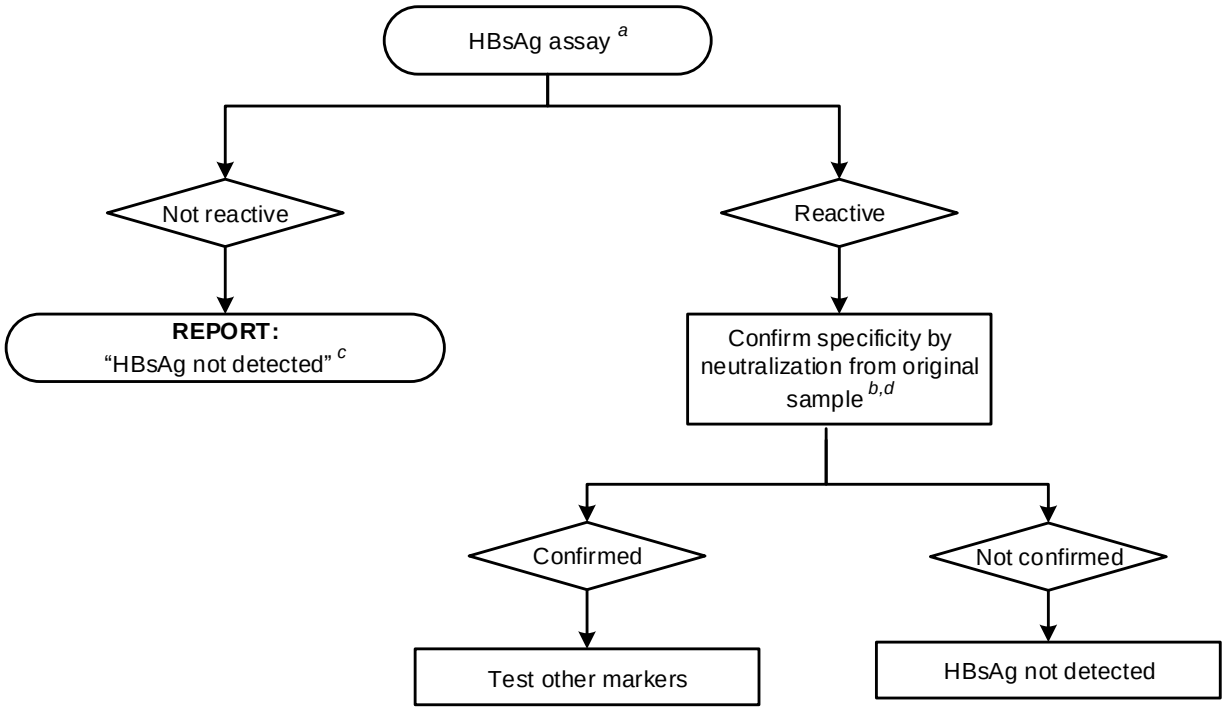
Specimens should be transported and processed as soon as possible[35].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[36].

6. Investigation

Algorithm 1: Hepatitis B surface antigen confirmation by neutralization

A text description of this algorithm is provided with this document.



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.

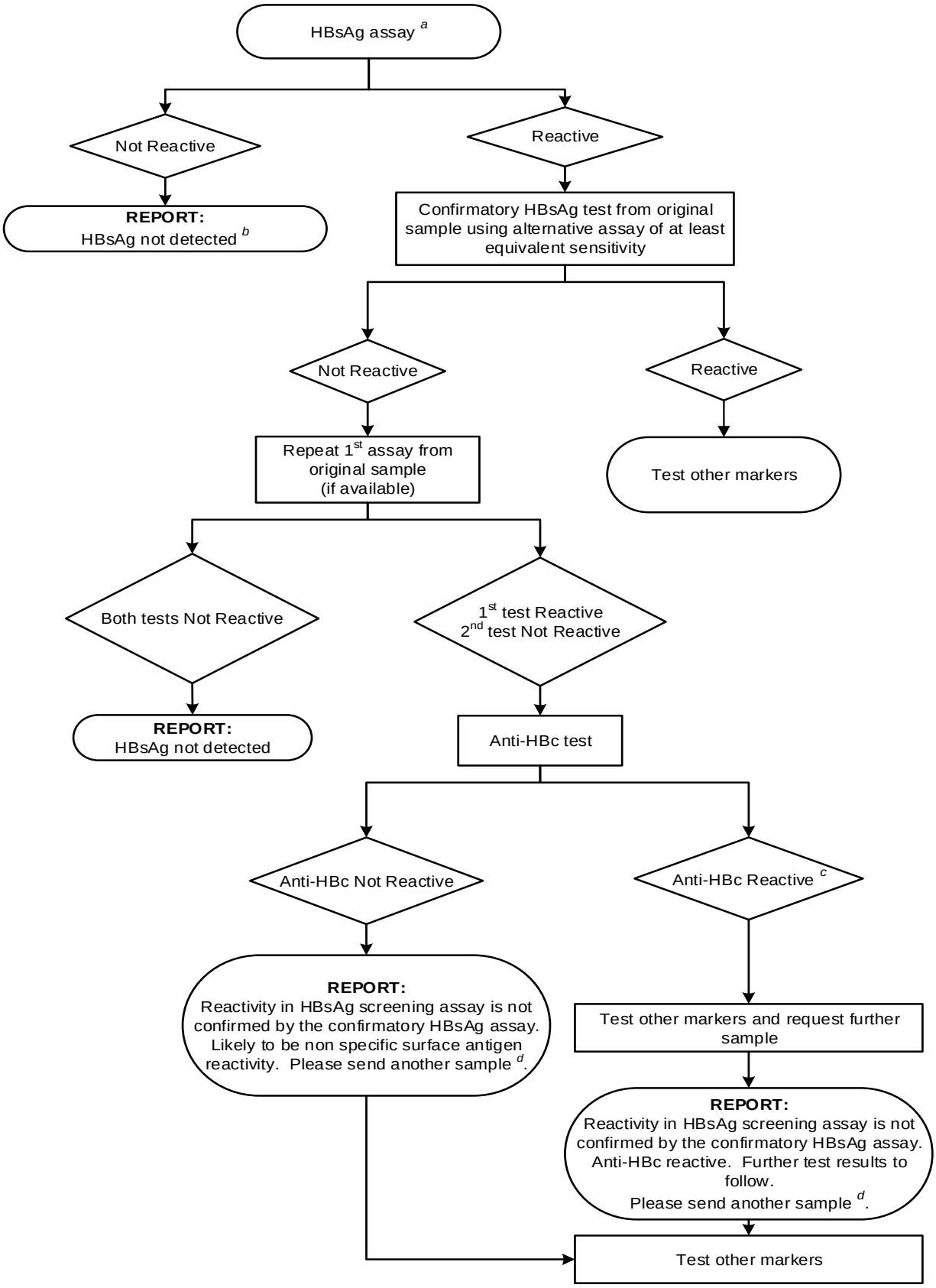
b) Haemolysed samples (for example, cadaver samples) may give non-neutralizable false reactive results.

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

d) 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. Further testing using an alternative surface antigen test of equivalent sensitivity or HBV DNA should be considered in this setting.

Algorithm 2: Hepatitis B surface antigen confirmation by an alternative assay

A text description of this algorithm is provided with this document



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 anti-HBc when found to be HBsAg negative.

c) When interpreting anti-HBc reactivity consider the possibility of false reactivity, especially if low level reactivity is observed[37].

d) Some laboratories may wish to test the original sample for HBV DNA instead of requesting a repeat sample.

7. Interpreting and reporting laboratory results

7.1 Hepatitis B reporting

The wording suggested here for reactive tests assumes this is the first sample received from this patient. Later samples may require modified report comments. There are other possible combinations of results, for example equivocal HBsAg with reactive HBeAg, which have not been tabled but which require individual comments based upon result profile and clinical scenario, along with a further sample. For assistance in the interpretation of results in pregnant people refer to NHS Infectious Diseases in Pregnancy Screening Programme Laboratory Handbook 2016-2017[32]: Interpretative comments should be provided on reports: refer to ISO 15189:2012.

NT = Not tested

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment	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.	For those with ongoing risk refer to the Green book
2	Not reactive	Reactive	NT	NT	NT	Reactive	NT	Consistent with past hepatitis B infection. Hepatitis B may reactivate in patients who are immunocompromised.	
3	Not reactive	Reactive	NT	NT	NT	Not reactive	NT	Consistent with past hepatitis B infection. Hepatitis B may reactivate in patients who are immunocompromised.	It is advisable to confirm isolated anti-HBc positive results with a second assay, as isolated anti-HBc sometimes represents false reactivity. Anti HBe positive can also confirm an anti-HBc positive result
4	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; anti-HBc (total) and anti-HBc (IgM) may

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment	Notes to aid report comments
									then be the only positive serological markers. Consider whether there is there a history of infection or recent jaundice.
5	Reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not reactive	Detected	Consistent with early acute infection with hepatitis B. 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 Hepatitis C, HIV and HDV. 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. Refer to specialist in liver disease.	Notify Health Protection team urgently. The detection of HBsAg without evidence of anti-HBc (total) and anti-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.
6	Reactive	Not reactive	Not reactive	Not reactive	Not reactive	Not reactive	NT	HBsAg detected. Send further sample in one week, or EDTA blood for HBV 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[38]. See also comments above regarding early infection and seek HBV DNA testing.

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment	Notes to aid report comments
7	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 vaccine can be detectable for about one week after vaccination[38].
8	Reactive	Not reactive	Not reactive	Reactive	Not reactive	Not reactive	NT or Detected	Consistent with early acute infection with hepatitis B. 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 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 Hepatitis C, HIV and HDV. Refer to specialist in liver disease.	Notify Health Protection team urgently. If pregnant, ensure appropriate treatment of baby or babies.
9	Reactive	Reactive	Reactive	Reactive	Not reactive	Not reactive	NT/ Detected	Consistent with recent infection with hepatitis B. Please send immediate repeat sample to confirm and notify Public health team urgently	Notify Health Protection team urgently. Interpretation depends on anti-HBc IgM level. Review clinical history and consider anti-HBc IgG avidity testing (at reference laboratory).

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment	Notes to aid report comments
								Immediate repeat and send 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 Hepatitis C, 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.
10	Reactive	Reactive	Not reactive	Reactive/ Not reactive	Reactive/ Not reactive	Not reactive or not tested	NT or not detected or 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	Notify Health Protection teams. If pregnant, ensure appropriate treatment of baby or babies.

	HBs Ag	Anti HBc (total)	HBc IgM	HBe Ag	Anti HBe	Anti HBs	Hep B DNA	Suggested wording of report comment	Notes to aid report comments
								excluded, including co-infections with Hepatitis C, HIV and HDV. Refer to specialist in liver disease.	
11	Reactive	Reactive	Reactive	Not reactive	Reactive	NT		Please interpret with clinical history and IgM levels. HBV DNA should be tested. 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 Hepatitis C, HIV and HDV. Refer to specialist in liver disease.	Notify Health Protection teams depending on clinical history. Request another sample now to confirm result. Resolving acute infection cannot be excluded. A flare in chronic hepatitis B cannot be excluded.

References

Modified GRADE table used by UK SMIs 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 SMIs 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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