



[diagnostics.roche.com](https://diagnostics.roche.com)



HEV infographic

# Diagnosis of Hepatitis E Virus Infection: Current Capabilities and Shortcomings

---

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide. It was responsible for an estimated 20 million infections, 3 million symptomatic cases of hepatitis, and 70,000 fatalities each year.<sup>1</sup> As a result of a still-evolving landscape of diagnostic tools, inadequate physician awareness, and incomplete implementation of clinical testing guidelines, the global burden of HEV infection is likely an underestimate. For similar reasons, individuals presenting with hepatitis may be misdiagnosed.

This review will provide an overview of HEV virology and epidemiology, the available approaches for diagnosis of HEV infection, and areas where improvement of diagnostic tools is needed.

## Summary

- HEV infection is difficult to diagnose based on clinical manifestations and liver function abnormalities alone.
- Accurate diagnosis relies on HEV-specific diagnostic testing, including tests for anti-HEV antibodies, viral RNA, and viral antigens.
- There are three important applications of tests for HEV:
  - Diagnosis of individual patients
  - Screening of blood donations
  - Seroprevalence studies
- Anti-HEV antibody tests are the mainstay of HEV diagnosis, but the performance of currently available assays is variable.
- Variations in sensitivity can lead to different conclusions when testing specimens for anti-HEV antibodies using multiple assays, while the use of assays with suboptimal sensitivity could lead to misdiagnosis.

## Content Overview

HEV Virology.....	3
Epidemiology.....	3
Clinical features of HEV infection.....	4
Immune response.....	5
Treatment and prevention.....	6
Diagnostic markers.....	6
Anti-HEV antibodies.....	6
Viral RNA.....	6
Viral antigen.....	6
Use cases for HEV testing.....	7
Individual patient diagnosis.....	7
Screening of blood and organ donors.....	9
Seroprevalence studies.....	9
Unmet needs in HEV diagnostic tools.....	9
Conclusions.....	9
References.....	10

## HEV Virology

HEV belongs to the *Hepeviridae* family of viruses (subfamily: *Orthohepevirinae*, genus: *Paslahepevirus*).<sup>2</sup> HEV particles are small, between 27 and 34nm in size, and icosahedral in shape. **Eight distinct genotypes of HEV have been identified, four of which (genotypes 1 to 4) are responsible for the vast majority of human infections** (details in the *Epidemiology* section). Isolated infection with HEV genotype 7 has been reported.<sup>3</sup> Each genotype can be further subclassified into a variety of subtypes based on phylogenetic analysis.<sup>4,5</sup> The four genotypes of HEV that commonly infect humans belong to the same serotype.

The HEV genome is a single molecule of capped, positive-sense RNA, 6.4 to 7.2 kb in length. The genome of all genotypes of HEV has three open reading frames (ORF) which are translated from genomic or subgenomic forms of viral mRNA. ORF1 encodes seven non-structural proteins needed for genome replication, while ORF2 encodes the structural capsid protein, and ORF3 encodes a single phosphoprotein. In HEV genotype 1, a fourth ORF has been reported.<sup>6</sup>

Detailed understanding of HEV replication is lacking, partly because a robust *in vitro* viral growth system is not available. Nonetheless, it is known that **infection is initiated in intestinal epithelial cells following ingestion of contaminated food or water, followed by dissemination of the virus in blood to hepatocytes in the liver.**

*Hepeviridae* infect liver cells via clathrin-mediated endocytosis, followed by translation of the genomic RNA to produce the ORF1 non-structural proteins needed for RNA replication in the infected cell cytoplasm. Following synthesis of a negative-sense RNA intermediate, new positive-sense genomic and subgenomic mRNAs are transcribed. Capsid proteins made from ORF2 in the subgenomic mRNA assemble into new virions, which package nascent copies of genomic RNA and are released via multivesicular bodies. Nascent virus particles released into the blood have an exosomal

membrane surrounding the capsid structure (quasi-enveloped particles). The envelope is lost following passage through the bile duct, resulting in uncoated viral particles entering the gut and being excreted in the bile and feces. Loss of the exosomal membrane is associated with an increase in infectivity.<sup>7</sup> For more details regarding HEV replication, see Himmelsbach et al.<sup>8</sup>

## Epidemiology

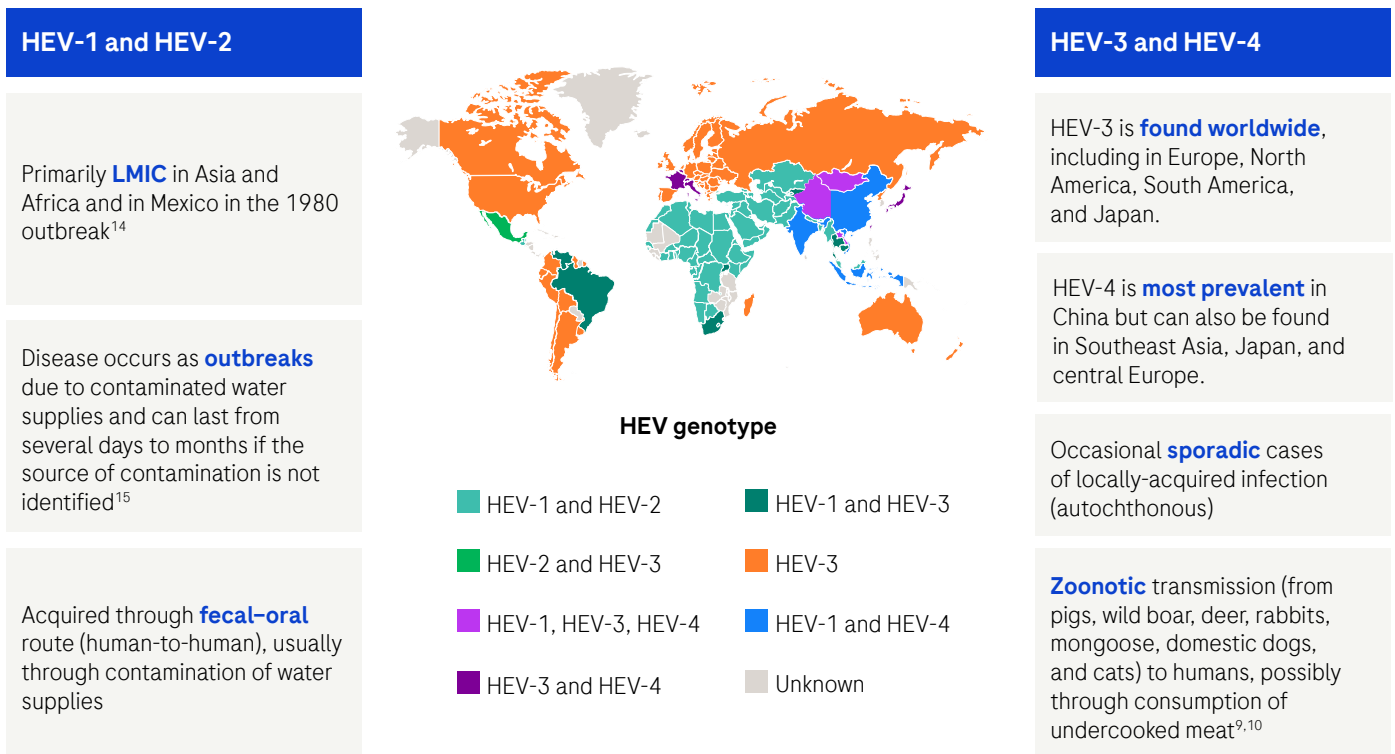
HEV genotype is strongly associated with the primary mode of transmission, geographic distribution, and degree of endemicity of infections.

HEV-1 and HEV-2 only infect humans and predominantly in low- and middle-income countries in Africa and Asia (HEV-1 and 2) and in Mexico (HEV-2). They are transmitted via the fecal-to-oral route, predominantly in resource-limited settings with poor sanitation; person-to-person (including mother-to-child) transmission is also possible. HEV-1 and 2 cause seasonal outbreaks as well as sporadic infections, which are usually acquired through drinking water contaminated with sewage.

HEV-3 and 4 are found in North and South America (HEV-3), Europe (HEV-3 and 4), and Asia (HEV-3 and 4) (**Figure 1**). They have a broader host range and cause sporadic zoonotic infections in humans. Animal hosts for HEV-3 and 4 include pigs, wild boar, rabbits, deer, mongoose, and domestic dogs and cats.<sup>9,10</sup> Farm workers and animal handlers in close contact with infected animals are at a relatively high risk of acquiring HEV infection in endemic areas.<sup>11</sup> HEV can also be acquired by people consuming undercooked meat (primarily pork, but also boar, rabbit, and deer), milk, or raw shellfish.



HEV is found globally, but HEV genotypes are region-specific<sup>12,13\*</sup>



**Figure 1.** HEV geographical distribution. Infographic generated based on data from references 12 and 13. Data collected until 2020. HEV, hepatitis E virus; LMIC, low- and middle-income countries.

Less frequently, and mostly in endemic areas, all four genotypes of HEV can also be transmitted parenterally through blood transfusion, organ transplantation, or vertically from infected mothers to their infants.<sup>16-18</sup>

## Clinical features of HEV infection

The clinical manifestations of HEV infection are variable in humans, ranging from asymptomatic in the majority of healthy individuals to life-threatening in certain vulnerable populations (Figure 2). The proportion of infections that are symptomatic is dependent on the genotype: HEV-1 and 2 are associated with a higher rate of symptomatic hepatitis than HEV-3 and 4 (about 2%).<sup>19,20</sup> The majority of symptomatic infections are characterized by self-limiting acute hepatitis, indistinguishable from other types of viral hepatitis, with fever, anorexia, nausea, myalgia, malaise, and jaundice, accompanied by elevated transaminase and bilirubin levels in

serum.<sup>19,20</sup> When they occur, symptoms usually become apparent approximately 2 to 8 weeks after infection and last for between 1 day and 6 weeks.<sup>21,22</sup>

A small fraction (0.5–4%) of HEV-infected individuals develop acute liver failure. Patients with existing chronic liver diseases are at increased risk of liver failure, and mortality can be as high as 67%. Pregnant women are at a high risk of developing symptomatic disease following an HEV-1 infection during the second and third trimesters. Many of these women progress to acute liver failure, hemorrhage, and eclampsia, and the mortality rate can reach 15–25%. HEV-1 infection during pregnancy is also associated with more frequent miscarriages, preterm deliveries, stillbirths, and perinatal mortality.

In immunocompromised or immunosuppressed individuals (including solid organ transplant recipients, people living with

HIV/AIDS, and patients receiving cancer chemotherapy), HEV-3 and 4 can also establish a chronic infection.<sup>23,24</sup> Initially, chronically infected individuals are asymptomatic or have mild, nonspecific symptoms and lack clinical signs of hepatitis. Over time, about 10% of chronically infected patients can progress to decompensated cirrhosis resulting in fatal liver failure.

**HEV is implicated in a variety of pathologies outside the liver** as well, the nature of which may be influenced by the genotype.<sup>25-27</sup> Such extrahepatic manifestations include neurological and renal disease, hematological disorders, pancreatitis, polyarthritits, myocarditis, and thyroiditis. The underlying mechanisms of how HEV infection leads to extrahepatic manifestations, including whether they are a result of viral replication in tissues outside of the liver versus secondary effects of liver pathogenesis, are not well defined.

HEV infection is generally self-limited in healthy immunocompetent individuals.<sup>12,28,29</sup>

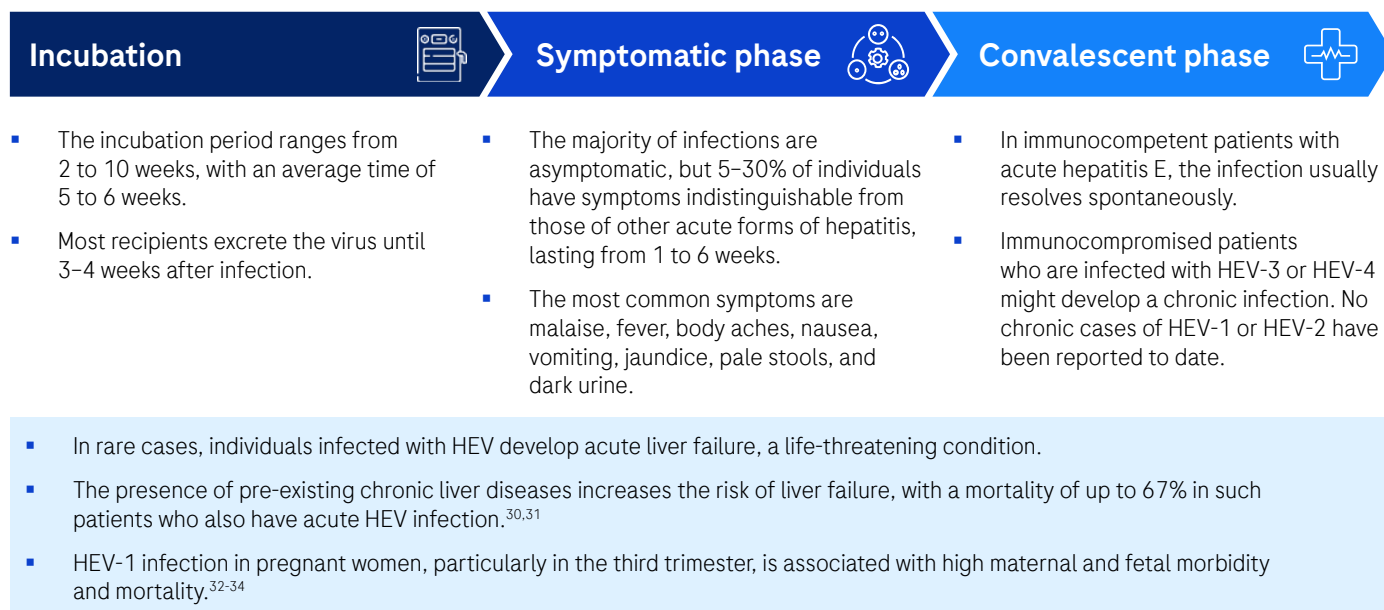


Figure 2. Course of HEV acute infection. HEV, hepatitis E virus.

## Immune response

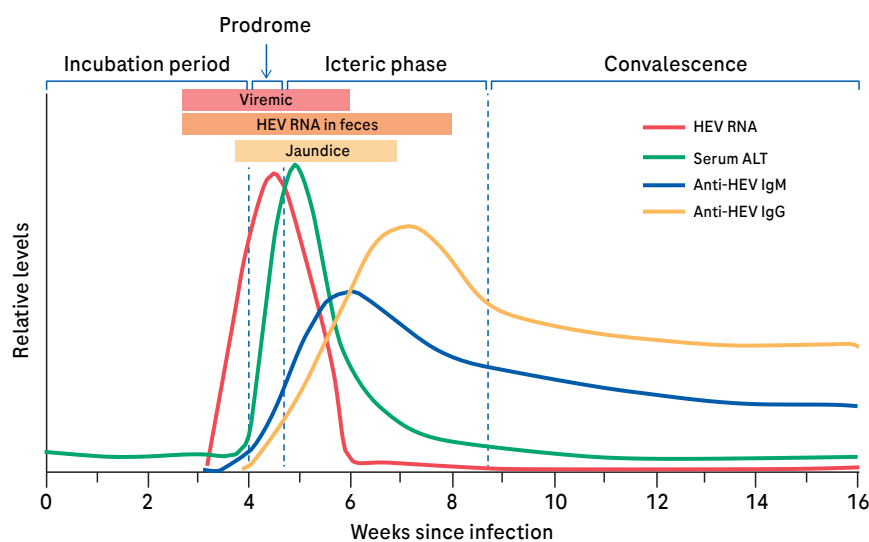
The adaptive immune response to HEV infection in immunocompetent individuals is typically characterized by development of anti-HEV IgM antibodies at around the time of symptom onset; these IgM antibody concentrations then drop to lower levels but can remain detectable for 6

months or longer.<sup>20,35,36</sup> Secretory IgA antibodies can also be measured in serum with kinetics that are similar to those of IgM.<sup>37</sup> Anti-HEV IgG antibodies become predominant a few weeks after IgM levels peak and can be detected for years after the infection has been cleared.<sup>20,35,36</sup> A conceptual diagram illustrating the dynamics of

antibody response and serum alanine transaminase (ALT) is shown in (Figure 3).

**HEV infection also induces a broad cytotoxic T-cell response in immunocompetent hosts. This response is likely important for long-lived immunity and protection from disease.<sup>39</sup>**

## Typical self-limited HEV infection profile



HEV RNA and capsid antigen become detectable in blood and stool ~2 weeks after viral exposure and persist for ~4 weeks and ~6 weeks in blood and stool, respectively.

Anti-HEV IgM is detectable in blood from the 4<sup>th</sup> week after viral exposure and lasts a maximum of 6–9 months.

Anti-HEV IgG appears shortly after IgM and persists for several years in blood.

ALT levels, an indicator of liver damage, generally increase 4 weeks after initial viral exposure.

In immunocompromised patients who develop **chronic HEV infection**, HEV RNA persists in blood, stool, and body fluids for **more than 3 months**.

Immunocompromised patients are likely to have low or undetectable levels of anti-HEV antibodies.<sup>40</sup>

Figure 3. A typical self-limited HEV infection profile.<sup>20,35,36</sup> Figure adapted from 20. HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; ALT, alanine transaminase.

## Treatment and prevention

In immunocompetent individuals, most HEV infections, whether symptomatic or not, are cleared by the immune system. However, antiviral therapy for HEV infection may be indicated for some acutely infected patients (e.g., pregnant women and immunosuppressed or immunocompromised patients) and in chronically infected patients.<sup>40</sup>

The first intervention recommended in response to confirmed HEV infection in immunosuppressed patients is to reduce the intensity of the immunosuppressive therapy if possible.<sup>40,41</sup> **While no HEV-specific antivirals have been tested in large randomized clinical trials or approved for clinical use, sustained viral response (or viral clearance) has been achieved through the use of ribavirin**, in spite of its important hematological side effects that can limit its tolerability and toxicity, especially in pregnant women.<sup>42-44</sup> **Pegylated interferon- $\alpha$  therapy has been used for treatment of chronic HEV infection**,<sup>45,46</sup> mostly in patients who cannot tolerate ribavirin; however, it cannot be used in transplant recipients and has significant side effects. **Sofosbuvir**, a nucleoside polymerase inhibitor approved for treatment of hepatitis C virus infection, **has anti-HEV activity *in vitro***<sup>47,48</sup> **but no proven efficacy in clinical studies.**<sup>49</sup>

**An effective vaccine against HEV infection (called HEV 239 or Hecolin<sup>®</sup>) was approved for use in China in 2010 and is undergoing clinical study for use in Bangladesh and the United States (US).**<sup>50-52</sup> The vaccine antigen is a fragment of the HEV-1 capsid (ORF2) protein, produced in bacteria. The recombinant protein self-assembles into virus-like particles. Several other candidate vaccines are in development.<sup>53</sup>

**In addition, there are several prevention strategies that can be employed to limit HEV infection.** These include ensuring access to clean public water supplies, general food safety measures, and the proper disposal of human excreta. Individuals can also avoid exposure by keeping good personal hygiene.

## Diagnostic markers

In general, clinical manifestations and liver function abnormalities associated with HEV infection are not distinguishable from those caused by other infectious hepatitis viruses, drug-induced liver injury (DILI), or autoimmune disease. Therefore, **accurate diagnosis of HEV infection relies heavily on diagnostic testing. Tests for HEV are based on the detection and/or quantitation of host antibody responses, viral RNA, or viral antigen (Figure 4).**

### Anti-HEV antibodies

Tests that detect the presence or measure the concentration of circulating anti-HEV antibodies are the **mainstay of HEV diagnosis owing to their relative simplicity and low cost** compared to HEV RNA tests. Most HEV serological tests described to date detect anti-HEV IgG or IgM antibodies in serum or plasma. Anti-HEV tests are commercially available from several different manufacturers, some of which are CE-IVD marked, but none has been approved by the US FDA. Antibody tests can be laboratory-based, including enzyme-linked immunoassays (ELISA), or in lateral-flow rapid test format. The performance characteristics of anti-HEV tests are variable, especially with regard to sensitivity.<sup>54-61</sup> Calibration of anti-HEV IgG assays using the WHO reference reagent for antibodies to HEV<sup>62,63</sup> has improved the situation somewhat.<sup>36</sup>

### Viral RNA

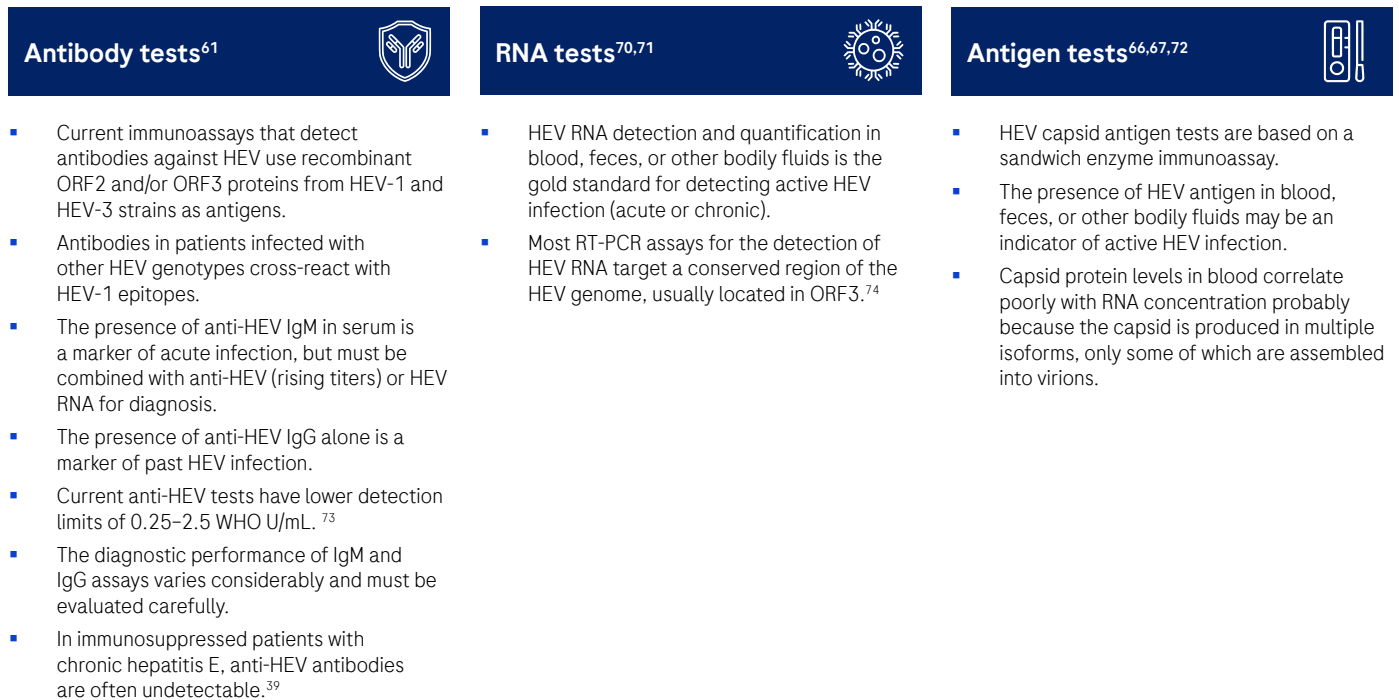
Detection of viral RNA in blood is considered to be the **gold standard for evidence of active viral replication.** Most assays for HEV RNA are based on quantitative real-time reverse transcription PCR (qRT-PCR) and are very sensitive. Since qRT-PCR tests are designed to amplify and detect specific sequences in the viral genome, they are at risk of decreased sensitivity or even false negative results in patients infected with viral variants bearing sequence changes that abrogate primer or probe binding. Targeting of highly conserved regions and use of degenerate primers are strategies that can mitigate this risk.

Viral RNA is the **first biomarker to become detectable after the incubation period following infection**, followed by viral antigen. Anti-HEV IgM antibodies then appear in serum, followed by IgG (**Figure 3**). IgM concentrations decrease more rapidly than IgG, usually becoming undetectable 3 to 4 months after they are first detected. Thus, simultaneous testing for both antibody types, as well as viral RNA or antigen, can give an indication of the duration of the infection (**Table 1**). If multiple measurements over time can be obtained, assessment of whether IgM or IgG levels are increasing or decreasing can also help determine the duration of HEV infection. However in immunocompromised individuals, antibody production may be delayed or not occur, making detection of viral RNA necessary to diagnose HEV infection. Also, anti-HEV IgM antibodies may persist for several months in some patients. In contexts where access to RNA testing is limited, a multifactorial model for identification of acute infection that uses anti-HEV IgM, viral antigen, and ALT levels has been proposed.<sup>64</sup>

### Viral antigen

The viral capsid (ORF2) protein is the **major component of the virus particle and an important antigen against which host antibodies are directed.** Capsid protein in blood or plasma **may reach detectable concentrations prior to anti-HEV antibodies** and can be measured by ELISA. Since capsid is produced during active replication of HEV in infected hepatocytes, its presence in blood may be a useful marker of acute infection.<sup>65</sup> However, capsid protein levels in blood correlate poorly with RNA concentration.<sup>66</sup> This may be related to the production of capsid in multiple isoforms, only some of which are assembled into virions.<sup>67,68</sup>

## Accurate diagnosis of HEV infection heavily relies on diagnostic testing.<sup>69</sup>



**Figure 4.** Diagnostic tools for HEV. HEV, hepatitis E virus; ORF, open reading frame; WHO, World Health Organization; U, units; IgG, immunoglobulin G; IgM, immunoglobulin M; RT-PCR, reverse transcription polymerase chain reaction.

### Use cases for HEV testing

Diagnostic testing strategies for HEV should be chosen based on the goal of testing and the clinical context. At least three **applications of HEV tests are important to consider: testing of individual patients with hepatitis, screening of blood supply to prevent transfusion-associated infection, and population-level surveillance.**

#### Individual patient diagnosis

**For individual patient diagnosis and monitoring, the European Association for the Study of the Liver (EASL) recommends that *all people with hepatitis* be tested for HEV.<sup>29</sup>**

This includes:

- All people with symptoms consistent with acute hepatitis.
- Travelers with hepatitis returning from areas endemic for HEV genotype 1 or 2.
- Patients with unexplained flares of chronic liver disease.

- Patients with abnormal liver function tests after receiving blood products.
- All immunosuppressed patients with unexplained abnormal liver function tests.
- Patients presenting with suspected DILI. In elderly patients, many of whom are on multiple medications, HEV infection is often misdiagnosed as DILI.<sup>75-77</sup> Before diagnosing hepatitis as DILI, HEV infection should be ruled out.
- Patients with symptoms possibly related to extrahepatic effects of HEV, including neuralgic amyotrophy, Guillain-Barré syndrome, encephalitis/myelitis, and unexplained acute neurological findings.
- Patients treated with ribavirin – test for HEV RNA in serum and stool at the end of a scheduled period of ribavirin therapy (usually 12 weeks).

According to the guidelines published by EASL,<sup>41</sup> European Centre for

Disease Prevention and Control (ECDC),<sup>78</sup> and Public Health England,<sup>79</sup> **individual diagnosis of HEV infection in immunocompetent patients should consist of serological tests for anti-HEV antibodies supplemented in some cases by nucleic acid tests (NAT). Anti-HEV antibody development may be delayed or may not be detectable in immunocompromised patients (e.g., transplant recipients), so increased reliance is placed on NAT results.** In immunocompetent patients with elevated liver function test results, an active HEV infection can be diagnosed based only on serology if IgM antibodies are present and the concentrations of IgG are increasing.<sup>79</sup> Patterns of IgG and IgM antibodies may also help distinguish between early (IgM positive, IgG negative) and late (both positive) infections. If HEV RNA is detected over more than a 3-month interval, the patient is diagnosed as having a chronic infection. See **(Table 1)** for a summary of biomarker patterns and association with different stages of infection.

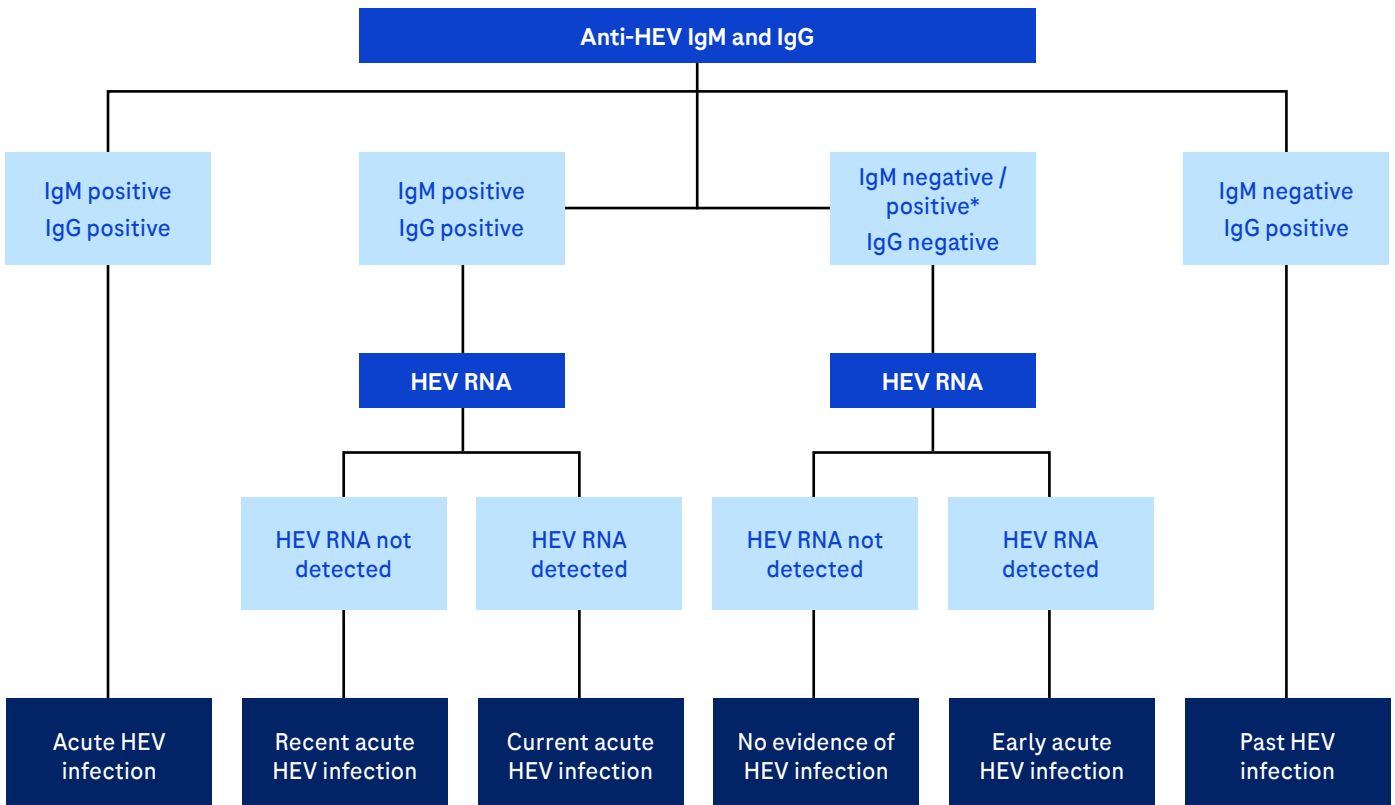
Laboratory test interpretation				
RNA	IgM	IgG	Infection phase	Virus replication
—	—	—	Not infected or incubation period	None or undetected
+	—	—	Acute (early)	✓
+	+	—	Acute (mid)	✓
+	+	+	Acute (late)	✓
—	+	+	Early recovery phase	✗
—	—	+	Late recovery phase	✗
+(≥3 months)	-/+	-/+	Chronic	✓

**Table 1.** Patterns of HEV infection biomarkers and association with stages of infection. Table adapted from 41. HEV, hepatitis E virus.

The Public Health England guidelines include an algorithm for interpretation of HEV testing results in support of individual diagnosis of immunocompetent patients<sup>79</sup> (Figure 5). Starting with antibody test results, a positive diagnosis can be made if both IgG and IgM are positive

even in the absence of NAT, which help discriminate between a previous infection (RNA negative) versus a current acute infection (RNA positive). If antibodies are absent, NAT can enable the detection of an early-stage acute infection or can rule out HEV infection if it is negative.

The presence of anti-HEV IgG but not IgM indicates a past infection. The presence of anti-HEV IgM but not IgG is not diagnostic in the absence of NAT, because IgM positivity on its own is not sufficient to diagnose an HEV infection.



\* Detection of anti-HEV IgM alone does not diagnose HEV infection

**Figure 5.** Interpretation of testing for HEV infection in immunocompetent patients. Adapted from 76. HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; RNA: ribonucleic acid.



## Screening of blood and organ donors

Several studies have reported that HEV can be detected in donated blood and that this can lead to **HEV infection of transfusion recipients**.<sup>16,17,54,56,58,80-86</sup> To reduce the risk of transfusion-associated transmission of HEV, the ECDC recommends that blood donor services screen blood donors for **HEV RNA**, informed by local risk assessment and cost-effectiveness studies.<sup>78</sup> RNA testing is preferred because of its greater sensitivity and association with infectivity compared to antibody testing.

Similarly, **HEV can be transmitted via transplantation of organs from HEV viremic donors**.<sup>16,43</sup> Importantly, because of the use of immunosuppressive therapy in transplant recipients to prevent organ rejection, HEV exposure can lead to chronic infection. **HEV testing of organ donors is recommended in international guidelines**.<sup>40,87</sup>

## Seroprevalence studies

Population-level surveillance of the prevalence of HEV infection is an **important aspect of our understanding of the epidemiology and infection prevention strategies for HEV**. Seroprevalence surveys are based on the detection of **anti-HEV IgG in serum or plasma and provide important information about differences in HEV prevalence** according to geography, trends over time, and demographic attributes such as age, sex, residential status (urban vs. rural), occupation, exposure to animal reservoirs, etc. They can also be used to compare assays, given a large enough sample size to control for other variables. There are many examples of HEV seroprevalence studies from countries all around the world that illustrate the utility of this type of data.<sup>1,83,88-104</sup> However, it is important to note that prevalence data from studies that use different methods for measurement of antibody levels should not be compared to each other, as the assay characteristics (i.e., sensitivity and specificity) are likely to be different. Seroprevalence surveys have also

been performed in animals in order to characterize the risk associated with occupational exposure to animals with high prevalence.<sup>10,93,94,103,105</sup>

## Unmet needs in HEV diagnostic tools

Retrospective assessments of the performance of diagnostic methods employed for the different use cases described above have illuminated **some areas where assay performance has been suboptimal. The most important of these is low and variable assay sensitivity**. Testing of panels of specimens for anti-HEV antibodies using multiple assays frequently demonstrates that variation in sensitivity can lead to different conclusions.<sup>55-61,96,106,107</sup> Assay variability influences estimation of the window period in asymptomatic individuals and comparison to symptomatic patients<sup>37</sup> as well as calculation of seroprevalence.<sup>54,91,96,101,108</sup> **The use of assays with suboptimal sensitivity could lead to misdiagnosis**, including not recognizing cases of HEV infection in individuals with low antibody titers or misclassification based on not detecting anti-HEV IgM, for example. **Comparability of results between different tests is an important consideration**, for example, when monitoring antibody or RNA concentrations over time in a particular patient, especially if the patient changes clinic location or if the laboratory changes assay provider. It is also crucial when comparing quantitative data between studies that use different tests. **Calibration using an accepted standard material**, such as those provided by the World Health Organization or its laboratory partners,<sup>62</sup> is the **best way to minimize the impact of interassay variability**.

## Conclusions

HEV infection is difficult to diagnose based on clinical manifestations and liver function abnormalities alone. **Accurate diagnosis relies on HEV-**

**specific diagnostic testing**, including tests for anti-HEV antibodies, viral RNA, and viral antigen.

**HEV testing strategies should be chosen based on the goal of testing and the clinical context**. There are three important applications of HEV tests: testing of individual patients with hepatitis, screening of the blood supply to prevent transfusion-associated infection, and population-level surveillance.

**Diagnosis of individual patients with hepatitis is based on serological tests for anti-HEV antibodies, NAT for HEV RNA, or a combination of both. In immunocompromised individuals, detection of viral RNA is necessary to diagnose HEV infection.**

**Screening of blood donations by testing for HEV RNA helps reduce the risk of transfusion-associated transmission of HEV**. As HEV can also be transmitted via transplantation of organs from HEV viremic donors, **HEV RNA testing of organ donors is also recommended**.

**Seroprevalence surveys help gain a better understanding of HEV epidemiology and prevention strategies based on the detection of anti-HEV IgG in serum.**

Anti-HEV antibody tests are the mainstay of HEV diagnosis because of their simplicity and low cost, but the performance characteristics of currently available assays are variable. Retrospective assessments of anti-HEV diagnostic methods have shown that **low and variable assay sensitivity is a major issue. Variations in sensitivity can lead to different conclusions when testing panels of specimens for anti-HEV antibodies using multiple assays, while the use of assays with suboptimal sensitivity could lead to misdiagnosis.**

## References

1. Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. 2012; 55(4):988-97.
2. Purdy MA, Drexler JF, Meng XJ, Norder H, Okamoto H, Van der Poel WHM, et al. ICTV Virus Taxonomy Profile: Hepeviridae 2022. *J Gen Virol*. 2022; 103(9):001778.
3. Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, et al. Chronic Infection With Camelid Hepatitis E Virus in a Liver Transplant Recipient Who Regularly Consumes Camel Meat and Milk. *Gastroenterology*. 2016; 150(2):355-7 e3.
4. Smith DB, Purdy MA, Simmonds P. Genetic variability and the classification of hepatitis E virus. *J Virol*. 2013;87(8):4161-9.
5. Primadharsini PP, Nagashima S, Okamoto H. Genetic Variability and Evolution of Hepatitis E Virus. *Viruses*. 2019;11(5):456.
6. Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, et al. Endoplasmic Reticulum Stress Induced Synthesis of a Novel Viral Factor Mediates Efficient Replication of Genotype-1 Hepatitis E Virus. *PLoS Pathog*. 2016;12(4):e1005521.
7. Yin X, Feng Z. Hepatitis E Virus Entry. *Viruses*. 2019; 11(10):883.
8. Himmelsbach K, Bender D, Hildt E. Life cycle and morphogenesis of the hepatitis E virus. *Emerg Microbes Infect*. 2018;7(1):196.
9. Pavio N, Meng XJ, Renou C. Zoonotic hepatitis E: animal reservoirs and emerging risks. *Vet Res*. 2010;41(6):46.
10. Caballero-Gomez J, Rivero-Juarez A, Jurado-Tarifa E, Jimenez-Martin D, Jimenez-Ruiz E, Castro-Scholten S, et al. Serological and molecular survey of hepatitis E virus in cats and dogs in Spain. *Transbound Emerg Dis*. 2022;69(2):240-8.
11. Kmush BL, Nelson KE, Labrique AB. Risk factors for hepatitis E virus infection and disease. *Expert Rev Anti Infect Ther*. 2015;13(1):41-53.
12. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. *Nat Rev Dis Primers*. 2017;3:17086.
13. Pallerla SR, Harms D, Johne R, Todt D, Steinmann E, Schemmerer M, et al. Hepatitis E Virus Infection: Circulation, Molecular Epidemiology, and Impact on Global Health. *Pathogens*. 2020;9(10):856.
14. Velazquez O, Stetler HC, Avila C, Ornelas G, Alvarez C, Hadler SC, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986-1987. *JAMA*. 1990;263(24):3281-5.
15. Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. A large waterborne viral hepatitis E epidemic in Kanpur, India. *Bull World Health Organ*. 1992;70(5):597-604.
16. Janahi EM, Parkar SFD, Mustafa S, Eisa ZM. Implications of Hepatitis E Virus in Blood Transfusions, Hemodialysis, and Solid Organ Transplants. *Medicina (Kaunas)*. 2020;56(5):206.
17. Cheung CKM, Wong SH, Law AWH, Law MF. Transfusion-transmitted hepatitis E: What we know so far? *World J Gastroenterol*. 2022;28(1):47-75.
18. Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet*. 1995;345(8956):1025-6.
19. Kamar N, Pischke S. Acute and Persistent Hepatitis E Virus Genotype 3 and 4 Infection: Clinical Features, Pathogenesis, and Treatment. *Cold Spring Harb Perspect Med*. 2019;9(7):a031872.
20. Aggarwal R, Goel A. Natural History, Clinical Manifestations, and Pathogenesis of Hepatitis E Virus Genotype 1 and 2 Infections. *Cold Spring Harb Perspect Med*. 2019;9(7):a032136.
21. Raji YE, Toung OP, Taib NM, Sekawi ZB. Hepatitis E Virus: An emerging enigmatic and underestimated pathogen. *Saudi J Biol Sci*. 2022;29(1):499-512.
22. Goel A, Aggarwal R. Hepatitis E: Epidemiology, Clinical Course, Prevention, and Treatment. *Gastroenterol Clin North Am*. 2020;49(2):315-30.

23. Ma Z, de Man RA, Kamar N, Pan Q. Chronic hepatitis E: Advancing research and patient care. *J Hepatol*. 2022;77(4):1109-23.
24. Damiris K, Aghaie Meybodi M, Niazi M, Pyrsopoulos N. Hepatitis E in immunocompromised individuals. *World J Hepatol*. 2022;14(3):482-94.
25. Wu J, Xiang Z, Zhu C, Yao Y, Bortolanza M, Cao H, et al. Extrahepatic manifestations related to hepatitis E virus infection and their triggering mechanisms. *J Infect*. 2021;83(3):298-305.
26. Fousekis FS, Mitselos IV, Christodoulou DK. Extrahepatic manifestations of hepatitis E virus: An overview. *Clin Mol Hepatol*. 2020;26(1):16-23.
27. Horvatits T, Pischke S. Extrahepatic manifestations and HEV, the genotype matters. *EBioMedicine*. 2018;36:3-4.
28. Horvatits T, Schulze Zur Wiesch J, Lutgehetmann M, Lohse AW, Pischke S. The Clinical Perspective on Hepatitis E. *Viruses*. 2019;11:617.
29. World Health Organization. Hepatitis E 2023. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-e>.
30. Blasco-Perrin H, Madden RG, Stanley A, Crossan C, Hunter JG, Vine L, et al. Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study. *Aliment Pharmacol Ther*. 2015;42:574-81.
31. Kumar A, Saraswat VA. Hepatitis E and Acute-on-Chronic Liver Failure. *J Clin Exp Hepatol*. 2013;3(3):225-30.
32. Yadav KK, Kenney SP. Hepatitis E Virus Immunopathogenesis. *Pathogens* 2021;10:1180.
33. Aslan AT, Balaban HY. Hepatitis E virus: Epidemiology, diagnosis, clinical manifestations, and treatment. *World J Gastroenterol* 2020;26: 5543-5560.
34. Wu C, et al. Hepatitis E virus infection during pregnancy. *Virol J* 2020;17:73.
35. Webb GW, Dalton HR. Hepatitis E: an underestimated emerging threat. *Ther Adv Infect Dis*. 2019;6:2049936119837162.
36. Lhomme S, Legrand-Abravanel F, Kamar N, Izopet J. Screening, diagnosis and risks associated with Hepatitis E virus infection. *Expert Rev Anti Infect Ther*. 2019;17(6):403-18.
37. Vollmer T, Diekmann J, Eberhardt M, Knabbe C, Dreier J. Hepatitis E in blood donors: investigation of the natural course of asymptomatic infection, Germany, 2011. *Euro Surveill*. 2016;21(35).
38. Al-Ayoubi J, Behrendt P, Bremer B, Suneetha PV, Gisa A, Rinker F, et al. Hepatitis E virus ORF 1 induces proliferative and functional T-cell responses in patients with ongoing and resolved hepatitis E. *Liver Int*. 2018;38(2):266-77.
39. Anastasiou OE, Thodou V, Berger A, Wedemeyer H, Ciesek S. Comprehensive Evaluation of Hepatitis E Serology and Molecular Testing in a Large Cohort. *Pathogens*. 2020;9:137.
40. McPherson S, Elsharkawy AM, Ankcorn M, Ijaz S, Powell J, Rowe I, et al. Summary of the British Transplantation Society UK Guidelines for Hepatitis E and Solid Organ Transplantation. *Transplantation*. 2018;102:15-20.
41. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. *J Hepatol*. 2018;68:1256-71.
42. Kamar N, Abravanel F, Behrendt P, Hofmann J, Pageaux GP, Barbet C, et al. Ribavirin for Hepatitis E Virus Infection After Organ Transplantation: A Large European Retrospective Multicenter Study. *Clin Infect Dis*. 2020;71(5):1204-11.
43. Markakis GE, Papatheodoridis GV, Cholongitas E. Epidemiology and treatment of hepatitis E in the liver transplantation setting: A literature review. *J Viral Hepat*. 2022;29(9):698-718.

44. Lhomme S, Marion O, Abravanel F, Izopet J, Kamar N. Clinical Manifestations, Pathogenesis and Treatment of Hepatitis E Virus Infections. *J Clin Med*. 2020; 9(2):331.
45. Haagsma EB, Riezebos-Brilman A, van den Berg AP, Porte RJ, Niesters HG. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl*. 2010;16(4): 474-7.
46. Kamar N, Abravanel F, Garrouste C, Cardeau-Desangles I, Mansuy JM, Weclawiak H, et al. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. *Nephrol Dial Transplant*. 2010;25(8): 2792-5.
47. Netzler NE, Enosi Tuipulotu D, Vasudevan SG, Mackenzie JM, White PA. Antiviral Candidates for Treating Hepatitis E Virus Infection. *Antimicrob Agents Chemother*. 2019;63(6):e00003-19.
48. Lampejo T. Sofosbuvir in the Treatment of Hepatitis E virus Infection: A Review of *in vitro* and *in vivo* Evidence. *J Clin Exp Hepatol*. 2022;12(4):1225-37.
49. Cornberg M, Pischke S, Muller T, Behrendt P, Piecha F, Benckert J, et al. Sofosbuvir monotherapy fails to achieve HEV RNA elimination in patients with chronic hepatitis E - The HepNet SofE pilot study. *J Hepatol*. 2020;73(3):696-9.
50. Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet*. 2010;376(9744):895-902.
51. Zaman K, Dudman S, Stene-Johansen K, Qadri F, Yunus M, Sandbu S, et al. HEV study protocol : design of a cluster-randomised, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV 239 (Hecolin) in women of childbearing age in rural Bangladesh. *BMJ Open*. 2020;10(1):e033702.
52. ClinicalTrials.gov. 2021. Available from: <https://clinicaltrials.gov/study/NCT03827395>.
53. Li Y, Huang X, Zhang Z, Li S, Zhang J, Xia N, et al. Prophylactic Hepatitis E Vaccines: Antigenic Analysis and Serological Evaluation. *Viruses*. 2020;12:109.
54. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol*. 2010;82(5):799-805.
55. Al-Absi ES, Al-Sadeq DW, Younis MH, Yassine HM, Abdalla OM, Mesleh AG, et al. Performance evaluation of five commercial assays in assessing seroprevalence of HEV antibodies among blood donors. *J Med Microbiol*. 2018;67(9):1302-9.
56. Cattoir L, Van Hoecke F, Van Maerken T, Nys E, Ryckaert I, De Boule M, et al. Hepatitis E virus serology and PCR: does the methodology matter? *Arch Virol*. 2017;162(9):2625-32.
57. Sommerkorn FM, Schauer B, Schreiner T, Fickenscher H, Krumbholz A. Performance of Hepatitis E Virus (HEV)-antibody tests: a comparative analysis based on samples from individuals with direct contact to domestic pigs or wild boar in Germany. *Med Microbiol Immunol*. 2017;206(3):277-86.
58. Zafrullah M, Zhang X, Tran C, Nguyen M, Kamili S, Purdy MA, et al. Disparities in detection of antibodies against hepatitis E virus in US blood donor samples using commercial assays. *Transfusion*. 2018; 58(5):1254-63.
59. Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF, Osterhaus AD, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. *J Clin Virol*. 2013; 58(4):629-34.
60. Drobeniuc J, Meng J, Reuter G, Greene-Montfort T, Khudyakova N, Dimitrova Z, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis*. 2010;51(3):e24-7.
61. Norder H, Karlsson M, Mellgren A, Konar J, Sandberg E, Lasson A, et al. Diagnostic Performance of Five Assays for Anti-Hepatitis E Virus IgG and IgM in a Large Cohort Study. *J Clin Microbiol*. 2016; 54(3):549-55.
62. Ferguson M, Walker D, Mast E, Fields H. Report of a collaborative study to assess the suitability of a reference reagent for antibodies to hepatitis E virus. *Biologicals*. 2002;30(1):43-8.

63. National Institute for Biological Standards and Control. WHO Reference Reagent for Hepatitis E Virus Antibody, Version 4.0. 2013. Available from: <https://www.nibsc.org/documents/ifu/95-584.pdf>.
64. Lu J, Huang Y, Wang P, Li Q, Li Z, Jiang J, et al. Dynamics of Hepatitis E Virus (HEV) Antibodies and Development of a Multifactorial Model To Improve the Diagnosis of HEV Infection in Resource-Limited Settings. *J Clin Microbiol*. 2021;59(2):e02321-20.
65. Soothill G, Hessey S, Erotocritou M, Griffiths P, Ijaz S, Thorburn D, et al. Diagnostic utility of hepatitis E virus antigen-specific ELISA versus PCR testing in a cohort of post liver transplant patients in a large university hospital. *J Clin Virol*. 2018;106:44-8.
66. Lapa D, Brega C, Mammone A, Zaccarelli M, Capobianchi MR, Garbuglia AR. Diagnostic performance of hepatitis E virus antigen assay in hepatitis E virus acute infection. *New Microbiol*. 2016;40(4):246-50.
67. Montpellier C, Wychowski C, Sayed IM, Meunier JC, Saliou JM, Ankavay M, et al. Hepatitis E Virus Lifecycle and Identification of 3 Forms of the ORF2 Capsid Protein. *Gastroenterology*. 2018; 154(1):211-23 e8.
68. Yin X, Ying D, Lhomme S, Tang Z, Walker CM, Xia N, et al. Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. *Proc Natl Acad Sci U S A*. 2018;115(18):4773-8.
69. Al-Sadeq DW, Majdalawieh AF, Mesleh AG, Abdalla OM, Nasrallah GK. Laboratory challenges in the diagnosis of hepatitis E virus. *J Med Microbiol*. 2018; 67(4):466-80.
70. Abravanel F, Chapuy-Regaud S, Lhomme S, Dubois M, Peron JM, Alric L, et al. Performance of two commercial assays for detecting hepatitis E virus RNA in acute or chronic infections. *J Clin Microbiol*. 2013;51(6):1913-6.
71. Abravanel F, Sandres-Saune K, Lhomme S, Dubois M, Mansuy JM, Izopet J. Genotype 3 diversity and quantification of hepatitis E virus RNA. *J Clin Microbiol*. 2012;50(3):897-902.
72. Tremeaux P, Lhomme S, Chapuy-Regaud S, Peron JM, Alric L, Kamar N, et al. Performance of an antigen assay for diagnosing acute hepatitis E virus genotype 3 infection. *J Clin Virol*. 2016;79:1-5.
73. Chevaliez S, Pawlotsky JM. Hepatitis Viruses. In: Cohen J, Powderly WG, Opal SM, editors. *Infectious Diseases 2*. 4th ed: Elsevier; 2017. p. 1417-25.
74. Thodou V, Bremer B, Anastasiou OE, Cornberg M, Maasoumy B, Wedemeyer H. Performance of Roche qualitative HEV assay on the cobas 6800 platform for quantitative measurement of HEV RNA. *J Clin Virol*. 2020;129:104525.
75. Dalton HR, Fellows HJ, Stableforth W, Joseph M, Thurairajah PH, Warshow U, et al. The role of hepatitis E virus testing in drug-induced liver injury. *Aliment Pharmacol Ther*. 2007;26(10):1429-35.
76. Davern TJ, Chalasani N, Fontana RJ, Hayashi PH, Protiva P, Kleiner DE, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology*. 2011; 141(5):1665-72 e1-9.
77. Sanabria-Cabrera J, Sanjuan-Jimenez R, Clavijo E, Medina-Caliz I, Gonzalez-Jimenez A, Garcia-Cortes M, et al. Incidence and prevalence of acute hepatitis E virus infection in patients with suspected Drug-Induced Liver Injury in the Spanish DILI Registry. *Liver Int*. 2021;41(7):1523-31.
78. European Centre for Disease Prevention and Control. Options for national testing and surveillance for hepatitis E virus in the EU/EEA. 2019. Available from: <https://www.ecdc.europa.eu/en/publications-data/options-national-testing-and-surveillance-hepatitis-e-virus-eueea-operational>.
79. Public Health England. Public health operational guidelines for hepatitis E: Health protection response to reports of hepatitis E infection. 2019. Available from: [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/845090/Public\\_Health\\_Operational\\_Guidelines\\_for\\_Hepatitis\\_E-protection\\_response.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/845090/Public_Health_Operational_Guidelines_for_Hepatitis_E-protection_response.pdf).
80. Bi H, Yang R, Wu C, Xia J. Hepatitis E virus and blood transfusion safety. *Epidemiol Infect*. 2020;148:e158.
81. Domanovic D, Tedder R, Blumel J, Zaaijer H, Gallian P, Niederhauser C, et al. Hepatitis E and blood donation safety in selected European countries: a shift to screening? *Euro Surveill*. 2017; 22(16):pii=30514.

82. Gallian P, Pouchol E, Djoudi R, Lhomme S, Mouna L, Gross S, et al. Transfusion-Transmitted Hepatitis E Virus Infection in France. *Transfus Med Rev.* 2019; 33(3):146-53.
83. Goel A, Vijay HJ, Katiyar H, Aggarwal R. Prevalence of hepatitis E viraemia among blood donors: a systematic review. *Vox Sang.* 2020;115(3):120-32.
84. Izopet J, Lhomme S, Chapuy-Regaud S, Mansuy JM, Kamar N, Abravanel F. HEV and transfusion-recipient risk. *Transfus Clin Biol.* 2017;24(3):176-81.
85. Westholter D, Hiller J, Denzer U, Polywka S, Ayuk F, Rybczynski M, et al. HEV-positive blood donations represent a relevant infection risk for immunosuppressed recipients. *J Hepatol.* 2018; 69(1):36-42.
86. Niederhauser C, Widmer N, Hotz M, Tinguely C, Fontana S, Allemann G, et al. Current hepatitis E virus seroprevalence in Swiss blood donors and apparent decline from 1997 to 2016. *Euro Surveill.* 2018; 23(35):pii=1700616.
87. Christopeit M, Schmidt-Hieber M, Sprute R, Buchheidt D, Hentrich M, Karthaus M, et al. Prophylaxis, diagnosis and therapy of infections in patients undergoing high-dose chemotherapy and autologous haematopoietic stem cell transplantation. 2020 update of the recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO). *Ann Hematol.* 2021;100(2):321-36.
88. Sayed IM, Abdelwahab SF. Is Hepatitis E Virus a Neglected or Emerging Pathogen in Egypt? *Pathogens.* 2022;11(11):1337.
89. Okagbue HI, Adamu MO, Bishop SA, Oguntunde PE, Odetunmbi OA, Opanuga AA. Hepatitis E Infection in Nigeria: A Systematic Review. *Open Access Maced J Med Sci.* 2019;7(10):1719-22.
90. Fernandez Villalobos NV, Kessel B, Rodiah I, Ott JJ, Lange B, Krause G. Seroprevalence of hepatitis E virus infection in the Americas: Estimates from a systematic review and meta-analysis. *PLoS One.* 2022;17(6):e0269253.
91. Wenzel JJ, Preiss J, Schemmerer M, Huber B, Jilg W. Test performance characteristics of Anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. *J Infect Dis.* 2013;207(3):497-500.
92. Horn J, Hoodgarzadeh M, Klett-Tammen CJ, Mikolajczyk RT, Krause G, Ott JJ. Epidemiologic estimates of hepatitis E virus infection in European countries. *J Infect.* 2018;77(6):544-52.
93. Mrzljak A, Dinjar-Kujundzic P, Jemersic L, Prpic J, Barbic L, Savic V, et al. Epidemiology of hepatitis E in South-East Europe in the "One Health" concept. *World J Gastroenterol.* 2019;25(25):3168-82.
94. Mrzljak A, Jemersic L, Savic V, Balen I, Ilic M, Jurekovic Z, et al. Hepatitis E Virus in Croatia in the "One-Health" Context. *Pathogens.* 2021;10(6):3168.
95. Li P, Liu J, Li Y, Su J, Ma Z, Bramer WM, et al. The global epidemiology of hepatitis E virus infection: A systematic review and meta-analysis. *Liver Int.* 2020; 40(7):1516-28.
96. Wilhelm B, Waddell L, Greig J, Young I. Systematic review and meta-analysis of the seroprevalence of hepatitis E virus in the general population across non-endemic countries. *PLoS One.* 2019; 14(6):e0216826.
97. Chatziprodromidou IP, Dimitrakopoulou ME, Apostolou T, Katopodi T, Charalambous E, Vantarakis A. Hepatitis A and E in the Mediterranean: A systematic review. *Travel Med Infect Dis.* 2022; 47:102283.
98. Qashqari FS. Seroprevalence of Hepatitis E Virus Infection in Middle Eastern Countries: A Systematic Review and Meta-Analysis. *Medicina (Kaunas).* 2022;58(7):905.
99. Saade MC, Haddad G, El Hayek M, Shaib Y. The burden of Hepatitis E virus in the Middle East and North Africa region: a systematic review. *J Infect Dev Ctries.* 2022;16(5):737-44.
100. Horvatits T, Ozga AK, Westholter D, Hartl J, Manthey CF, Lutgehetmann M, et al. Hepatitis E seroprevalence in the Americas: A systematic review and meta-analysis. *Liver Int.* 2018;38(11):1951-64.
101. Pisano MB, Campbell C, Anugwom C, Re VE, Debes JD. Hepatitis E virus infection in the United States: Seroprevalence, risk factors and the influence of immunological assays. *PLoS One.* 2022; 17(8):e0272809.
102. Pisano MB, Martinez-Wassaf MG, Mirazo S, Fantilli A, Arbiza J, Debes JD, et al. Hepatitis E virus in South America: The current scenario. *Liver Int.* 2018; 38(9):1536-46.

103. Moraes D, Mesquita JR, Dutra V, Nascimento MSJ. Systematic Review of Hepatitis E Virus in Brazil: A One-Health Approach of the Human-Animal-Environment Triad. *Animals (Basel)*. 2021;11(8): 2290.
104. Raji YE, Toung OP, Mohd Taib N, Sekawi ZB. A systematic review of the epidemiology of Hepatitis E virus infection in South - Eastern Asia. *Virulence*. 2021;12(1):114-29.
105. Meester M, Tobias TJ, Bouwknecht M, Kusters NE, Stegeman JA, van der Poel WHM. Infection dynamics and persistence of hepatitis E virus on pig farms - a review. *Porcine Health Manag*. 2021;7(1):16.
106. Avellon A, Morago L, Garcia-Galera del Carmen M, Munoz M, Echevarria JM. Comparative sensitivity of commercial tests for hepatitis E genotype 3 virus antibody detection. *J Med Virol*. 2015;87(11): 1934-9.
107. Khudyakov Y, Kamili S. Serological diagnostics of hepatitis E virus infection. *Virus Res*. 2011; 161(1):84-92.
108. Koyuncu A, Mapemba D, Ciglenecki I, Gurley ES, Azman AS. Setting a Course for Preventing Hepatitis E in Low and Lower-Middle-Income Countries: A Systematic Review of Burden and Risk Factors. *Open Forum Infect Dis*. 2021; 8(6):ofab178.

© 2023 Roche

Roche Diagnostics International Ltd

[diagnostics.roche.com](https://diagnostics.roche.com)