NATIONAL LABORATORY HANDBOOK

Laboratory Testing for Suspected Viral Hepatitis in Adults

Document reference number	**CSPD010/2018**	**Document developed by**	National Clinical Programme for Pathology
Revision number	Version 1.	**Document approved by**	National Clinical Programme for Pathology. Clinical Advisory Group for Pathology. National Clinical Advisor and Group Lead.
Approval date	May 2018	**Responsibility for implementation**	Acute Hospital Division
Next Revision date	May 2021	**Responsibility for review and audit**	National Clinical Programme for Pathology

National Clinical & Integrated Care Programmes
Person-centred, co-ordinated care

PATHOLOGY

ROYAL COLLEGE OF PHYSICIANS OF IRELAND

Authors

Cillian De Gascun, Consultant Virologist & Laboratory Director, UCD National Virus F Laboratory, Dublin 4.
Maeve Doyle, Consultant Microbiologist, University Hospital Waterford, Waterford.
Bartley Cryan, Consultant Medical Microbiologist, Cork University Hospital, Cork.
Jeff Connell Assistant Director, Principal Clinical Scientist at National, Virus Reference Laboratory, Dublin 4.
Colm McDonnell, Chief Medical Scientist, University Hospital Limerick, Limerick.
Niamh O'Sullivan, Consultant Microbiologist, Our Lady's Children's Hospital Crumlin, Dublin 12.
Richard Drew, Consultant Microbiologist, The Rotunda Hospital, Dublin 1.
Susan Knowles, Consultant Microbiologist, National Maternity Hospital, Holles Street, Dublin 2.
Deirbhile Keady, Consultant Microbiologist, Galway University Hospital, Galway.
Adele Habbington, Senior Medical Scientist, Our Lady's Children's Hospital Crumlin, Dublin 12.
Sinead McDermott, Consultant Microbiologist, St Vincent's University Hospital, Dublin 4.
Martin Cormican, Consultant Microbiologist, Galway University Hospital, Galway.
Brendan Crowley, Consultant Microbiologist, St James's Hospital, Dublin 8.

Date

June 2017

Scope

The aim of this guideline is to provide a minimum national standard for practising professionals in the field of laboratory medicine and infection specialties in Ireland. These guidelines apply to adult (as defined by individuals aged at least 16 years), non-pregnant patients. It is envisaged that standardisation of the diagnostic process will help to assure the equivalence of investigation strategies in different laboratories nationally. This benefits public health surveillance, service development, and research activity.

Key Recommendations for Clinical Users

- Testing for Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), is recommended in patients in whom an abnormal liver chemistry profile (LCP) has been recorded, assuming no other obvious cause, and in those displaying signs or symptoms of acute hepatitis.

- Testing for Hepatitis E Virus (HEV) is recommended – in addition to the above – for all cases of acute symptomatic hepatitis e.g. acute jaundice, and those cases requiring hospitalisation.

- Testing for primary Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) infection may be considered as alternative viral causes of hepatitis, in cases where the results of the first-line screen are negative.

- Abnormal LCP can be defined as an increase of twice the upper limit of the normal (ULN) range[1].

- Individuals testing positive for acute or chronic HBV infection should also be screened for the presence of the Hepatitis Delta agent (HDV) [2] .

- New diagnoses of Hepatitis A, B, C, and E are notifiable to public health: HDV is not currently a notifiable infection[3].

- All individuals newly diagnosed with chronic HBV or HCV should be referred to a consultant hepatologist or infectious diseases physician for further assessment[2, 4].

- The severity of acute viral hepatitis is variable, ranging from asymptomatic to fulminant.

- If at the time of presentation, the LCP abnormalities are known to be present for more than 6 months in an immunocompetent individual, then testing for HBV and HCV only is reasonable (with reflex testing for HDV if HBV infected).

Key Recommendations for Laboratories

- Testing is recommended in consultation with clinicians in patients in whom an abnormal liver chemistry profile (LCP) has been recorded.

- Laboratories should be accredited to the ISO15189:2012 standard for Medical Laboratories – Requirements for Quality and Competence.

- Laboratories should perform accredited CE marked assays insofar as is possible.

- Laboratories should participate in ISO accredited external quality assurance (EQA) schemes.

- Individuals testing positive for acute or chronic HBV infection should also be screened for the presence of the Hepatitis Delta agent (HDV) in consultation with clinicians.

Background & Epidemiology

Viral hepatitis globally is responsible for an estimated 1.4 million deaths per year from acute infection and hepatitis-related liver cancer and cirrhosis – a toll comparable to that of HIV and tuberculosis. Of those deaths, approximately 47% are attributable to hepatitis B virus, 48% to hepatitis C virus, and the remainder to hepatitis A virus and hepatitis E virus[5,6]. In Ireland, (per www.hpsc.ie) for the last full year for which data are available (2015), there were 36 notified cases of Hepatitis A Virus (HAV), 549 cases of Hepatitis B Virus (HBV), and 675 cases of Hepatitis C Virus (HCV). There are no data available for Hepatitis Delta, and no 2015 data for Hepatitis E, which was only made notifiable in December of that year. However, there were 86 HEV notifications to the HPSC's CIDR (computerised infectious disease reporting) information system in 2016.

Despite the relatively small numbers reported, there is a significant amount of diagnostic testing for viral hepatitis performed each year in Ireland. In addition, anecdotally, it would appear that a significant amount of unnecessary, or duplicate, testing is also performed, especially for those hepatitis viruses that cause chronic infection, HBV and HCV. It is probable that a significant proportion of this unnecessary testing is the result of the lack of national guidance in the area. That being said, some of the duplicate testing is almost certainly the result of the absence of a

national medical laboratory information system. This latter issue is expected to be addressed over the next 1-3 years.

Testing

The most common infectious hepatitis is of viral aetiology[1]. In addition, the clinical features and course of uncomplicated acute viral hepatitis are similar among the several potential aetiological agents. As such, testing for HAV (fig.1), HBV (fig.2), and HCV (fig.3) is recommended (with reflex testing for HDV (fig.5) if HBV infected) for all individuals presenting with unexplained elevations in their liver chemistry profile. Testing for HEV is recommended in those individuals with acute symptomatic hepatitis, and in those individuals requiring hospitalisation.

Of note, acute viral hepatitis may result from infections other than those listed above, such as Cytomegalovirus (CMV), or Epstein Barr Virus (EBV): testing for these viruses can be considered second-line, assuming initial investigations are negative.

Who to Test

- Testing is recommended in all patients in whom an abnormal LCP has been recorded, with abnormal LCP defined as an increase of twice the upper limit of the normal range (ULN).
- Testing for HBV is recommended as part of the routine antenatal screen: this is intended to provide the opportunity to prevent the vertical transmission of HBV from the mother to the child[7].
- Testing for HCV in those who do not necessarily have an abnormal LCP should be performed in accordance with the National HCV Screening Guidelines (2017)[8].

Who Not to Test

- Assuming the presence of an abnormal LCP, or a clinical presentation consistent with acute hepatitis, there are no exclusions to this guidance: see Figures for pathogen-specific notes.

Who to Re-Test

- Please refer to Figures for pathogen-specific notes

Of note, minimum retesting intervals are for guidance only, and in particular clinical situations clinical judgement may mandate more frequent testing. In these cases the requesting clinician should discuss with the laboratory.

Specimen and Ordering Information

- All requests (electronic and paper) and specimens must adhere to the laboratories standard requirements. In order to comply with accreditation standards, laboratories cannot accept or process samples which do not meet minimum standards.
- All tests described herein should be performed on serum.
- Serum for testing should be processed and stored in accordance with the manufacturers' instructions for the assays being employed.

How to Test

- The diagnosis of acute HAV infection (fig.1) is based primarily on the detection of HAV-specific IgM class antibody (anti-HAV IgM).
- The diagnosis of HBV infection (fig.2) is based primarily on the detection of HBV surface antigen (HBsAg).
- The diagnosis of HCV infection (fig.3) is based primarily on the detection of HCV-specific IgG class antibody (anti-HCV).
- The diagnosis of HEV infection (fig.4) is based primarily on the detection of HEV-specific IgM class antibody (anti-HEV IgM).
- The diagnosis of Hepatitis Delta (fig.5) is based primarily on the detection of HDV-specific IgG class antibody (anti-HDV).

Interpretation of tests

- Please refer to Figures for pathogen-specific notes

Figure 1: Hepatitis A Virus (HAV) Acute Infection Serology (*Immunocompetent*)

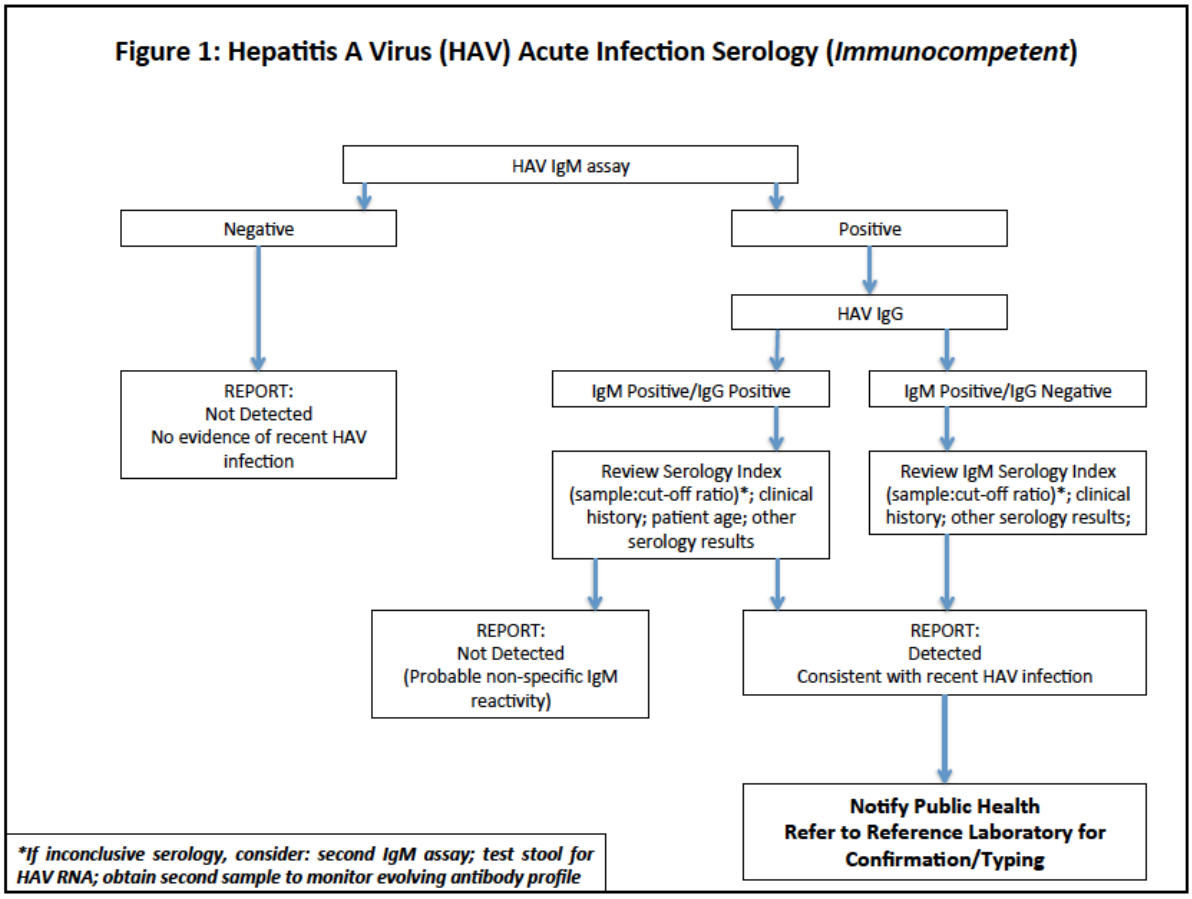


Figure 1 Notes:

i. HAV IgM might not be reliable in those who are significantly immunocompromised: consider referring for HAV RNA testing[9].

ii. Specificity of HAV IgM assays can be suboptimal: as such, results should ideally be interpreted in conjunction with clinical details, patient age, HAV-specific risk factors, and the results of other serology assays e.g. HAV IgG, EBV VCA IgM[10].

iii. When reviewing positive HAV IgM results, the strength of the reactivity i.e. the *serology index* should be taken into account. The serology index refers to the strength of reactivity in the patient sample, compared with the threshold for positivity for the assay. This is also known as the sample:cut-off ratio. Whilst it is not possible to include a single value in the algorithm, as all assays are different, in general true IgM positives would be expected to yield sample:cut-off ratios of at least 3-5.

iv. Laboratories – insofar as is feasible – should be able to provide information to clinicians on the level of reactivity observed in the IgM assay: e.g. through the use of *equivocal* or *weak positive* ranges. Of note, any such ranges should be in keeping with the manufacturers' instructions for reporting results.

v. HAV IgG testing should be performed on all patients in whom IgM testing is positive.

vi. The presence of a convincing HAV IgG result in the setting of a weakly reactive IgM result raises the possibility of non-specificity within the IgM assay.

vii. HAV IgM might be reactive after recent vaccination[11].

viii. A negative HAV IgM result on a sample taken within 5 days of onset of symptoms does not exclude recent HAV infection[11].

ix. False positive IgM results are more common in older adults, and those from developing countries, as they are likely to have had HAV infection previously[12].

x. Interpret positive HAV IgM results in those over 55 years of age with caution as they are likely to have been infected previously.

xi. HAV IgM can remain detectable for 6 months following primary infection[13].

xii. Follow-up serum samples should be obtained from those patients whose initial specimens yield inconclusive results: consideration may also be given to testing stool for the presence of HAV RNA.

xiii. Laboratories may consider including a second IgM assay in their local algorithm as this may be useful in confirming true positives, and/or identifying false positives.

xiv. All positive HAV samples should be referred to the National Virus Reference Laboratory (NVRL) so typing can be attempted.

xv. Please note that HAV IgM testing is not indicated if assessing immunity to HAV for the purposes of informing vaccination policy: HAV IgG testing only is recommended. However, per the Immunisation Guidelines for Ireland, (www.immunisation.ie) universal pre-vaccination testing is not indicated (see point xvi below).

xvi. For those aged over 50 years or with a history of jaundice, haemophilia or residence in a high-risk area, pre-vaccination testing for immunity to hepatitis A may be considered in order to reduce costs.

xvii. Post-vaccination testing for anti-HAV is not routinely indicated.

xviii. There is currently no recommendation for HAV vaccine booster doses in the immunisation guidelines as the duration of the vaccine induced immune response has been demonstrated to protect for at least 15 years. It is likely that at least 95% and 90% of subjects will remain seropositive 30 and 40 years after vaccination, respectively[14].

Figure 2: Hepatitis B Virus (HBV) Diagnostic Serology

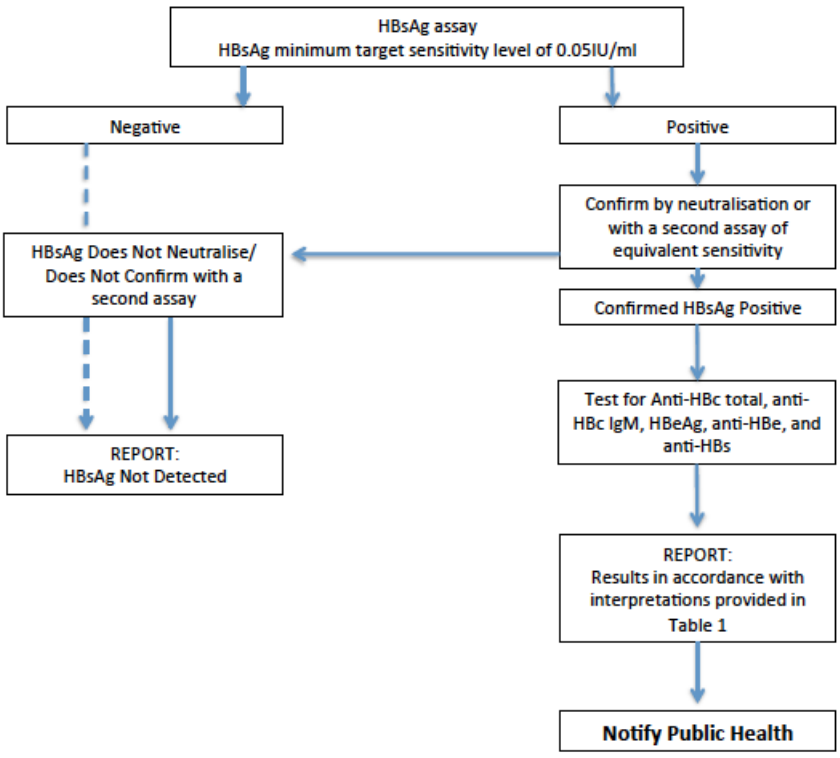


Figure 2 Notes:

i. It is recommended that only HBsAg assays capable of detecting immune/vaccine escape variants should be used[15].

ii. Haemolysed samples are prone to yield non-neutralisable false reactive results[15].

iii. All newly diagnosed individuals with chronic hepatitis should be referred to a hepatologist or an Infectious Diseases physician.

iv. All newly diagnosed individuals with HBV infection (acute or chronic) should be tested for the presence of antibody to Hepatitis Delta (HDV): laboratories – in consultation with clinicians – may consider the introduction of reflex testing for HDV (Figure 5) to ensure this practice is observed.

v. A second sample should be obtained from all individuals newly diagnosed with HBV infection to confirm their status.

vi. HBV DNA testing is not required to confirm a diagnosis of acute or chronic HBV infection: as such, it is not routinely indicated at the time of diagnosis.

vii. Consideration should be given to performing HBV genotyping in acute HBV infection, for public health purposes (contact tracing, outbreak investigation etc.).

viii. Once an individual is confirmed to be chronically infected, subsequent routine testing should comprise only HBsAg and HBeAg/anti-HBe (based on whether the patient is HBeAg positive or negative).

ix. Anti-HBc and anti-HBc IgM testing are not routinely indicated in the monitoring of known chronically infected individuals.

x. As a general rule, annual HBV serology (comprising HBsAg and HBeAg/anti-HBe) is sufficient when monitoring chronically infected individuals: however, those individuals receiving antiviral treatment may require more frequent testing e.g. 3 or 6 monthly. As such, no single absolute rule applies. Nonetheless, where possible, local agreements should be established with hepatologists, infectious disease (ID) physicians, or clinical nurse specialists/advanced nurse practitioners to mimimise unnecessary testing.

xi. Recent vaccination can lead to a positive HBsAg result if testing is performed within 7-10 days of vaccine administration[16].

Table 1: Interpretation of HBV serology

HBsAg	HBsAg (confirmation)	HBeAg	Anti-HBe	Anti-HBc Total	Anti-HBc IgM	Anti-HBs	Interpretation
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Susceptible to HBV
POS	Neg	Neg	Neg	Neg	Neg	Neg	False positive HBsAg; negative HBV serology
POS	POS	Neg	Neg	Neg	Neg	Neg	Early HBV infection; recent HBV vaccine; or non-specificity within the assay. Obtain follow-up sample promptly to clarify status
POS	POS	POS	Neg	POS	POS	Neg	Acute HBV infection. Obtain second sample to confirm; notify public health; consider genotyping. Screen and vaccinate household contacts
POS	POS	POS/Neg	POS/Neg	POS	Neg	Neg	Chronic HBV infection. Obtain second sample to confirm. Hepatology/ID referral recommended. Notify public health. Screen and vaccinate household contacts
Neg	Neg	Neg	POS/Neg	POS	Neg	POS/Neg	Resolved HBV infection
Neg	Neg	Neg	Neg	Neg	Neg	POS	Response to HBV vaccine

Table 1 Notes:

i. Anti-HBc IgM may be detectable in recent acute infection, during a flare of viral replication, or at a low level in chronic HBV infection: the level of anti-HBc IgM can be useful to distinguish between these scenarios. (Acute cases are more likely to have levels over 200 Paul Ehrlich Units/ml; flares typically have levels of less than 50.)[15]

ii. It is advisable to confirm isolated anti-HBc positive results with a second assay, ideally of a different format, as isolated anti-HBc sometimes represents false reactivity: anti-HBs testing might also aid interpretation if the vaccine history is available.

iii. Isolated anti-HBc positive results should also be confirmed as the administration of IVIG, blood, or blood products can lead to a transient positive anti-HBc result in the recipient. In this case, repeat testing to clarify anti-HBc status should be performed > 21days after the last administration.

iv. The detection of HBsAg in isolation can reflect early acute infection, false reactivity, or the detection of recombinant HBsAg from vaccine. If suspecting acute infection, HBV DNA testing is recommended to confirm the HBsAg is genuine.

v. Household contacts of HBV infected individuals should be screened for evidence of infection, and vaccinated if non-immune[15].

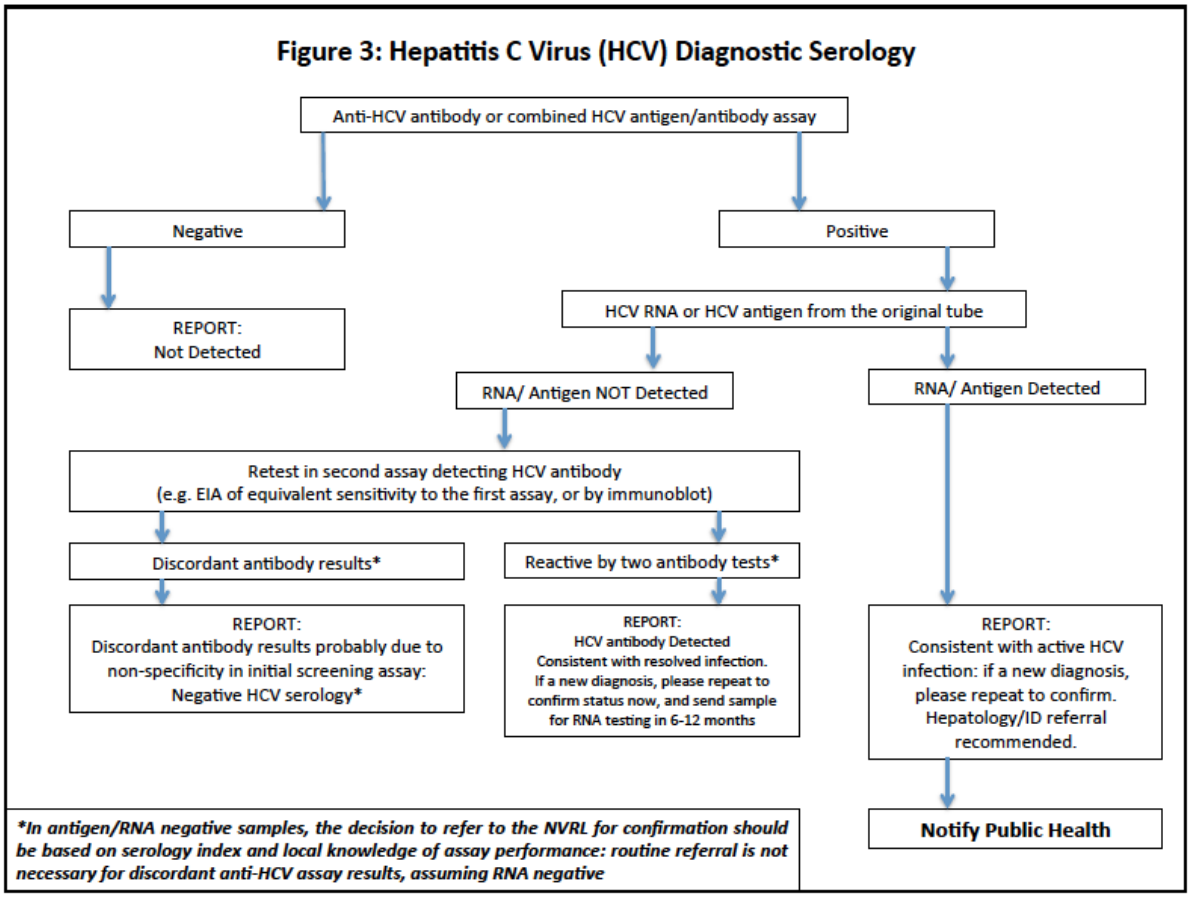


Figure 3 Notes:

i. A second sample should be obtained from all individuals newly diagnosed with HCV infection, active or resolved, to confirm their status.

ii. In cases of recent HCV contact or exposure risk, RNA testing after 6 weeks is preferred to serology. However, the majority of immunocompetent individuals will have detectable anti-HCV by 12 weeks post-exposure[17].

iii. Individuals infected with HCV typically remain anti-HCV positive for life: as such, repeat antibody testing is unnecesary once the patient's HCV status has been determined.

iv. If anti-HCV reactivity is low in screening assays, consideration should be given to checking specificity with an HCV immunoblot in those individuals who are antigen/RNA negative: this should distinguish true past infection from non-specific reactives[17].

v. An alternative approach to weakly reactive anti-HCV results is to obtain a second sample for repeat serology in 4-6 weeks, as this could minimise costs by reducing unnecessary RNA testing. An increase in the serology index (sample:cut-off ratio) between the two specimens might suggest an evolving – hence genuine – anti-HCV response.

vi. Individuals with persistent non-progressing low level reactivity in the anti-HCV screening assays over two or more samples should be referred to the NVRL for additional testing to distinguish between resolved infection and false positive anti-HCV results.

vii. Of note, it is not necessary to perform an HCV immunoblot in those individuals who have detectable antibody and antigen/RNA at diagnosis.

viii. Depending on the serology index (sample:cut-off ratio) of the anti-HCV result available in the local laboratory, it is not necessarily essential for all samples with evidence of resolved infection to be referred to the NVRL for HCV immunoblot. However, a second EIA must be performed if not referring for immunoblot.

ix. If testing anti-HCV positive individuals who are at risk of HCV reinfection, HCV RNA or HCV antigen testing alone should be performed: there is no need to repeat anti-HCV testing.

x. The frequency of screening at-risk individuals for HCV reinfection depends on the nature of the ongoing exposure risk, and should be carried out in accordance with national guidelines[8].

Figure 4: Hepatitis E Virus (HEV) Acute Infection Serology (*Immunocompetent*)

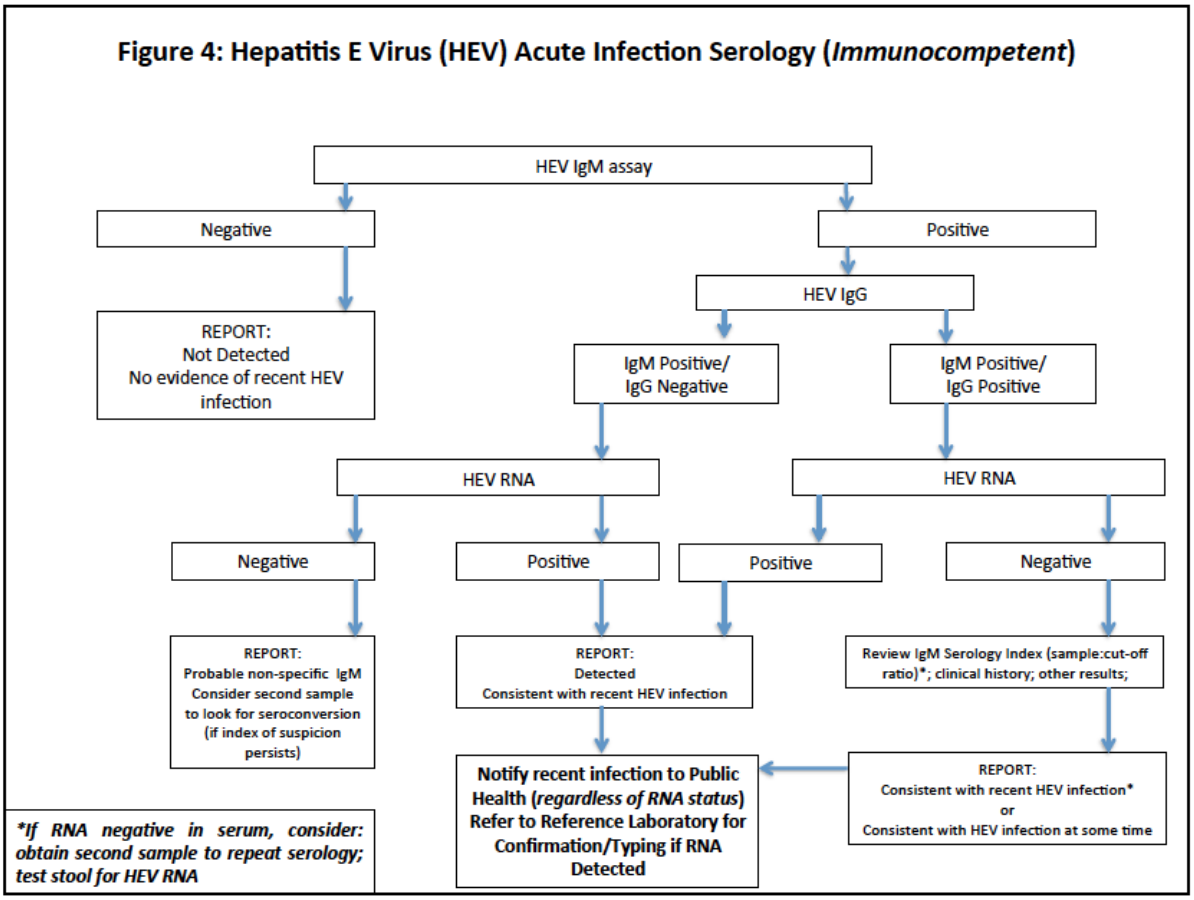


Figure 4 Notes:

i. HEV genotyping is recommended for public health reasons to distinguish imported infections from those acquired locally.

ii. In immunocompromised patients, HEV serology might be unreliable, so HEV RNA testing should be considered if suspecting HEV infection[18].

iii. HEV can cause chronic infection in immunocompromised individuals, and should be considered if individuals in those groups have chronic abnormal LCPs[18].

iv. HEV IgG testing should be performed in all individuals testing positive for HEV IgM to assist with the interpretation of results.

v. When reporting results, the serology index (sample:cut-off ratio) for the IgM and IgG should be reviewed in the context of the clinical presentation.

vi. A negative HEV RNA in serum does not exclude the diagnosis of HEV in the setting of convincing serology, as the period of viraemia in HEV is short (<3 weeks)[18].

vii. HEV testing is not required in all cases of abnormal liver chemistry profile: however, as a notifiable infection, it should be considered in cases of acute symptomatic hepatitis, in individuals requiring hospitalisation, in returning travellers, and in those individuals with known occupational risk factors, specifically farmers, food handlers, and those with exposure to pork or pork products.

Figure 5: Hepatitis Delta Diagnostic Serology

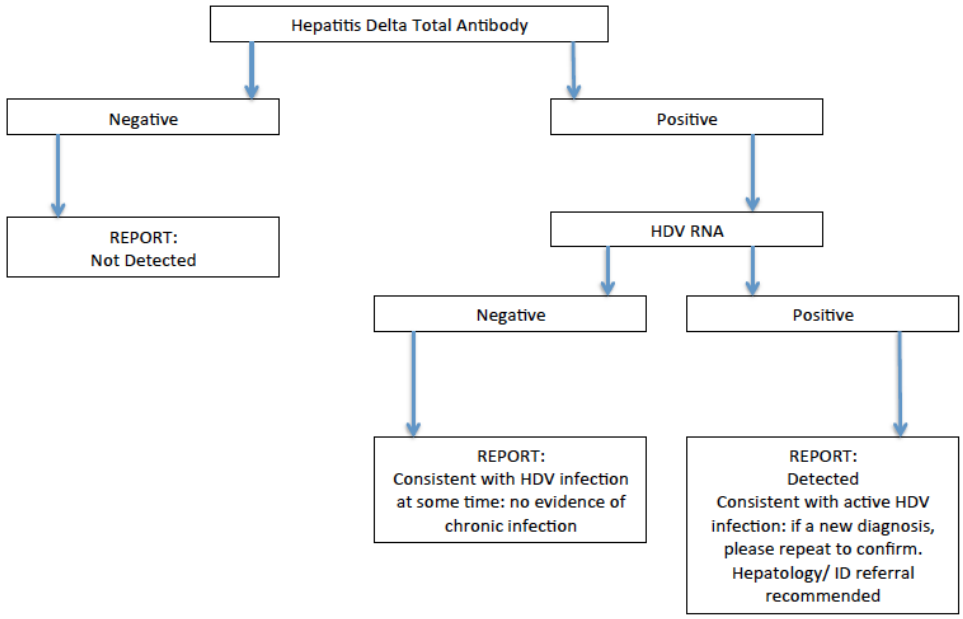


Figure 5 Notes:

i. HDV testing should be performed in all individuals newly diagnosed with HBV infection, as defined by being HBsAg positive: the presence of HDV will affect the choice of antiviral therapy if these individuals are being considered for treatment[2].

ii. Long-term follow-up HDV RNA monitoring is recommended for all treated patients, as long as HBsAg remains in serum, as late-stage relapses can occur.

iii. HDV testing is not indicated in individuals who test negative for HBsAg.

iv. HDV testing should be performed in those individuals with chronic HBV infection who experience an unexplained elevation in LCP.

Recommendations for National Laboratory Information System (MedLIS)

MedLIS should include the following categories for suspected viral hepatitis:

1. Suspected acute viral (symptomatic) hepatitis:
Test for HAV IgM, HBsAg, HCV antibody/antigen, and HEV IgM.

2. Suspected chronic viral hepatitis:

Test for HBsAg, and HCV antibody.

3. Hepatitis Immunity Screen (indicated either post-vaccination, or pre-iatrogenic immunosuppression)

Test for HAV IgG, anti-HBc, anti-HBs, and HCV antibody.

4. Viral hepatitis screen in the immunocompromised patient

Test for HAV RNA, HBsAg, HCV RNA, and HEV RNA.

Information for Patients

The Hepatitis viruses A, B, C, D, and E are members of different virus families that can cause infection and inflammation of the liver. Hepatitis A virus (HAV) and Hepatitis E virus (HEV) are acquired through eating or drinking contaminated water or food stuffs, and in those individuals with an otherwise healthy immune system, typically cause only a short self-limiting infection that does not cause any long-term damage to the individual or the liver. There is no specific antiviral treatment for HAV or HEV. However, there is a vaccine available for the prevention of HAV and it is recommended that individuals intending to travel to countries where HAV is common should be vaccinated. There are fewer than 100 cases of HAV and HEV reported in Ireland each year.

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are more serious infections as they have the capacity to cause chronic (ongoing) infection in the liver, that can over time result in fibrosis (scar tissue formation in the liver), cirrhosis (severe fibrosis), and liver cancer. That being said, the majority (95%) of adults who acquire HBV as adults will resolve the infection themselves. Similarly, between 60% and 70% of adults who acquire HCV infection will also resolve the infection themselves. In contrast to HAV and HEV, HBV and HCV are acquired through exposure to the blood and body fluids of other infected individuals. This is why these infections are more common in persons who inject drugs, men who have sex with men, and prisoners. There are between 500 and 600 cases of HBV and HCV reported in Ireland each year. For those individuals infected with HBV or HCV, antiviral treatment is available, and for HCV treatment is essentially curative. The treatment for HBV is also very effective but will not cure all those infected. Fortunately however, for HBV there is a very safe and effective vaccine that was introduced into the universal immunisation schedule for children in Ireland in July 2008. There is currently no vaccine available for HCV.

Hepatitis delta (HDV) is an unusual virus, or more accurately, a sub-viral particle, as it is incapable of infecting individuals on its own. HDV requires the presence of HBV to cause infection in humans, and as such is only seen in those individuals who also have HBV infection. As a result, HDV can cause both acute and chronic infection, and like HBV and HCV, is acquired through contact with the blood and bodily fluids of those already infected. As HDV is not currently a notifiable infection in Ireland, we do not know exactly how many cases occur each year. However, it would appear to be a small number based on our clinical experience. That being said, it is important to look for HDV in those individuals infected with HBV, as it does have an impact on the

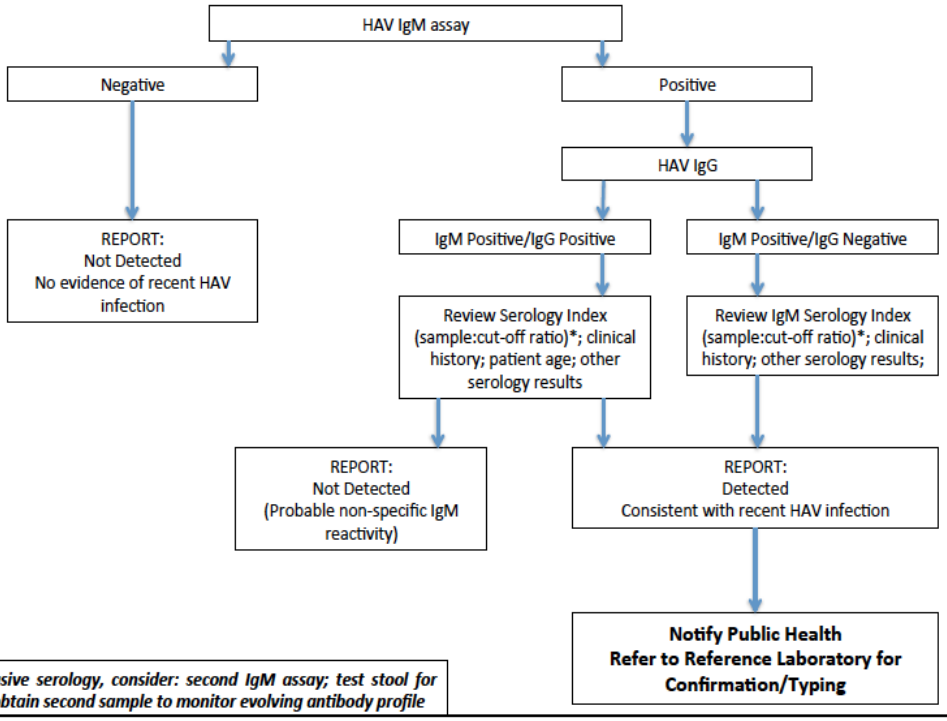
choice of antiviral therapy for HBV. Although there is no vaccine against HDV, the vaccine against HBV also provides protection against HDV on account of the relationship between the two viruses.

References

1. Public Health England. 2014. Acute Infective Hepatitis. UK Standards for Microbiology Investigations. S 1 Issue 1.3. *https://www.gov.uk/guidance/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories*.
2. European Association for the Study of the Liver. 2017. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370-398.
3. Health Protection Surveillance Centre. 2016. List of Notifiable Diseases. *http://www.hpsc.ie/notifiablediseases/listofnotifiablediseases/File,678,en.pdf*. [Accessed July 2017].
4. European Association for the Study of the Liver. 2017. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol 2017;66:153-194.
5. WHO Guidelines on hepatitis B and C testing. Geneva: World Health Organisation; 2017. Licence: CC BY-NC-SA 3.0 IGO.
6. Global Hepatitis Report 2017. Geneva: World Health Organisation; 2017. Licence: CC BY-NC-SA 3.0 IGO.
7. American Academy of Pediatrics [Hepatitis B]. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. Red Book: 2015 Report of the Committee on Infectious Diseases. 30th Ed. Elk Grove Village, IL: American Academy of Pediatrics;2015:[400-423].
8. Department of Health (2017). Hepatitis C Screening (NCEC National Clinical Guideline No. 15). http://health.gov.ie/national-patient-safety-office/ncec/national-clinical-guidelines/.
9. Chakvetadze C, MalletV, Gaussec L, Hannoun L, Pol S. 2011. Acute hepatitis A virus infection without IgM antibodies to hepatitis A virus. Ann Intern Med 2011;154:507-508.
10. Thomas L and the Hepatitis A Guidelines Group. 2009. Guidance for the prevention and control of hepatitis A virus infection. Health Protection Agency.
11. Advisory Committee on Immunization Practices. 1999. Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1999;48:1-37.
12. Center for Disease Control and Prevention. 2005. Positive test results for acute hepatitis A virus infection among persons with no recent history of acute hepatitis, United States, 2002-2004. MMWR Wkly Rep 2005;54:453-6.
13. Kao HW, Ashcavai M, Redeker AG. 1984. The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. Hepatology 1984;4;933-6.
14. Royal College of Physicians of Ireland. Immunisation Guidelines for Ireland [Hepatitis A]. http://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/CH8_Hepatitis_A.pdf.
15. Public Health England. 2014. Hepatitis B Diagnostic Serology in the Immunocompetent (including Hepatitis B in Pregnancy). UK Standards for Microbiology Investigations. V 4 Issue 5.3. *https://www.gov.uk/guidance/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories*.
16. Dow BC, Yates P, Galea G, Munro H, Buchanan I, Ferguson K. 2002. Hepatitis B vaccinees may be mistaken for confirmed hepatitis B surface antigen-positive blood donors. Vox Sang 2002;82:15-7.
17. Public Health England. 2014. Investigation of Hepatitis C Infection by Antibody Testing or Combined Antigen/Antibody Assay. UK Standards for Microbiology Investigations. V 5 Issue 6.2. *https://www.gov.uk/guidance/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories*.
18. Kamar N, Dalton HR, Abravanel F, Izopet J. 2014. Hepatitis E Virus Infection. Clin Microbiol Rev 2014;27(1):116. DOI:10.1128/CMR.00057-13.

Appendices

Figure 1: Hepatitis A Virus (HAV) Acute Infection Serology (*Immunocompetent*)



**If inconclusive serology, consider: second IgM assay; test stool for HAV RNA; obtain second sample to monitor evolving antibody profile*

Figure 1 Notes:

i. When reviewing positive HAV IgM results, the strength of the reactivity i.e. the serology index / sample cut-off ratio should be taken into account.

ii. The presence of a convincing HAV IgG result in the setting of a weakly reactive IgM result raises the possibility of non-specificity within the IgM assay.

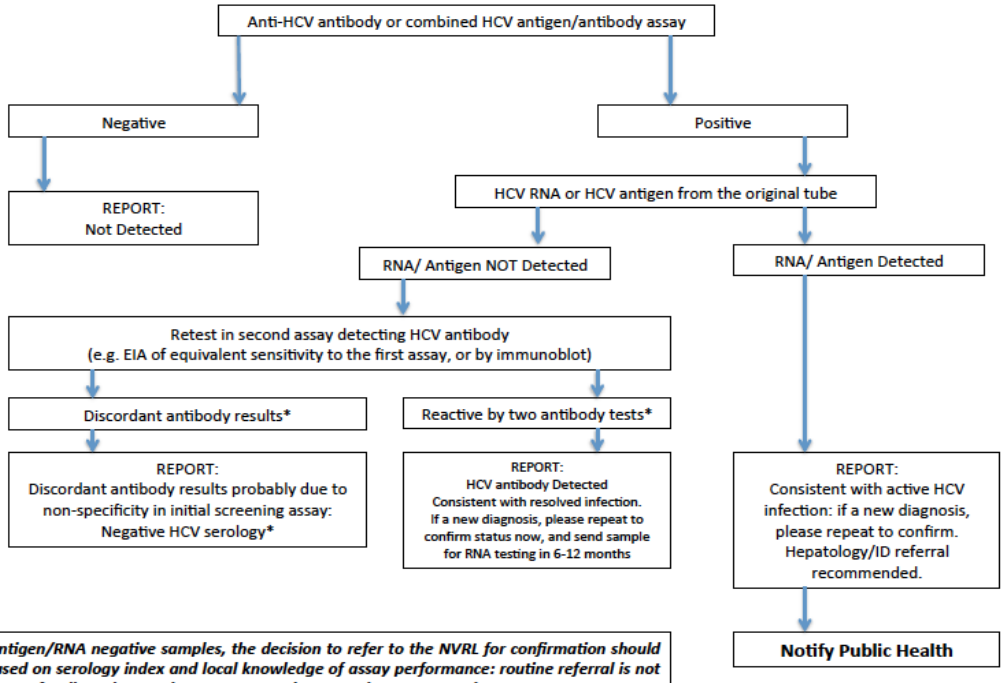
iii. HAV IgM might be reactive after recent vaccination

iv. A negative HAV IgM result on a sample taken within 5 days of onset of symptoms does not exclude recent HAV infection.

v. Interpret positive HAV IgM results in those over 55 years of age with caution as they are likely to have been infected previously.

vi. HAV IgM can remain detectable for 6 months following primary infection.

Figure 3: Hepatitis C Virus (HCV) Diagnostic Serology



**In antigen/RNA negative samples, the decision to refer to the NVRL for confirmation should be based on serology index and local knowledge of assay performance: routine referral is not necessary for discordant anti-HCV assay results, assuming RNA negative*

Figure 3 Notes:

i. Individuals infected with HCV typically remain anti-HCV positive for life: as such, repeat antibody testing is unnecesary once the patient's HCV status has been determined.

ii. If anti-HCV reactivity is low in screening assays, consideration should be given to checking specificity with an HCV immunoblot in those individuals who are antigen/RNA negative: this should distinguish true past infection from non-specific reactives.

iii. If testing anti-HCV positive individuals who are at risk of HCV reinfection, HCV RNA or HCV antigen testing alone should be performed: there is no need to repeat anti-HCV testing.

Figure 2: Hepatitis B Virus (HBV) Diagnostic Serology

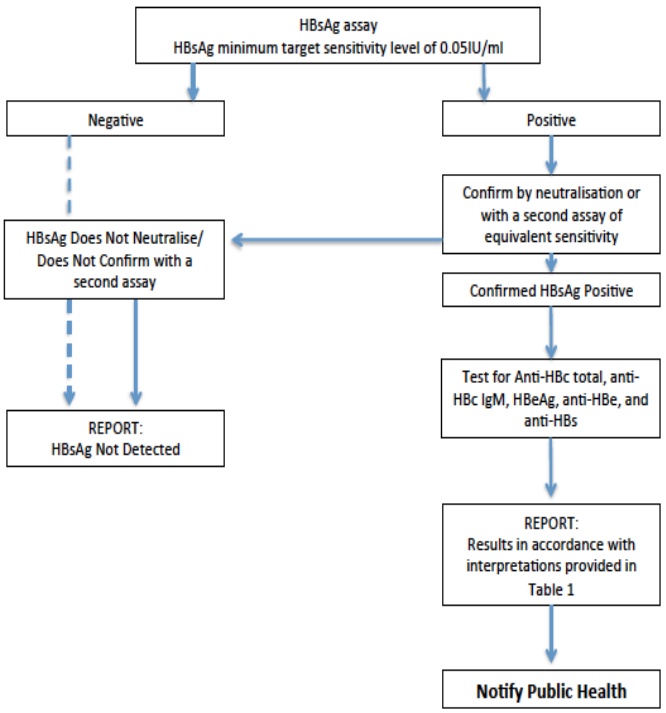


Figure 2 Notes:

i. All newly diagnosed individuals with HBV infection (acute or chronic) should be tested for the presence of antibody to Hepatitis Delta (HDV).

ii. HBV DNA testing is not required to confirm diagnosis of acute or chronic HBV infection.

iii. Consideration should be given to performing HBV genotyping in acute HBV infection, for public health purposes (contact tracing, outbreak investigation etc).

iv. In those chronically HBV infected, monitoring should comprise only HBsAg and HBeAg/anti-HBe (based on patient status).

v. Anti-HBc and anti-HBc IgM testing are not routinely indicated in the monitoring of known chronically infected individuals.

Table 1: Interpretation of HBV serology

HBsAg	HBsAg (confirmation)	HBeAg	Anti-HBe	Anti-HBc Total	Anti-HBc IgM	Anti-HBs	Interpretation
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Susceptible to HBV
POS	Neg	Neg	Neg	Neg	Neg	Neg	False positive HBsAg; negative HBV serology
POS	POS	Neg	Neg	Neg	Neg	Neg	Early HBV infection; recent HBV vaccine; or non-specificity within the assay. Obtain follow-up sample promptly to clarify status
POS	POS	POS	Neg	POS	POS	Neg	Acute HBV infection. Obtain second sample to confirm; notify public health; consider genotyping. Screen and vaccinate household contacts
POS	POS	POS/Neg	POS/Neg	POS	Neg	Neg	Chronic HBV infection. Obtain second sample to confirm. Hepatology/ID referral recommended. Notify public health. Screen and vaccinate household contacts
Neg	Neg	Neg	POS/Neg	POS	Neg	POS/Neg	Resolved HBV infection
Neg	Neg	Neg	Neg	Neg	Neg	POS	Response to HBV vaccine

Table 1 Notes:

i. Anti-HBc IgM may be detectable in recent acute infection, during a flare of viral replication, or at a low level in chronic HBV infection.

ii. It is advisable to confirm isolated anti-HBc positive results with a second assay, ideally of a different format, as isolated anti-HBc sometimes represents false reactivity: anti-HBs testing might also aid interpretation if the vaccine history is available.

iii. Isolated anti-HBc positive results should also be confirmed on a second sample as the administration of IVIG, blood, or blood products can lead to a transient positive anti-HBc result in the recipient. In this case, repeat testing to clarify anti-HBc status

Figure 4: Hepatitis E Virus (HEV) Acute Infection Serology (*Immunocompetent*)

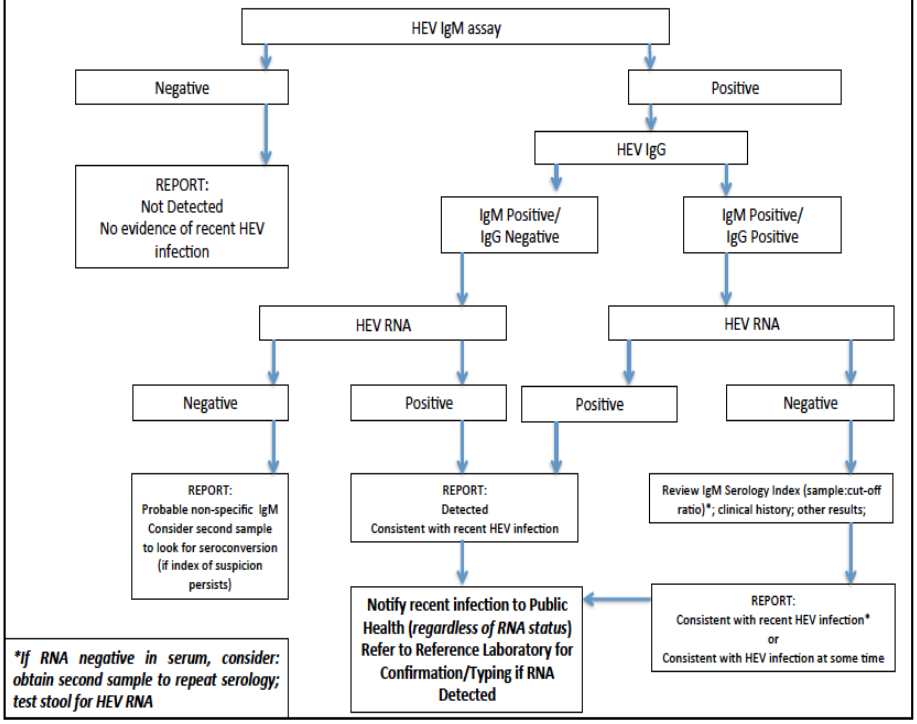


Figure 4 Notes:

i. HEV genotyping is recommended for pub health reasons to distinguish imported infec from those acquired locally.

ii. HEV IgG testing should be performed in individuals testing positive for HEV IgM to a with the interpretation of results.

iii. When reporting results, the serology ind (sample:cut-off ratio) for the IgM and IgG sh be reviewed in the context of the clinical presentation.

iv. A negative HEV RNA in serum does not exclude the diagnosis of HEV in the setting convincing serology, as the period of viraer HEV is short (<3 weeks).

Figure 5: Hepatitis Delta Diagnostic Serology

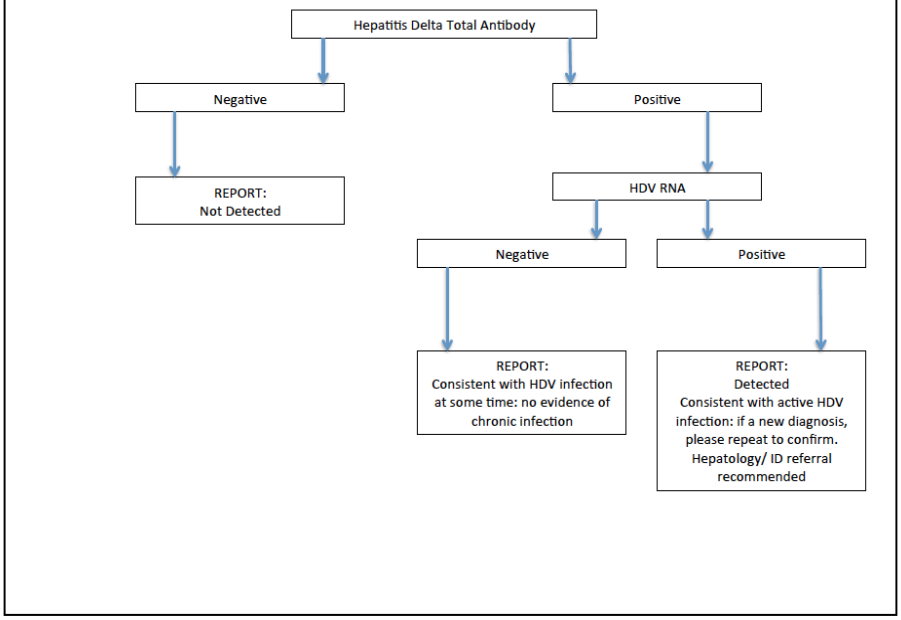


Figure 5 Notes:

i. HDV testing should be performed in all individuals newly diagnosed with HBV infection, as defined by being HBsAg positive: the presence of HDV will affect the choice of antiviral therapy if these individuals are being considered for treatment.

ii. Long-term, follow-up HDV RNA monitoring is recommended for all treated patients, as long as HBsAg remains in serum, as late-stage relapses can occur.

iii. HDV testing is ***not*** indicated in individuals who test negative for HBsAg.