Hepatitis E (*Hepatitis E virus*)

Laboratory case definition

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

Version: 1.0
Authorisation: PHLN
Consensus date: January 2015

---

1 PHLN Summary Laboratory Definition

1.1 Condition:

Hepatitis E virus infection

1.1.1 Definitive Criteria

1. Detection of *hepatitis E virus* nucleic acid in blood or tissue specimens, or
2. Isolation of *hepatitis E virus* in cell culture, with confirmation by a nucleic acid detection test, or
3. Seroconversion of IgG or total antibody titres against *hepatitis E virus*, or
4. A four-fold or greater rise in IgG or total antibody titres against *hepatitis E virus* during or after a compatible clinical illness

1.1.2 Suggestive Criteria

1. Detection of IgM directed against *hepatitis E virus* in a single specimen

Note: Any cases believed to be acquired in Australia (i.e. no history of recent travel to a country with known hepatitis E activity) should be confirmed by a second reference laboratory.

2 Introduction

*Hepatitis E virus (*HEV) is a positive-sense single-stranded RNA virus with an icosahedral capsid and no envelope, classified as a species in the unclassified family *Hepeviridae*, that can infect a wide range of mammalian species. Related species of virus, that have not yet been shown to cause human disease, have been found in birds and bats.

Human infections have been documented in most countries of the world. Epidemic disease is confined to developing countries and is caused by genotypes 1 and 2, while milder sporadic autochthonous disease due to genotypes 3 and 4 is found in both developing and developed countries [9]. The latter form of hepatitis E has been found in Australia from consumption of contaminated pork (http://www.health.nsw.gov.au/Infectious/factsheets/Pages/Hepatitis_E.aspx). However, the majority of HEV hepatitis in Australia occurs in people who have travelled to developing countries.

In humans, HEV infection generally causes a self-limiting syndrome of acute hepatitis, similar to hepatitis A virus [11]. There are some important clinical differences - disease in pregnant women is often fulminant, and chronic infection in immunocompromised patients is recognised [12]. Also, unlike hepatitis A, person-to-person spread is rare. Infection is usually acquired by ingestion of contaminated food or water and outbreaks usually result from a common point source [9]. There is also the possibility of transmission through food prepared from animals that are infected with the virus, especially pork products. Cured meats have been implicated as a vehicle for transmission between species. Transmission via blood transfusion has occurred rarely, and may be under-diagnosed.

There are at least 4 genotypes of HEV. The epidemic genotypes (1 and 2) are associated with outbreaks of human infection in developing countries, whilst the zoonotic genotypes (3 and 4) are associated with infection in other mammals in addition to autochthonous human infection. The genome size is 7.2 kilobases, and includes three open reading frames (ORFs). ORF1 encodes a non-structural protein with several functions including RNA-dependent RNA polymerase activity, and ORF2 encodes the capsid. ORF3 is smaller, overlaps ORFs 1 and 2, and encodes a phosphoprotein involved in virion formation and release. Tissues with viral replication include lymph nodes and intestine, in addition to liver [19]. Cell lysis is not essential for virus release and the virus is relatively non-cytopathic [11].

2.1 Clinical Presentation

The incubation period is 2 to 8 weeks. Patients typically present with acute hepatitis with fever and jaundice, clinically resembling hepatitis A virus infection. Elevated plasma liver enzymes, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are the most sensitive markers for hepatitis during acute HEV infection. In many cases there are additional abnormalities in liver function, commonly hyperbilirubinaemia and in severe cases abnormal coagulation profiles and elevated blood ammonia levels. Subclinical infection and chronic infection of immunocompromised patients may occur. A small proportion of patients with biochemical evidence of hepatitis believed to be caused by drug reactions were found to have evidence of current or recent HEV infection [6]. Although previous HEV infection generally leads to lifelong immunity in immunocompetent patients, it is possible that self-limiting infection with virus shedding in stool may occur in seropositive patients who are re-exposed to HEV. Chronic infection lasting months or years is well-described in severely immunocompromised patients such as transplant recipients. In these patients, antibody tests are unreliable and diagnosis relies on nucleic acid detection in blood or faeces.

In animal models, severity of disease is correlated with the magnitude of the infective dose. During pregnancy, severity of disease also appears to be linked to viral load [5]. However hepatic damage is believed to arise mostly as a result of the immune response to HEV infection. Maximum viraemia, viral

shedding in faeces and transmissibility occurs during the incubation period, shortly before the onset of symptoms and before the start of the antibody response.

2.2 Treatment

In general there is no proven specific treatment for HEV infection. Interferon-α and/or ribavirin have been used, particularly in immunocompromised patients [9].

2.3 Prevention

There is no TGA- or FDA-approved vaccine. Two vaccines have undergone randomised controlled efficacy trials. The GSK vaccine [16] uses a recombinant HEV antigen expressed using a baculovirus system. The Xiamen Biologicals vaccine [20] uses a bacterially expressed recombinant HEV antigen. In both trials, vaccine was administered to adults in a 3-dose regimen with alum adjuvant.

3 Tests

At present there are no FDA-approved *in vitro* diagnostic (IVD) tests for hepatitis E virus. However, there is one TGA-approved IVD, described below, and a number of CE-marked assays.

3.1 Culture

Efficient cell culture systems for HEV have been developed [17]. Several cell lines, including PLC/PRF/5, A549 and HepG2/C3A, can support the growth of HEV. However, cell culture is rarely used for routine diagnostic purposes.

3.2 Nucleic Acid Testing

The most highly conserved portion of the HEV genome comprises ~300 nucleotides at the 5'-end of ORF2. There is a second shorter and somewhat less conserved region near the 5'-end of ORF1.

There are currently no TGA-approved IVD kits for HEV RNA detection. CE-marked kits are available from several manufacturers and there is a WHO International Standard (PEI 6329/10) for HEV RNA developed using HEV genotype 3 [Baylis2013].

3.2.1 Specimens

Typical specimens for HEV nucleic acid testing are serum, plasma and faeces, but it can also be performed on liver and other tissue samples.

3.2.2 Test Sensitivity

The clinical sensitivity of HEV NAT is not known. HEV RNA is detectable in blood and faeces about 2 weeks after infection, and precedes the onset of illness, and RT-PCR appears to be more sensitive than IgM early in the course of infection [18]. It remains detectable for 2-3 months in the serum and 3-4 months in the faeces [9]. As described in Section 2.1, maximum viraemia occurs during the incubation period and falls as symptoms commence.

3.2.3 Test Specificity

The clinical specificity of HEV NAT is not known. In general, with a well-designed assay and good laboratory procedures, the specificity for a PCR-based nucleic acid test should be >95-97%. Positive results can be confirmed by sequencing if necessary.

3.2.4 Suitable Test Verification/Validation Criteria

All testing should be performed in accordance with NPAAC "*Requirements for the Development and Use of In-House* In Vitro *Diagnostic Medical Devices*" and "*Requirements for Medical Testing of Microbial Nucleic Acids*". Commercial kit performance should be verified in accordance with NATA requirements. It is recommended that all diagnostic test results are traceable to the WHO International Standard (PEI 6329/10), with additional controls and participation in EQAS or inter-laboratory exchange where possible.

3.2.5 Suitable External QC Programme

Neither RCPA QAP nor NRL offer a HEV nucleic acid EQAS module. Internationally, Quality Control for Molecular Diagnostics (QCMD) and Instand offer HEV RNA in their EQAS programmes.

3.3 Serological Diagnosis

Although there are four genotypes that infect humans, the antigenic epitopes from ORF2 and ORF3 are shared, so HEV comprises a single serotype.

Anti-HEV IgM and IgG antibodies appear about 2 weeks after infection, and IgM persists for 3-12 months, or longer in up to 25% of cases [13]. Anti-HEV IgG persists for life. For this reason, the diagnosis of current or recent HEV infection based on IgM detection is only reliable if there is a concordance of epidemiological, clinical and laboratory findings. Recent HEV infection can also be indicated by either seroconversion from IgG negative to IgG positive between acute and convalescent sera, or by detecting a significant (eg. four-fold or greater) rise in anti-HEV titres. Presence of anti-HEV IgG without IgM in a single sample is consistent with previous HEV infection.

Anti-HEV antibodies can be detected using commercial or in-house EIA or using immunochromatographic (ICT) assays. Supplemental testing by Western blot can be performed if necessary, particularly where there is suspected acquisition within Australia.

Western blot assays are more specific that the EIA and ICT assays and are particularly useful for confirming specificity of IgG and/or IgM in circumstances where the EIA/ICT results are unclear and/or the patient has no history of travel to a developing country.

HEV antigen detection has been studied in serum derived from a blood donor population [18]: this successfully detected a subset of patients who had positive HEV nucleic acid tests (NAT) and negative antibody tests who may have been early in the course of the illness. However, antigen detection appeared to be less sensitive than NAT.

A WHO reference reagent (International Biological Reference Preparation) of lyophilised anti-HEV serum is held as NIBSC 95/584 [8].

3.3.1 Specimens

Serum for HEV antibody or antigen testing can be obtained using blood collected into a plain (clotted) tube. Plasma is also an acceptable sample for some test methods, and this can be obtained using a gel separator tube.

3.3.2 Test Sensitivity

The clinical sensitivity of anti-HEV antibodies has not been well described as most published studies do not use a well-defined sample of patients and reference standard. Evaluation of various assays for IgG showed sensitivity varied from 17% to 100%, the peptide-based assays showing inferior performance [2].

In one study, an in-house anti-HEV IgM assay (developed for a vaccine trial) had a sensitivity of 92-97%, depending upon cut-off, and demonstrated superior performance to a widely used commercial assay [15]. In another study, the sensitivity of six IgM assays ranged between 72 and 98% [7]. In an outbreak, the sensitivity of five assays ranged between 72 and 91% for symptomatic cases, but was only 39-51% for asymptomatic cases with HEV RNA detected by RT-PCR [14]. A single measurement of total antibody had a sensitivity of 86% [10]. Sensitivity of antibody-based assays is lower in immunocompromised patients; in one small study the sensitivity of IgM in this setting was 85-88% [1].

3.3.3 Test Specificity

The clinical specificity of anti-HEV antibodies has not been well described as most published studies do not use a well-defined sample of patients and reference standard. In one study, an in-house anti-HEV IgM assay had a specificity >95%, depending upon cut-off, and demonstrated superior performance to a widely used commercial assay [15]. In another study, specificity for six IgM assays ranged between 78 and 96% [7]. In an outbreak setting, the specificity of 5 different assays ranged between 74 and 100% [14]. A single measurement of total antibody had a specificity of 89% [10].

3.3.4 Suitable Test Verification/Validation Criteria

All testing should be performed in accordance with NPAAC "*Requirements for the Development and Use of In-House* In Vitro *Diagnostic Medical Devices*", where relevant. Commercial kit performance should be verified in accordance with NATA requirements. It is recommended that all diagnostic test results are traceable to the WHO International Biological Reference Preparation (NIBSC 95/584). Laboratories should participate in an EQAS.

3.3.5 Suitable External QC programme

RCPA QAP Serology offers a HEV antibody module in the Hepatitis program. NRL do not offer any HEV modules at this time.

3.4 Electron Microscopy

HEV was first discovered using immune electron microscopy [3]. Virions are icosahedral and ~32nm in diameter and can be found in faeces. Immune electron microscopy remains a valid method for confirming a diagnosis of HEV infection, but has very limited availability and is substantially less sensitive than NAT [2]. However, electron microscopy is no longer routinely used for diagnosis. It may

be considered in special circumstances (eg. small-volume liver biopsies from patients with hepatitis of unknown origin).

4 Agreed Typing and Subtyping Methods

HEV comprises a single serotype. Genotyping is usually performed by sequencing an amplified region of the HEV genome. The most common region to be sequenced is the highly conserved 5'-end of ORF2; however finer discrimination between strains is possible when additional regions with more variation are included in sequencing, or the entire genome is sequenced.

4.1 SNOMED CT concepts

SNOMED CT Code	SNOMED CT concept
235867002	Acute hepatitis E (disorder)
450880008	Chronic hepatitis E (disorder)
17092007	Hepatitis E antibody measurement (procedure)
36446003	Hepatitis E virus measurement (procedure)
60992009	Hepatitis E antigen measurement (procedure)
64629004	Antibody to hepatitis E virus (substance)
78475006	Hepatitis E virus (organism)
401241007	Hepatitis E antibody level (procedure)
401242000	Hepatitis E IgM level (procedure)
401243005	Hepatitis E IgG level (procedure)

5 References

1. Abravanel, Florence, Sabine Chapuy-Regaud, Sébastien Lhomme, Marcel Miedougé, Jean-Marie Peron, Laurent Alric, Lionel Rostaing, Nassim Kamar, and Jacques Izopet. 2013. “Performance of Anti-HEV Assays for Diagnosing Acute Hepatitis E in Immunocompromised Patients.” *Journal of Clinical Virology* 58 (4): 624–28. doi:10.1016/j.jcv.2013.10.003.
2. Aggarwal, R. 2013. “Diagnosis of hepatitis E.” *Nat. Rev. Gastroenterol. Hepatol.* 10, 24–33. doi:10.1038/nrgastro.2012.187.
3. Balayan, M.S., A.G. Andjaparidze, S.S. Savinskaya, E.S Ketiladze, D.M. Braginsky, A.P. Savinov, and V.F. Poleschuk. 1983. “Evidence for a Virus in Non-A, Non-B Hepatitis Transmitted via the Fecal-Oral Route.” *Intervirology* 20 (1): 23–31. doi:10.1159/000149370.
4. Baylis, Sally A., Johannes Blümel, Saeko Mizusawa, Keiji Matsubayashi, Hidekatsu Sakata, Yoshiaki Okada, C. Micha Nübling, Kay-Martin O. Hanschmann, and the HEV Collaborative Study Group. 2013. “World Health Organization International Standard to Harmonize Assays for Detection of Hepatitis E Virus RNA.” *Emerging Infectious Diseases* 19 (5): 729–35. doi:10.3201/eid1905.121845.
5. Borkakoti, Jayanta, Rajib Kishore Hazam, Asim Mohammad, Ashok Kumar, and Premashis Kar. 2013. “Does High Viral Load of Hepatitis E Virus Influence the Severity and Prognosis of Acute Liver Failure during Pregnancy?” *Journal of Medical Virology* 85 (4): 620–26. doi:10.1002/jmv.23508.
6. Davern, Timothy J., Naga Chalasani, Robert J. Fontana, Paul H. Hayashi, Petr Protiva, David E. Kleiner, Ronald E. Engle, et al. 2011. “Acute Hepatitis E Infection Accounts for Some Cases of Suspected Drug-Induced Liver Injury.” *Gastroenterology* 141 (5): 1665–72.e9. doi:10.1053/j.gastro.2011.07.051.
7. Drobeniuc, Jan, Jihong Meng, Gábor Reuter, Tracy Greene-Montfort, Natasha Khudyakova, Zoya Dimitrova, Saleem Kamili, and Chong-Gee Teo. 2010. “Serologic Assays Specific to Immunoglobulin M Antibodies against Hepatitis E Virus: Pangenotypic Evaluation of Performances.” *Clinical Infectious Diseases* 51 (3): e24–27. doi:10.1086/654801.
8. Ferguson, Morag, Dawn Walker, Eric Mast, and Howard Fields. 2002. “Report of a Collaborative Study to Assess the Suitability of a Reference Reagent for Antibodies to Hepatitis E Virus.” *Biologicals* 30 (1): 43–48. doi:10.1006/biol.2001.0315.
9. Hoofnagle JH, Nelson KE, Purcell RH. 2012. “Hepatitis E”. *N Engl J Med* 2012;367:1237-44.
10. Innis, Bruce L., Jitvimol Seriwatana, Robin A. Robinson, Mrigendra P. Shrestha, Patrice O. Yarbough, Charles F. Longer, Robert M. Scott, David W. Vaughn, and Khin Saw Aye Myint. 2002. “Quantitation of Immunoglobulin to Hepatitis E Virus by Enzyme Immunoassay.” *Clinical and Diagnostic Laboratory Immunology* 9 (3): 639–48. doi:10.1128/CDLI.9.3.639-648.2002.
11. Kamar, Nassim, Harry R. Dalton, Florence Abravanel, and Jacques Izopet. 2014. “Hepatitis E Virus Infection.” *Clinical Microbiology Reviews* 27 (1): 116–38. doi:10.1128/CMR.00057-13.
12. Krain, Lisa J., Kenrad E. Nelson, and Alain B. Labrique. 2014. “Host Immune Status and Response to Hepatitis E Virus Infection.” *Clinical Microbiology Reviews* 27 (1): 139–65. doi:10.1128/CMR.00062-13.

13. Myint KSA, Endy TP, Shrestha MP, Shrestha SK, Vaughn DW, Innis BL, et al. 2006. “Hepatitis E antibody kinetics in Nepalese patients.” *Trans R Soc Trop Med Hyg*. 100(10):938–41. doi:10.1016/j.trstmh.2005.12.005
14. Myint, Khin Saw Aye, Timothy P. Endy, Robert V. Gibbons, Kanti Laras, Mammen P. Mammen, Endang R. Sedyaningsih, Jitvimol Seriwatana, Jonathan S. Glass, Sumitda Narupiti, and Andrew L. Corwin. 2006. “Evaluation of Diagnostic Assays for Hepatitis E Virus in Outbreak Settings.” *Journal of Clinical Microbiology* 44 (4): 1581–83. doi:10.1128/JCM.44.4.1581-1583.2006.
15. Seriwatana, Jitvimol, Mrigendra P. Shrestha, Robert M. Scott, Sergei A. Tsarev, David W. Vaughn, Khin Saw Aye Myint, and Bruce L. Innis. 2002. “Clinical and Epidemiological Relevance of Quantitating Hepatitis E Virus-Specific Immunoglobulin M.” *Clinical and Diagnostic Laboratory Immunology* 9 (5): 1072–78. doi:10.1128/CDLI.9.5.1072-1078.2002.
16. Shrestha, Mrigendra Prasad, Robert McNair Scott, Durga Man Joshi, Mammen P. Mammen, Gyan Bahadur Thapa, Narbada Thapa, Khin Saw Aye Myint, et al. 2007. “Safety and Efficacy of a Recombinant Hepatitis E Vaccine.” *New England Journal of Medicine* 356 (9): 895–903. doi:10.1056/NEJMoa061847.
17. Tanaka, Toshinori, Masaharu Takahashi, Eiji Kusano, and Hiroaki Okamoto. 2007. “Development and Evaluation of an Efficient Cell-Culture System for Hepatitis E Virus.” *Journal of General Virology* 88 (3): 903–11. doi:10.1099/vir.0.82535-0.
18. Vollmer, T., C. Knabbe, and J. Dreier. 2014. “Comparison of Real-Time PCR and Antigen Assays for Detection of Hepatitis E Virus in Blood Donors.” *Journal of Clinical Microbiology* 52 (6): 2150–56. doi:10.1128/JCM.03578-13.
19. Williams, T. P. E., C. Kasorndorkbua, P. G. Halbur, G. Haqshenas, D. K. Guenette, T. E. Toth, and X. J. Meng. 2001. “Evidence of Extrahepatic Sites of Replication of the Hepatitis E Virus in a Swine Model.” *Journal of Clinical Microbiology* 39 (9): 3040–46. doi:10.1128/JCM.39.9.3040-3046.2001.
20. Zhu, Feng-Cai, Jun Zhang, Xue-Feng Zhang, Cheng Zhou, Zhong-Ze Wang, Shou-Jie Huang, Hua Wang, et al. 2010. “Efficacy and Safety of a Recombinant Hepatitis E Vaccine in Healthy Adults: A Large-Scale, Randomised, Double-Blind Placebo-Controlled, Phase 3 Trial.” *The Lancet* 376 (9744): 895–902. doi:10.1016/S0140-6736(10)61030-6.