cobas®

Roche

Elecsys® Anti-HEV IgG

Immunoassay for the quantitative determination of IgG antibodies to the hepatitis E virus (HEV) in human serum and plasma

Summary

HEV is the etiological agent of hepatitis E and is regarded as an emerging pathogen of global public health concern.[1,2] HEV, which is classified as species *Paslahepevirus balayani* in the family Hepeviridae, genus *Paslahepevirus*, includes 8 genotypes, of which HEV 1-4 are the most frequently detected globally. HEV-1 and HEV-2 infect only humans, whereas HEV-3 and HEV-4 infect humans but also several animal species such as pig, boar, rabbit and deer.[3-9] It is estimated that HEV-1 and HEV-2 account for approximately 20.1 million HEV infections, 3.4 million symptomatic cases, 70,000 deaths, and 3000 stillbirths annually.[10,11]

HEV is an icosahedral, non-enveloped, positive-sense, single-stranded RNA virus with a diameter of 27-34 nm. The RNA genome of 7.2 kb has 3 open reading frames (ORFs): ORF1, encoding non-structural proteins involved in viral replication; ORF2, encoding the viral capsid protein important for virion assembly and immunogenicity; and ORF3, encoding a protein essential for virus release.[1,3,12-16]

HEV-1 and HEV-2 are commonly found in developing countries with poor sanitation,[1,4,17] and they are typically transmitted through the fecal-oral route. HEV-3 and HEV-4 are prevalent in both developing and developed nations and they are transmitted zoonotically.[1,13,18] In several countries, occasional HEV-3 transmission through blood transfusion has been reported.[1,12,19]

HEV infection usually causes a mild or subclinical infection with a self-limiting illness that lasts from 2 to 6 weeks.[20,21] Symptomatic hepatitis E is similar to other acute hepatitis infections (fatigue, nausea, vomiting as well as jaundice and elevated liver enzymes).[20] High-risk populations are immunocompromised patients,[16,20,22,23] patients with underlying liver conditions, and elderly people and pregnant women.[3,12,14,24-26] In pregnant women, HEV-1 infection may lead to severe clinical outcomes with a mortality rate of up to 30%.[5,14,20,27,24,25] Vertical transmission from mother to fetus can cause premature birth and perinatal mortality.[28-31] Acute and chronic HEV-3 and HEV-4 infections have also been associated with extra-hepatic manifestations, especially neurological and renal disorders.[16,20,26,31]

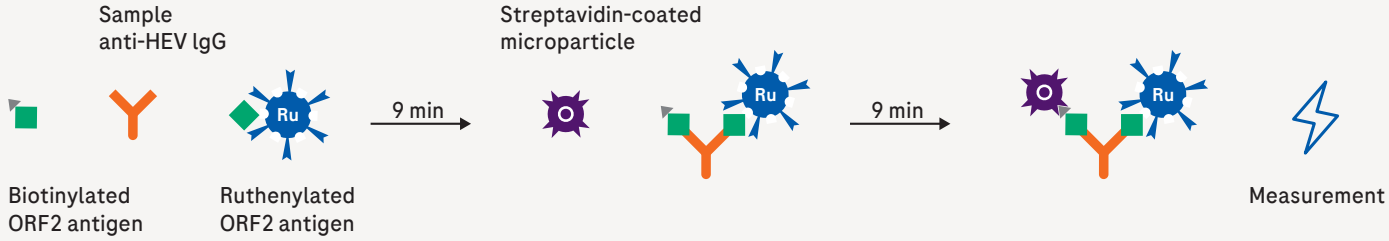
Around 3 weeks post-infection, HEV RNA becomes detectable in blood and stool, with viremia lasting approximately 3-6 weeks, and shedding of virus in stool for approximately 4-6 weeks.[31] IgM antibodies against the HEV capsid protein are detectable in serum after 1-4 weeks for up to 6-9 months post infection, and are a key marker of recent or current infection.[3,12,16,25,26,31,32] Anti-HEV IgG antibodies appear around the same time or soon after anti-HEV IgM antibodies. They are an indicator of recent and past infection and usually persist for several years.[12,25,26,31,33]

Testing for hepatitis E is recommended in all patients presenting symptoms consistent with acute hepatitis, patients with unexplained flares of chronic hepatitis, in all immunosuppressed patients with unexplained abnormal liver function tests, and in case of suspected drug-induced liver injury (DILI).[16,31] In pregnant women, antenatal screening for HEV antibodies should be considered.[34] Acute HEV infection can be diagnosed by the detection of anti-HEV antibodies (IgG, IgM, or both) in serum or plasma, in combination with testing for HEV RNA. Serological testing alone relies upon the combined detection of anti-HEV IgM and rising anti-HEV IgG titers.[31] Past infection is determined by the presence of anti-HEV IgG.[20,31,35,36]

The Elecsys® Anti-HEV IgG assay uses recombinant proteins based on structural domains of HEV ORF2 (genotype 1 and 3) as antigens in a double-antigen sandwich assay format for the quantitative determination of IgG antibodies to HEV. The quantitative assay result is also qualitatively interpreted for the detection of anti-HEV IgG. Measurement of anti-HEV IgG is intended as an aid, in conjunction with other laboratory results and clinical information, in the diagnosis of acute HEV infection in combination with detection of anti-HEV IgM or HEV RNA, as part of the differential diagnosis of acute hepatitis to enable timely initiation of medical interventions, in assessing the immune status to HEV, in estimating the risk of HEV reinfection, or in detecting past HEV infections in seroepidemiological studies.[37]

Electrochemiluminiscence immunoassay (ECLIA)[37]

Test principle: double-antigen sandwich assay (testing time: 18 mins)



1st incubation (9 minutes)
20 µL*/12 µL** of sample, biotinylated recombinant HEV ORF2 antigen, and recombinant HEV ORF2 antigen labeled with a ruthenium complex form a sandwich complex.

2nd incubation (9 minutes)
After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Measurement
The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

* on **cobas**® e 411 analyzer and **cobas** e 601 / 602 modules ** on **cobas** e 402 and **cobas** e 801 analytical modules

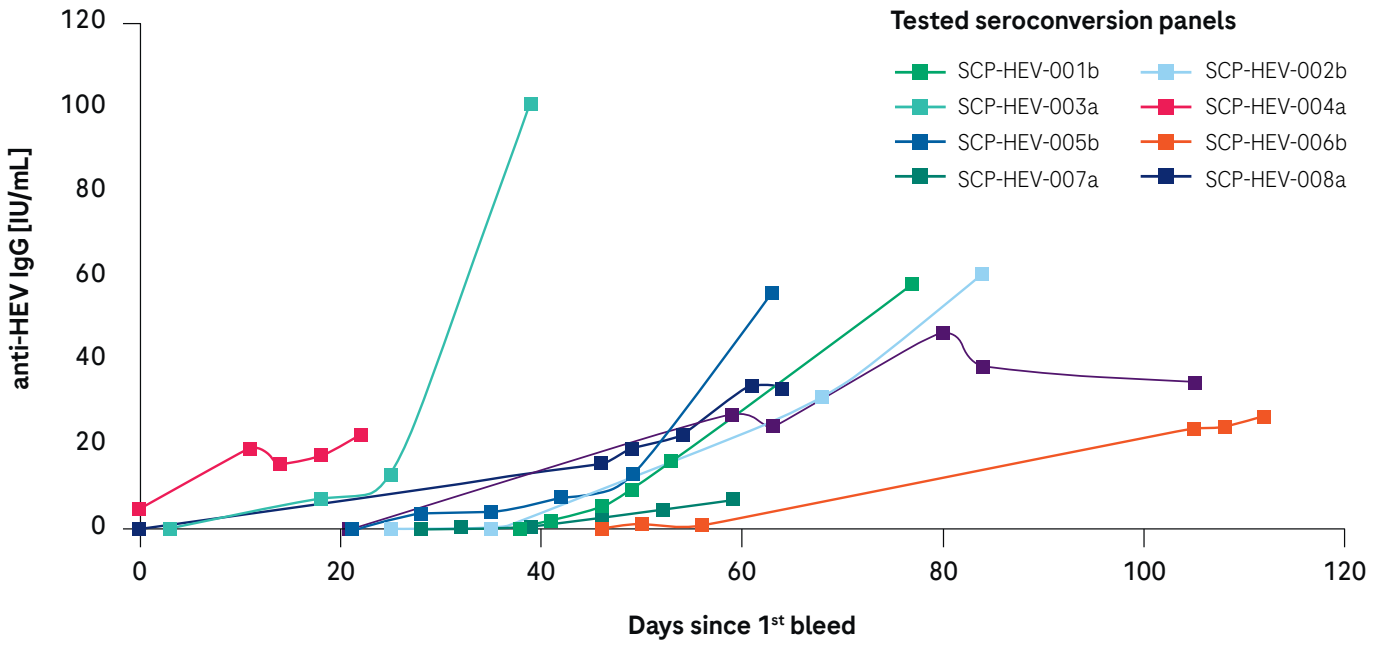
Elecsys Anti-HEV IgG assay characteristics[37]

Systems	**cobas**® e 411 analyzer **cobas** e 601 / **cobas** e 602 modules	**cobas** e 402 analytic module **cobas** e 801 analytic module
Testing time	18 minutes	
Test principle	double-antigen sandwich assay, quantitative	
Calibration	Individual 2-point calibration	
Traceability	WHO Reference Reagent for Hepatitis E Virus Antibody (NIBSC code: 95/584)	
Measuring range	0.05-25 U/mL; Limit of Detection = 0.025 U/mL	
Interpretation	< 0.15 U/mL = non-reactive for anti-HEV IgG ≥ 0.15 U/mL = reactive for anti-HEV IgG	
Specimen types	Serum collected using standard sampling tubes or tubes containing separating gel. Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA and Na-citrate plasma. Plasma tubes containing separating gel can be used.	
Sample volume	20 µL	12 µL
Onboard stability	8 weeks	16 weeks
Intermediate precision in positive samples	**cobas** e 411: CV* 2.1 – 2.7 % **cobas** e 601/602: CV 1.0 – 1.3 %	CV 1.1 – 1.3 %

* coefficient of variation

Seroconversion sensitivity[37]

Seroconversion sensitivity of the Elecsys Anti-HEV IgG assay was shown by testing 9 commercial seroconversion panels in comparison to 5 other registered anti-HEV IgG assays. The Elecsys Anti-HEV IgG assay detected anti-HEV IgG in 84 out of a total of 112 panel members, while the comparison assays detected 76 (+ 1 borderline, Mikrogen recomWell HEV IgG), 79 (+ 1 borderline, Euroimmun Anti-HEV ELISA IgG), 80 (bioMérieux VIDAS Anti-HEV IgG2 and Wantai HEV IgG ELISA), and 81 (DiaSorin Liaison Murex Anti-HEV IgG), respectively. In addition, anti-HEV IgG titer assessment indicated rising antibody titers in the seroconversion panels over time.



Relative sensitivity[37]

A total of 596 samples (440 samples from patients with presumed acute HEV infection and 156 samples from patients recovered from a hepatitis E infection) were tested with the Elecsys Anti-HEV IgG assay and 3 commercially available anti-HEV IgG assays at 3 different study sites. Samples from patients with presumed acute hepatitis E infection included 252 samples from Europe (endemic for HEV genotype 3) and 188 samples from Vietnam and Bangladesh (endemic for HEV genotype 1). Additionally, 50 samples (confirmed genotype 4) were measured at one study site in China with the Elecsys Anti-HEV IgG assay and 3 anti-HEV IgG assays commercially available in China. Samples were considered positive if the result was reactive in all of the comparator assays.

Cohort	N	Confirmed positive samples	Elecsys Anti-HEV IgG congruent positive	Sensitivity (95 % CI)
Presumed acute*	440	380	375	98.7 % (97.0 – 99.6 %)
Presumed acute**	50	48	48	100 % (92.6 – 100 %)
Recovered from HEV infection	156	141	141	100 % (97.4 – 100 %)
Overall	**646**	**569**	**564**	**99.1 % (98.0 – 99.7 %)**

* included 252 samples from Europe (endemic for HEV-3) and 188 samples from Vietnam and Bangladesh (endemic for HEV-1);
** from China (confirmed genotype 4); CI: confidence interval, 2-sided

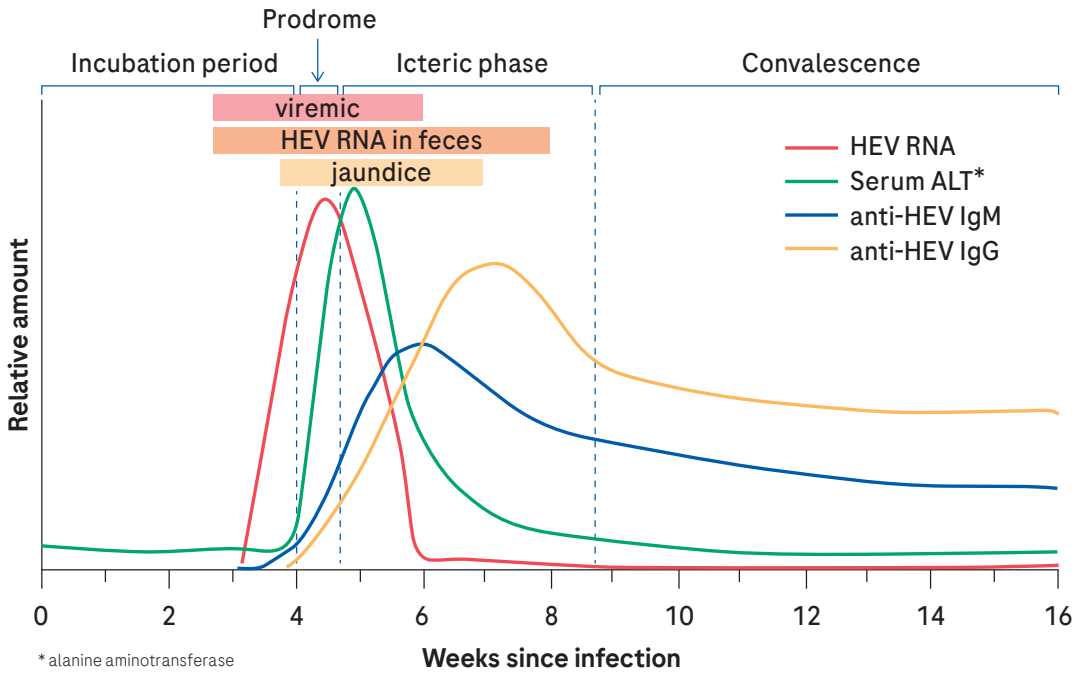
Relative specificity[37]

A total of 8011 samples from blood donors (n = 5040), diagnostic routine (n = 2427) and pregnant women (n = 544) were tested at 4 centers in Europe with the Elecsys Anti-HEV IgG assay and 3 commercially available anti-HEV IgG assays. Samples were considered negative for anti-HEV IgG if they were non-reactive in 2 out of 3 comparator assays. Discrepant Elecsys Anti-HEV IgG reactive samples were resolved using an in-house neutralization assay based on an alternative recombinant HEV antigen. Samples were considered true positives if, in the presence of the neutralizing HEV antigen, they showed a significantly lower anti-HEV IgG concentration in the Elecsys Anti-HEV IgG assay.

Cohort	N	Confirmed negative samples	Elecsys discrepant positive samples*	Specificity (95 % CI)
Blood donors	5040	4055	4	99.88 % (99.75 – 99.97 %)
Daily routine	2427	1434	9	99.37 % (98.81 – 99.71 %)
Pregnant women	544	435	1	99.77 % (98.73 – 99.99 %)
Overall	**8011**	**5924**	**14**	**99.76 % (99.60 – 99.87 %)**

* no significant reduction of anti-HEV IgG reactivity after neutralization with an alternative recombinant HEV antigen; CI: confidence interval, 2-sided

Course of HEV infection and diagnostic markers [2,7,16]



* alanine aminotransferase

Order information

Product	Material configuration	Material Number
Elecsys Anti-HEV IgG [a)]	100 tests	09 044 639 190
Elecsys Anti-HEV IgG [b)]	300 tests	09 044 647 190
PreciControl Anti-HEV IgG	16 × 1.3 mL	09 044 655 190

a) for use on the **cobas**® e 411 analyzer and the **cobas** e 601 / 602 modules; b) for use on the **cobas** e 402 and **cobas** e 801 analytical units

References

1 Khuroo MS, et al. Hepatitis E: Discovery, global impact, control and cure. World J Gastroenterol. 2016;22:7030-7045.

2 Webb GW, Dalton HR. Hepatitis E: an underestimated emerging threat. Ther Adv Infect Dis. 2019;6:2049936119837162.

3 Li P, et al. The global epidemiology of hepatitis E virus infection: A systematic review and meta-analysis. Liver Int. 2020;40(7):1516-1528.

4 Goel A, Aggarwal R. Hepatitis E: Epidemiology, Clinical Course, Prevention, and Treatment. Gastroenterol Clin North Am. 2020;49:315-330.

5 Raji YE, et al. Hepatitis E Virus: An emerging enigmatic and underestimated pathogen. Saudi J Biol Sci. 2022;29:499-512.

6 Kamar N, et al. Hepatitis E. Lancet. 2012;379:2477-2488.

7 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:a032136.

8 Smith DB, et al. Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). J Gen Virol. 2020;101:692-698.

9 Purdy MA, et al. ICTV Virus Taxonomy Profile: Hepeviridae. 2022. J Gen Virol 2022;103(9).

10 World Health Organization (WHO). Hepatitis E vaccine: WHO position paper. Wkly Epidemiol Rec. 2015;18:185-200.

11 Rein DB, et al. The global burden of hepatitis E genotypes 1 and 2 in 2005. Hepatology. 2012;55:988-997.

12 Al-Sadeq DW, et al. Laboratory challenges in the diagnosis of hepatitis E virus. J Med Microbiol. 2018;67(4):466-480.

13 Izopet J, et al. Hepatitis E virus infections in Europe. J Clin Virol. 2019;120:20-26.

14 Aggarwal R. Diagnosis of hepatitis E. Nat Rev Gastroenterol Hepatol. 2013;10:24-33.

15 Blasco-Perrin H, et al. Hepatitis E, the neglected one. Liver Int. 2016;36 Suppl 1:130-134.

16 Lhomme S, et al. Screening, diagnosis and risks associated with Hepatitis E virus infection. Expert Rev Anti Infect Ther. 2019;17:403-418.

17 Ruggeri FM, et al. Zoonotic transmission of hepatitis E virus in industrialized countries. New Microbiol. 2013;36:331-44.

18 World Health Organisation. Factsheet Hepatitis E. Last updated June 2022 [Internet, updated 2022 June 24, cited 2023 June 2]. Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-e.

19 Pérez-Gracia MT, et al. Hepatitis E: an emerging disease. Infect Genet Evol. 2014;22:40-59.

20 Kamar N, et al. Hepatitis E virus infection. Nat Rev Dis Primers. 2017;3:17086.

21 Lhomme S, et al. Hepatitis E Pathogenesis. Viruses. 2016;8:212.

22 Larrue H, et al. Hepatitis E, what's the real issue? Liver Int. 2020;40(Suppl 1):43-47.

23 Lhomme S, et al. Risk of zoonotic transmission of HEV from rabbits. J Clin Virol. 2013;58:357-362.

24 Zhao Q, et al. Antigenic determinants of hepatitis E virus and vaccine-induced immunogenicity and efficacy. J Gastroenterol. 2013;48:159-168.

25 Aggarwal R. Hepatitis E: clinical presentation in disease-endemic areas and diagnosis. Semin Liver Dis. 2013;33:30-40.

26 Velavan TP, et al. Hepatitis E: An update on One Health and clinical medicine. Liver Int 2021;41:1462-1473.

27 Wu C, et al. Hepatitis E virus infection during pregnancy. Virol J. 2020;17:73.

28 Jilani N, et al. Hepatitis E virus infection and fulminant hepatic failure during pregnancy. J Gastroenterol Hepatol. 2007;22(5):676-682.

29 Khuroo MS, et al. Clinical course and duration of viremia in vertically transmitted hepatitis E virus (HEV) infection in babies born to HEV-infected mothers. J Viral Hepat. 2009;16:519-523.

30 Li M, et al. Hepatitis E virus infection and its associated adverse feto-maternal outcomes among pregnant women in Qinhuangdao, China. J Matern Fetal Neonatal Med. 2020;33:3647-3651.

31 European Association of the Study of the Liver (EASL). Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol. 2018;68(6):1256-1271.

32 Kar P, Karna R. A Review of the Diagnosis and Management of Hepatitis E. Curr Treat Options Infect Dis. 2020;12:310-320.

33 Zhou Y. Immunobiology and Host Response to HEV. Adv Exp Med Biol. 2016;948:113-141.

34 Sultana R, et al. IgG antibody response demonstrates inverse correlation with viral load in Bangladeshi women with acute hepatitis E virus genotype 1 infection. Int J Infect Dis. 2021;104:482-490.

35 Bendall R, et al. 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;82:799-805.

36 European Centre for Disease Prevention and Control (ECDC). Options for national testing and surveillance for hepatitis E virus in the EU/EEA - operational guidance [Internet, updated 2019 Sep 5, cited 2023 June 2]. Available at: https://www.ecdc.europa.eu/en/publications-data/options-national-testing-and-surveillance-hepatitis-e-virus-eueea-operational.

37 Elecsys Anti-HEV IgG (Mat. Nos. 09044639190 & 09044647190) method sheets. V1 2023-09.

Published by
Roche Diagnostics International Ltd
CH-6343 Rotkreuz
Switzerland

diagnostics.roche.com